Effect of different bio-agents and botanicals on leaf spot (Colletotrichum capsici Syd.) of betelvine plants (Piper betel L.) in vitro

Anoop Kumar, Sunil Zacharia, Ghanshyam Kumar Pandey and Mohd Danish

Abstract
Betel vine is popularly known as Pan in India. Pan is the local vernacular Hindi name used for leaves of betel vine plant (Piper betel L.). The present research work was conducted at Department of Plant Pathology, SHUATS, Allahabad, U. P. during 2016-2017. The result revealed that treatments viz., Trichoderma viride, Bacillus subtilis, Neem oil, Tobacco leaf extract against leaf spot C. capsici of betelvine. While, per cent inhibition of the test pathogen ranged from 12.21 to 71.02 per cent. Among fungal bio-control agents maximum inhibition of 71.02 per cent was recorded with T. viride followed by T. harzianum (70.52%), T. viride (68.54%), T. subtilis (28.55%), and T. Neem oil (62.19%) found maximum inhibition. However, the minimum inhibition of per cent was recorded on Tobacco leaf extract (12.21%).

Keywords: Anthracnose, Bacillus subtilis, betelvine (Piper betel L.), copper oxychloride, neem oil, tobacco leaf extract, Trichoderma harzianum, and Trichoderma viride

Introduction
The deep green heart shaped leaves of betel vine are popularly known as Pan in India. Pan is the local vernacular Hindi name used for leaves of betel vine plant (Piper betel L.). It is a perennial creeper belonging to family Piperaceae. On account of its immense medicinal, social, religious and export value, betel vine is a cash crop of economic importance and is extensively grown on large scale in different parts of India. India is the largest producer and exporter of betel leaves in the world (Arulmozhiyan et al., 2005) [1]. In India, Madhya Pradesh is the leading State in betel vine production. Approximately 15 different varieties are cultivated in MP. The vine is a shade loving perennial root climber. There are about 100 varieties of betel vine in the world, of which about 40 are found in India and 30 in West Bengal (Guha, 1997; Maity, 1989; Samanta, 1994). The most probable place of origin of betel vine is Malaysia (Chattopadhyay and Maity, 1967).

Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, ringworm, swelling of gum, rheumatism, abrasion, cuts and injuries etc. as folk medicine while the root is known for its female contraceptive effects (Chopra et al., 1956; Khanna, 1997). Not only that, the betel leaves really do not have any match as a cheap, natural and easily available appetizer, digestive, mild stimulant, aphrodisiac and refreshing mastication. Chewing of betel leaves produce a sense of well-being, increased alertness, sweating, salivation, hot sensation and energetic feeling with exhilaration. It also increases the capacity to exercise physical and mental functions more efficiently for a longer duration but it may produce a kind of psychoactive effect causing a condition of mild addiction leading to habituation and withdrawal symptoms (Chu, 2001; Garg and Jain, 1996). The pan leaves are affected by common agents which introduce microbial pathogens onto leaf surface, they are agency of wind, its direction and presence of microbial load, agency of water and its properties, soil, its properties and during post-harvest stages, factors like packaging material, moisture content and finally water in which the leaves are submersed before they are converted into betel quid. The leaves, which are subjected to these factors, develop leaf diseases like ‘Leaf Spot’ and ‘Leaf Blight’ resulting in economic loss to distributors, shop keepers and people involved in this trade (CSIR, 1984).
Out of the diseases, anthracnose caused by *Colletotrichum capsici* (Syd.) Butler and Bisby and bacterial leaf spot caused by *Xanthomonas campestris* pv. *betlicola* (Patel, Kulkarni and Dhande) Dye, Phytophthora leaf and foot rot caused by *P. palmivora* and basal rot caused by *Sclerotium rolfsii* are the main yield limiting factors of the betelvine cultivation all over India. The pathogen *X. c. pv. betlicola* has been renamed as *Xanthomonas axonopodis* pv. *betlicola* (Vauterin et al., 1995). The leaf spot of betelvine has been also reported to be caused by a fungus – bacterium complex (Bhale et al., 1985; Deka et al., 2005). In such leaf spot, *C. capsici* is always associated with the bacterium *X. a. pv. betlicola*. Since, betel leaves are consumed fresh; considerable emphasis has been given to the less persistent and more eco-friendly means of managing betelvine disease. The diseases complex can be effectively controlled by the chemicals like 0.5% Bordeaux mixture or 0.1% copper – oxychloride (Yadav et al., 1993).

### Materials and Methods


For Pathogenicity test surface sterilized (0.1% HgCl₂) seeds of anthracnose susceptible betelvine Cv. *JS*-335 were sown (@ 10 seeds/pot) in the earthen pots (25 cm dia) filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1). Five healthy growing betelvine plants per pot were maintained, watered regularly and kept in the screen house for further growth. The mass multiplication of test pathogen (*Colletotrichum capsici*) was done on the PDA in petri-plates. Spore suspension of the test pathogen was prepared by harvesting freshly sporulating 7-8 days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore-cum-mycelial suspension was filtered through double-layered muslin cloth and filtrate obtained was suitably diluted with sterile distilled water to get inoculum concentration of 3-5 x 10⁶ spores/ml. Thirty days old seedlings of betelvine were artificially inoculated by spraying the conidial suspension (3-5 x 10⁶ conidial/ml) of the test pathogen with automizer. Seedlings sprayed with sterile water (without inoculum) were also maintained as suitable control. Inoculated plants were incubated in the screen house where high humidity (>80%) and optimum temperature (24±2 °C) were maintained for further development of anthracnose symptoms. Subsequently reisolation of the pathogen was done and identification and symptomatology were studied.

### Poisoned Food technique

The fungicides and plant extracts amended PDA was poured (15 - 20 ml/plates) in sterilized Petri plates (90 mm dia.) under aseptic conditions. Each treatment with respective concentration was replicated thrice. On solidification of PDA in Petri plates, all treatment plates were inoculated / seeded aseptically by placing in the center with 5.0 mm uniform, mycelial disc obtained from 7 days old culture of *Colletotrichum capsici* multiplied on agar plates (Nene and Thapliyal, 1993) [15].

### Dual culture technique

Disc (5 mm) of *Colletotrichum capsici* was placed at the center on a petri-plates containing solidified PDA medium and disc (5 mm) of *Trichoderma harzianum* was placed at opposite from center. A loopful of 24 hour old culture of *Pseudomonas fluorescens* was inoculated at 2 cm just opposite to the pathogen on each plate (Dennis and Webster, 1971) [14].

All the treatment (inoculated) and control petri plates where then incubated at 24 ± 2 °C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen. Observations on radial mycelial growth of *Colletotrichum capsici* were recorded in each treatment and replication and per cent growth inhibition of the test pathogen over control was worked out by following formula (Vincent, 1927).

\[
\text{Percent inhibition (I)} = \frac{C - T \times 100}{C}
\]

Where

- \( C \) = Growth of test fungus (mm) in control plate,
- \( T \) = Growth of test fungus (mm) in treatment plates

### Results

The results obtained are being presented as follows. Identification of pathogen is done through a piece of sporulating mycelium which was mounted with lactophenol cotton blue and observed under the light microscope. Based on typical symptoms on foliage and pods, cultural characteristic of the fungus on PDA and microscopic observations recorded such as, mycelium - hyaline, septate and branched. Acervuli - the acervuli were oval to conical and appeared single. It was dark brown to black in colour and measured 181.0 X 275.5μ in size, with numerous black, needles like intermixed long and short setae. Conidia - single celled, smooth, hyaline, curved and measured 21 to 23.5 X 3.8 to 4.1 μ in size. Conidiophores were simple and elongated. The measurements were recorded with the help of stage and ocular micrometer. The fungus was identified and confirmed with the help of available literature (Anisworth 1973) as *Colletotrichum capsici* Syd., causing anthracnose of betelvine.

### Symptomatology

Initial symptoms of anthracnose on foliage were noticed at 125-130 DAT on betelvine crop. The most prominent symptoms occurred on foliage were brown coloured patches with grey coloured centre on upper surface and scorched appearance on the lower surface. In advance stage necrosis of leaf vein, leaf rolling, petiole canker, and defoliation occurred. Acervuli on infected leaf resembled small pinkish coloured patches surrounded by the minute blackish brown setae. Infected leaves finally dried out prematurely with shriveled.

### In vitro evaluation of fungicides, botanicals and bioagents

capsici, the results of which are presented in Table No.1 and 2 respectively. The result revealed that all species of bio-agents exhibited antagonistic effect against C. capsici and were found to inhibit significantly the radial growth of the pathogen over check control and T. harzianum (67.17%) and control (0.00%).

Per cent inhibition of the test pathogen ranged from 12.21 to 71.02 per cent. Among fungal bio-control agents, maximum inhibition of 71.02 per cent was recorded with T. harzianum, followed by T. viride, (70.52%), T. frontalis (68.54%), T. - T. harzianum (67.17%), and T. Neem oil (62.19%), found maximum inhibition. The minimum inhibition of per cent was recorded on T. - Tobacco leaf extract (12.21%). Similar results were reported by (Jayakalshmi et al. 1998), (Patel and Joshi 2001) [13], who found the maximum inhibition of Colletotrichum gloeosporioides by Trichoderma viride. Also the maximum per cent inhibition of C. gloeosporioides was achieved due to T. viride as earlier observed by (Haralpatil, 2005) for anthracnose of Piper betle (Patel and Joshi, 2001) [13] for leaf spot of turmeric and (Bhave, 2005) [4] for leaf spot of black pepper.

These results thus support the present findings. Recent works have shown that common plant disease such as root rot disease, wilt, fruit rot and other plant diseases can be managed by Trichoderma spp. (Begum et al., 2010; El Komy et al., 2015; Howell, 2002; Mbarga et al., 2012) [3, 6, 8, 11]. The secondary metabolites secreted by Trichoderma spp. have proven its role in suppressing the growth of pathogenic microorganisms and stimulating the plant growth (Contreras-Cornejo et al., 2015a; Contreras-Cornejo et al., 2015b; Kubicek et al., 2001; Kulig et al., 2000) [9, 10]. Besides, the interaction between plant and Trichoderma spp. successfully regulate root architecture, increase the length of lateral and primary root that result in the effectiveness of nutrient uptake by the plant (Cai et al., 2013; Naseby et al., 2000; Yedidia et al., 2001) [5, 12].

Table 1: Antagonistic activity of bio-agent on Colletotrichum capsici by dual culture

<table>
<thead>
<tr>
<th>Treatments</th>
<th>2nd day (24hr)</th>
<th>3rd day (48hr)</th>
<th>4th day (72hr)</th>
<th>5th day (96hr)</th>
<th>6th day (120hr)</th>
<th>Mean Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radial growth (mm)</td>
<td>Inhibition (%)</td>
<td>Radial growth (mm)</td>
<td>Inhibition (%)</td>
<td>Radial growth (mm)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>Control (Inoculated)</td>
<td>8.33</td>
<td>0.00</td>
<td>19.17</td>
<td>0.00</td>
<td>28.50</td>
<td>0.00</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>7.5</td>
<td>9.96</td>
<td>11.00a</td>
<td>42.62</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>T. viride</td>
<td>7.00</td>
<td>7.92</td>
<td>11.67a</td>
<td>34.79</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>6.33</td>
<td>24.01</td>
<td>15.83</td>
<td>17.42</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>S. Ed. (±)</td>
<td>2.453</td>
<td>2.363</td>
<td>3.70a</td>
<td>4.68</td>
<td>0.00</td>
<td>12.21</td>
</tr>
<tr>
<td>C.D. (P = 0.05)</td>
<td>2.363</td>
<td>2.386</td>
<td>2.415</td>
<td>1.702</td>
<td>2.696</td>
<td>2.561</td>
</tr>
</tbody>
</table>

Table 2: Antifungal activity of fungicide and botanicals on Colletotrichum capsici by food poisoned technique

<table>
<thead>
<tr>
<th>Treatments</th>
<th>2nd day (24hr)</th>
<th>3rd day (48hr)</th>
<th>4th day (72hr)</th>
<th>5th day (96hr)</th>
<th>6th day (120hr)</th>
<th>Mean Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radial growth (mm)</td>
<td>Inhibition (%)</td>
<td>Radial growth (mm)</td>
<td>Inhibition (%)</td>
<td>Radial growth (mm)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>Control (Inoculated)</td>
<td>8.33</td>
<td>0.00</td>
<td>19.17</td>
<td>0.00</td>
<td>28.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Copper oxychloride</td>
<td>7.67</td>
<td>13.97</td>
<td>12.50a</td>
<td>39.12</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Neem oil</td>
<td>5.83</td>
<td>1.92</td>
<td>8.00c</td>
<td>11.32</td>
<td>8.83</td>
<td>15.79</td>
</tr>
<tr>
<td>Tobacco leaf extract</td>
<td>8.17a</td>
<td>30.01</td>
<td>17.00a</td>
<td>58.27</td>
<td>24.00b</td>
<td>69.02</td>
</tr>
<tr>
<td>S.Ed. (±)</td>
<td>0.589</td>
<td>0.770</td>
<td>0.789</td>
<td>0.896</td>
<td>0.867</td>
<td>0.867</td>
</tr>
<tr>
<td>C.D. (P = 0.05)</td>
<td>1.831</td>
<td>2.394</td>
<td>2.453</td>
<td>2.787</td>
<td>2.696</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions
Present research work concluded that the pathogen Colletotrichum capsici was found to be associated with anthracnose of betelvine and in vitro results were revealed that Bacillus subtilis grew quickly and inhibited pathogen mycelial growth by 71.02%, followed by T. harzianum (70.52%), but botanical Neem oil inhibited pathogen mycelial growth by 62.19%.

Acknowledgement
The author is grateful to the Head of Department of Plant Pathology for providing the necessary facilities to carry out the present research work.

References