BIOLOGICAL AND CHEMICAL MANAGEMENT OF PHYTOPHTHORA ROOT ROT / COLLAR ROT IN CITRUS NURSERY

R. M. GADE
Department of Plant Pathology,
Panjabrao Deshmukh Krishi Vidyapeeth,
Akola - 444 104 (MS) INDIA
e-mail: gadermg@gmail.com

KEYWORDS
Citrus jambhiri
Phytophthora parasitica
Trichoderma
Pseudomonas
Fungicides

ABSTRACT
Citrus jambhiri Lush is the most widely used root stock for most of citrus scion in India. Soil samples were collected from nurseries of Vidarbha region in India. Almost all samples were found associated with P. parasitica (28-46 cfu/g soil) when tested on PARPH medium. Range of reaction was found tolerant root stock whereas, Citrus jambhiri was found susceptible to the disease. In vitro antagonism showed that P. parasitica significantly inhibited by T. harzianum and T. virens (84.96%). However, intensity of antagonism was different as per medium. There was a continuous reduction in pathogen population from 41 to 8 propagules/g soil with reduction in root rot/collar rot in Citrus jambhiri. All Thirty seven native isolates of Pseudomonas spp. were found positive for production of IAA, HCN and Siderophore. Pf XXVI (16.80%) and Pf IV (24.10%) were found effective to manage the disease in addition to increased growth response under glass house condition. Among chemicals, seed treatment with Metalaxyl @2.5 g/kg seed f.b spraying of metalaxyl at 45 and 90 days after emergence was effective to manage the disease (8.36%).

INTRODUCTION
Phytophthora spp. are the causal agents of several serious diseases of citrus in India. Phytophthora parasitica, P. citrophthora and P. palmivora have been mostly involved in causing damping off, collar rot and root rot in citrus (Naqvi, 1988; Bowman et al., 2007; Shekari et al., 2012). However, Phytophthora parasitica and P. palmivora are the most prevalent species in citrus orchards of Vidarbha region (Das et al., 2011). It remains a threat and a persistent problem wherever, citrus is grown that can result in substantial tree loss particularly trees on susceptible root stock Whiteside (1974). Supply of poor quality sampling may result in root rot, root rot or gummosis in orchard. These are the most important soil borne diseases of citrus causing mortality of newly planted trees and a slow decline and yield loss of mature trees (Graham and Menge, 1999). Epidemics of Phytophthora on heavy black cotton soils play an important role in citrus root stock failure. Survey have been undertaken to see the association of soil borne diseases of citrus causing mortality of newly planted trees and to reduce propagules of Phytophthora in citrus (Koche, 2011). In the present study, objective was to identify resistant root stocks to

MATERIALS AND METHODS
Field nursery samples were taken from primary nursery of the citrus up to transplantation of seedling in secondary nursery beds. The number of samples collected varied with the size of nursery. At least 10 soil cores were taken from root zone from several rows of the field nursery. The samples were placed in plastic bags to maintain soil moisture, transported to laboratory, and assayed as described by Timmer et al. (1988). 10g soil
from each sample was diluted in 90mL water having 0.25 % agar. One mL aliquot was spread on each of 10 plates of PARPH selective medium (Kannwischer and Mitchell, 1978). The plates were incubated at 28°C for 2-3 days and no. of colonies of Phytophthora was counted. Soil in the second core was flooded with water, bailed with pieces of citrus leaves, and placed in the incubator for 48h (Grimm and Alexander, 1973). The leaves were transferred to petridishes and examined for the presence of papillate sporangia.

**Screening of root stocks**

Seeds of rough lemon (Citrus jambiri), cleopatra mandarin (C. reticula Blanco) and Rangpur lime (Citrus limonia) were sown in pots. Pure culture of Phytophthora parasitica was maintained on V-8 juice agar by serial transfer. Chlamydospores of each isolate were produced in V-8 juice broth by the method of Tsao (1971) for use as inoculum. The soil was mixed manually to produce an inoculum concentrate.

To determine the propagule concentration, 1g samples of the soil was mixed manually to produce an inoculum concentrate. To determine the propagule concentration, 1g samples of the inoculum concentrate were plated on a selective medium containing pimaricin, ampicillin, rifampcin, penicil hloronitrobenzene and hymexazol (PARPH) (Timmer, 1988). The inoculum concentrate of each isolate was added to autoclaved fine sand to provide a density of 31-33 propagules/g of soil. Non inoculated pots were treated as control. For all treatments, 10 single seedlings in each replication and seven replications per treatment were used for each of the rootstock and placed in a completely random design in the greenhouse. Each pot was watered to runoff twice a day. Greenhouse conditions ranged from 25 to 35°C and 60 to 100% relative humidity. Seedlings were evaluated each of the rootstock and placed in a completely random design. Data for the three rootstocks were analyzed in randomized block design.

**Growth inhibition test**

Purified cultures were maintained on corn meal agar. A 5 mm disc of Phytophthora culture was placed at one side of the previously plated 90mm diameter with 20mL of different media (V-8 juice, CMA, PDA and 2% Agar) and 5 mm disc of Trichoderma (individual species) was placed at opposite side of Phytophthora disc. These Petri plates were incubated at 25°C. Three plates were used for each replication and three replications were used for each treatment.

**Characterization of Pseudomonas**

**Siderophore production**

Production of siderophore by *P. fluorescens* was assessed by Plate assay method as described by Schwyn and Neilands (1987).

**Hydrocyanic acid production**

HCN production was tested by the method of Castic and Castric (1983).

**Indole acetic acid production**

Indole Acetic acid production was tested according to (Gorden and Webber, 1951).

**Gelatin liquefaction**

The test indicated utilization of protein and production of proteolytic enzymes by bacterium and to differentiate *Pseudomonas fluorescens* and *P.putida*

**Oxidase test**

Take an inoculating loop or toothpick. Then touch and spread a well isolated colony on an oxidase disk (Disk contains N, N-dimethyl-p-phenylenediamine oxalate and o-naphthpol).The reaction was observed within 2 minutes at 25-30°C. Deep purple blue indicate positive reaction.

**Arginine test**

For Arginine test media was made according to the method of Fay and Berry (1972). Purple colour indicates positive reaction and yellow colour or no colour change indicates negative reaction.

**Nitrate reduction**

The nitrate broth medium will be inoculated with the bacteria and inoculated at 37°C for 48h or until the next period. To each tube 1mL of sulphanillic acid and naphylamineacetate is to be added. Reduction of nitrite to nitrate is indicated by the production of distinct red colouration. Comparison was made with the blank. On the basis of characteristics and by dual culture test antifungal activity of *P. fluorescens* isolates were identified and selected on the basis of their inhibition activity for further study.

**Use of antagonist and fungicides for disease management**

*Citrus jambiri* seeds which is a susceptible root stock to Phytophthora bacterized with *P. fluorescens* isolates (Pf-I, Pf-IV, Pf-XXVI) @10g/kg seed and for *Trichoderma* spp. seeds were treated @4g/kg seed. For fungicides, seeds were treated with the formulation of metalaxyl @2.5 g/kg seed and 2g/L water for spraying similar concentrations were used for fosetyl - AL. Similar procedure was followed as described in screening of root stock for development of sickness in soil.

**RESULTS**

Survey of Amravati and Nagpur district citrus nurseries was done where nearly 80 lakhs grafts of citrus are being produced every year. All most all the samples in Nagpur and Amravati district were tested positive to Phytophthora. Propagule densities of soil was in the range of 28.00-38.67 cfu/g soil in Amravati and 29.11 to 46.33 cfu/g soil in Nagpur district. However, Phytophthora was not detected in each one of the field nursery of Amravati and Nagpur district. The leaf baiting technique detected more positive samples as compared to selective medium when the propagule density was high. It was failed to produce results when the propagule density was low (Table 1).

**Screening of root stock**

All three rootstock screened in this experiment were polyembryonic and produce high proportion of seedlings. Seedling emergence began at nearly 21 DAS. Post emergence seedling root rot were noted in all three rootstocks. In the first two weeks after emergence of the seedlings death was preceded by severe leaf yellowing and necrosis at collar region of the seedlings (Table 2). Per cent root rot was significantly
Table 1: Occurrence of Phytophthora among citrus nurseries in Amravati and Nagpur District of Vidarbha region

<table>
<thead>
<tr>
<th>Locations</th>
<th>District No. of samples</th>
<th>Selective Media</th>
<th>Positive in leaf baiting technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>of positive</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>No of Samples</td>
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<td>g soil</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>1. Amravati</td>
<td>09</td>
<td>08</td>
<td>28.00</td>
</tr>
<tr>
<td>2.</td>
<td>13</td>
<td>13</td>
<td>38.67</td>
</tr>
<tr>
<td>3.</td>
<td>06</td>
<td>04</td>
<td>31.50</td>
</tr>
<tr>
<td>4.</td>
<td>10</td>
<td>08</td>
<td>34.50</td>
</tr>
<tr>
<td>5.</td>
<td>04</td>
<td>04</td>
<td>29.67</td>
</tr>
<tr>
<td>6.</td>
<td>15</td>
<td>11</td>
<td>34.67</td>
</tr>
<tr>
<td>7.</td>
<td>08</td>
<td>06</td>
<td>32.83</td>
</tr>
<tr>
<td>8.</td>
<td>11</td>
<td>07</td>
<td>36.29</td>
</tr>
<tr>
<td>9.</td>
<td>06</td>
<td>05</td>
<td>28.67</td>
</tr>
<tr>
<td>10.</td>
<td>05</td>
<td>04</td>
<td>35.50</td>
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<td>11.</td>
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<td>07</td>
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<tr>
<td>12.</td>
<td>08</td>
<td>06</td>
<td>34.67</td>
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<td>13.</td>
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<td>0.00</td>
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<tr>
<td>14.</td>
<td>06</td>
<td>06</td>
<td>35.67</td>
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<tr>
<td>15.</td>
<td>10</td>
<td>07</td>
<td>35.29</td>
</tr>
<tr>
<td>Nagpur</td>
<td>08</td>
<td>08</td>
<td>35.33</td>
</tr>
<tr>
<td></td>
<td>06</td>
<td>05</td>
<td>30.00</td>
</tr>
<tr>
<td></td>
<td>04</td>
<td>02</td>
<td>34.67</td>
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<tr>
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<td>0.00</td>
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<tr>
<td></td>
<td>05</td>
<td>04</td>
<td>29.11</td>
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<td></td>
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<td>33.11</td>
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<td>04</td>
<td>36.50</td>
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<td>06</td>
<td>04</td>
<td>29.67</td>
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<td></td>
<td>03</td>
<td>01</td>
<td>46.33</td>
</tr>
<tr>
<td></td>
<td>03</td>
<td>03</td>
<td>30.67</td>
</tr>
</tbody>
</table>

Values in parenthesis are arc sin means

Table 2: Response of citrus root stock to Phytophthora parasitica

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Root Fresh wt (g)</th>
<th>Root Shoot Length (cm)</th>
<th>Root rot (%)</th>
<th>CD (P = 0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rangapur Lime</td>
<td>2.07</td>
<td>41.60</td>
<td>26.67 (29.76)</td>
<td>10.03</td>
</tr>
<tr>
<td>Rough lemon</td>
<td>2.19</td>
<td>38.57</td>
<td>33.11</td>
<td>0.96</td>
</tr>
<tr>
<td>Cleopatra mandarin</td>
<td>2.56</td>
<td>46.20</td>
<td>26.67</td>
<td>1.03</td>
</tr>
<tr>
<td>CD</td>
<td>0.10</td>
<td>1.03</td>
<td>2.74</td>
<td>1.44</td>
</tr>
</tbody>
</table>

Table 3: Assessment of Antagonism of Trichoderma spp. against Phytophthora parasitica on different medium

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>Mean colony diameter (mm)</th>
<th>2% Agar</th>
<th>Per cent inhibition</th>
<th>2% Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V-8 juice</td>
<td>CMA</td>
<td>PDA</td>
<td>V-8 juice</td>
</tr>
<tr>
<td>Trichoderma virens</td>
<td>16.70</td>
<td>17.40</td>
<td>10.30</td>
<td>5.20</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>17.30</td>
<td>18.00</td>
<td>10.60</td>
<td>6.10</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>17.30</td>
<td>17.60</td>
<td>10.60</td>
<td>6.00</td>
</tr>
<tr>
<td>Trichoderma hamatum</td>
<td>18.00</td>
<td>18.60</td>
<td>13.00</td>
<td>6.50</td>
</tr>
<tr>
<td>Control</td>
<td>90.00</td>
<td>90.00</td>
<td>70.50</td>
<td>8.30</td>
</tr>
<tr>
<td>CD (P = 0.01)</td>
<td>0.41</td>
<td>0.49</td>
<td>0.28</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table 4: Characteristics of P. fluorescens, P. putida and P. aeruginosa

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>P. fluorescens</th>
<th>P. putida</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Isolates</td>
<td>19</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>- ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+ ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Arginine test</td>
<td>+ ve</td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>IAA</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>HCN</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Siderophore</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
</tbody>
</table>

All Trichoderma spp and P. fluorescens significantly reduce root rot and stimulated the growth of the seedlings of Citrus jambhiri. T. virens was found effective to lower down propagule count of Phytophthora in the soil (8 cfu/g soil). Seed treatment with metalaxyl @ 2.5 g/kg seed f.b. spraying @ 0.2% at 45 and 90 DAE was found significantly superior to manage root rot per cent than all other treatments but at par with seed treatment with metalaxyl @ 2.5 g/kg seed f.b. spraying of allite @0.2% at 75 DAE. Amongst bioagents P. fluorescens XXVI (16.80%) was found effective to lower down the root rot incidence f.b. T. virens (17.50%). Root (2.92g) and shoot fresh wt. (8.51g) were found maximum in Cleopatra mandarin.

DISCUSSION

Field nurseries were found contaminated with Phytophthora. It may be due to rising of nurseries on same piece of land and

The mycelial growth of P. parasitica except control. T. virens was found significantly superior to inhibit the mycelial growth of P. parasitica on the entire medium (V-8 juice 16.70mm, CMA 17.40mm, PDA 10.30mm, 2% Agar 5.20mm) as compared to all other treatments.
to the roadside to attract the customer and also there is no restriction to any person to enter in the nursery. Shekari et al. (2012) determined the soil population of each species and found that 82 and 86% of the orchards were infested respectively with P. citrophthora and P. nicotianae with average over 10 propagules/g soil. Ridings et al., (1977) showed that even with strict sanitary practices, recontamination of disinfected areas occurred when it was present near to the nursery. The use of selective medium was as effective in the detection of Phytophthora as the leaf baiting technique. Therefore, selective medium would be useful for detection of Phytophthora spp. where laboratory facilities are available (Zitko et al., 1987). Three citrus rootstocks were screened to identify those that were highly resistant to root rot. Root rot incidence was observed low in Rangpur lime and Cleopatra mandarin. It suggests that Rangpur lime show potentiality as superior root stock because of their high tolerance to Phytophthora root rot (Armarkar, 2011). Cleopatra mandarin and sour orange are said to be highly resistant to infection by Phytophthora (Timmer et al., 1988). However, Cleopatra mandarin was recognized as susceptible to Phytophthora (Anonymous, 1991). CMA and V-8 Juice was found suitable medium for the growth of Phytophthora. Faster growth was observed on CMA, whereas, lowest growth rate was recorded in 2 % Agar (Naqvi, 2005). Meyer and Abdallah (1978) reported that Pseudomonas spp. are all members of the same intrageneric homology group. They include P. aeruginosa, P. putida and P. fluorescens. They are well known for production of broad spectrum antibiotics. It is proved to be a major mechanism involved in their biocontrol activity (O’ Sullivan and O’ Gara, 1992). HCN and siderophore produced by Pseudomonas spp. were also involved in their antifungal activity. Voisard et al., (1989) observed suppression of black rot of tobacco was due to the production of HCN by P. fluorescens and also HCN induced resistance in the host plant. In the present study, all selected antifungal Pseudomonas isolates were observed to produce HCN in vitro, which might have contributed for their biocontrol activity in addition to antibiotics (Gade and Armarkar, 2011). One of the proposed mechanisms of plant growth promotion by bacteria was production of IAA, cytokinin and Gibberellins (Glick, 1991). All four species of Trichoderma tested in the experiment provide significant disease control and enhanced plant growth (Jagtap et al., 2012). The reduction of root rot by T. spp. may be due to high antagonistic potential that includes antibiosis, parasitism and production of lytic enzymes (Singh et al., 2004). Trichoderma viride recorded minimum mean colony diameter on 2% agar and highest inhibition 85.39% of mycelial growth of P. nicotianae on PDA over untreated control followed by the bioagent T. viride and T. harzianum. P. fluorescens XXVI and IV were also found effective to manage the disease in addition to plant growth promotion activity. Yang et al., (1994) reported that Pseudomonas putida 6909 and Pseudomonas fluorescens 09906 suppressed population of Phytophthora parasitica in the citrus rhizosphere, suggesting these bacteria may be useful in control of citrus root rot (Gade et al., 2008). Amongst fungicides, seed treatment with metalaxyl @2.5g/kg seed f.b. spraying @0.2% at 45 and 90 DAE was found effective. Naqvi (1993) conducted an experiment to determine effect of certain systemic and non systemic fungicide on soil population of Phytophthora parasitica and found that metalaxyl was more promising than fosetyl-AL because metalaxyl directly kills the pathogen in vivo. However, Graham and Timmer (2003) reported that metalaxyl and fosetyl AL are highly effective against Phytophthora spp.; they are often used routinely by nurserymen to suppress Phytophthora populations and reduced root rot damage in citrus nursery stock. Gade and Giri (2005) observed significant reduction in population of Phytophthora sp. (11.25 cfu/g soil) in beds priorly treated with solarization and then drenched with metalaxyl @ 0.2% alternate at bimonth interval. Significant decrease in mortality was also recorded in same treatment (5.78%) with added benefit of increase in height and girth of Citrus jambhiri seedlings (Gade et al., 2008). Drenching of metalaxyl @ 0.2 per cent reduced mortality in citrus caused due to Phytophthora (13.9%) with added benefit of plant height (52.9cm) and girth (Gade et al., 2005).

From the survey of citrus nurseries it is observed that field nurseries must have an alternative which is found to be contaminated with Phytophthora. Root stocks study warranted the use of Citrus jambhiri which is found susceptible to Phytophthora. Production of antibiotics, IAA, HCN and siderophore by P. fluorescens and production metabolites by Trichoderma spp. will play major role in suppression of root rot and enhancement of plant growth in citrus nurseries.

### Table 5: Effect of bioagents and fungicides on occurrence of Phytophthora propagules /g soil, root and shoot fresh wt. and per cent root rot due to P. parasitica

<table>
<thead>
<tr>
<th>Bioagents/ Fungicides</th>
<th>Cfu/g soil</th>
<th>Root rot(%)</th>
<th>Root fresh wt (g)</th>
<th>Shoot fresh wt.(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichoderma virens</td>
<td>08</td>
<td>17.50 (24.73)</td>
<td>2.72</td>
<td>7.92</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>12</td>
<td>19.60 (26.26)</td>
<td>2.54</td>
<td>7.20</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>11</td>
<td>26.40 (30.91)</td>
<td>2.65</td>
<td>7.25</td>
</tr>
<tr>
<td>Trichoderma hamatum</td>
<td>21</td>
<td>32.70 (34.87)</td>
<td>2.36</td>
<td>6.93</td>
</tr>
<tr>
<td>Pseudomonas fluorescens I</td>
<td>22</td>
<td>29.20 (32.70)</td>
<td>2.33</td>
<td>6.10</td>
</tr>
<tr>
<td>Pseudomonas fluorescens IV</td>
<td>16</td>
<td>24.10 (29.38)</td>
<td>2.87</td>
<td>8.34</td>
</tr>
<tr>
<td>Pseudomonas fluorescens XXVI</td>
<td>12</td>
<td>16.80 (24.19)</td>
<td>2.92</td>
<td>8.51</td>
</tr>
<tr>
<td>Seed treatment with metalaxyl @2.5g/kg</td>
<td>11</td>
<td>8.56 (17.01)</td>
<td>2.56</td>
<td>7.16</td>
</tr>
<tr>
<td>Seed f.b. spraying @0.2% at 45 and 90 DAE</td>
<td>21</td>
<td>9.71 (18.14)</td>
<td>2.40</td>
<td>6.92</td>
</tr>
<tr>
<td>Seed treatment with metalaxyl @2.5g/kg</td>
<td>11</td>
<td>8.56 (17.01)</td>
<td>2.56</td>
<td>7.16</td>
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<tr>
<td>Seed f.b. spraying of Alliette @0.2% at 75 DAE</td>
<td>41</td>
<td>44.20 (41.67)</td>
<td>1.38</td>
<td>3.95</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>1.67</td>
<td>0.62</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Values in parenthesis are arc sin means; DAE- Days after emergence
REFERENCES


