In vitro efficacy of phyto-extracts against Fusarium oxysporum f. sp. udum causing wilt disease of pigeonpea

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Abstract
Fusarium oxysporum f. sp. udum is one of the most devastating soil-borne diseases causing wilt of Pigeonpea. The aim of present investigation was to evaluate the antifungal activities of phyto-extracts which can be used to control wilt disease of pigeonpea. Results revealed that all the 12 botanicals tested (each @ 10 and 20 µg/ml) exhibited a wide range of radial mycelial growth of F. udum and it was decreased considerably with increase in concentration of the test botanicals from 10 to 20 per cent. The phyto-extracts / botanicals viz, Allium cepa, Lantana camara, Osimum sanctum, Gliricidia sepium, Azadirachta indica, Allium sativum, Bougainvillea spectabilis, Moringa oleifera, Eucalyptus globulus, Pongamia pinnata, Vinca rosea and Asparagus racemosus were evaluated in vitro for their antifungal activities against wilt of pigeonpea, F. udum using poison food technique. Average mycelial growth inhibition recorded in the test botanicals was ranged from 28.89 (Bougainvillea spectabilis) to 77.23 per cent (Azadirachta indica). However it was significantly highest in Azadirachta indica (77.23%) followed by Allium sativum (76.11%), Osimum sanctum (64.08%), Allium cepa (59.26%), Eucalyptus globulus (60.19%), Lantana camara (57.41%), Pongamia pinnata (56.30%), Vinca rosea (53.71%), Asparagus racemosus (40.00%), Glycerridae maculate (39.63%), Moringa oleifera (36.48%) and Bougainvillea spectabilis (28.89%).

Keywords: Pigeonpea wilt, Fusarium oxysporum f. sp. udum in vitro, phyto-extracts, botanicals, Azadirachta indica, Allium sativum

Introduction
Pigeonpea [Cajanus cajan (L.) Millsapugh] is known by more than 350 vernacular names, the most popular being arhar, yellow dhal, red gram, tur (India), congo pea, gandul, guandu (Brazil), angola pea (United Kingdom), catjang pea, ambrevade, pois d’angdie (French-speaking West Africa), quinchochoncho (Venezuela). Archaeological finds of pigeonpea dating about to 3400 years ago (14th century BC) have been found at Neolithic sites in Karnataka state of India (Sanganekallu) and its border areas (Tuljapur Garhi in Maharashtra state and Gopalpur in Orissa state) and also the south Indian states such as Kerala, where it is called Tomara Payaru. From India it traveled to East Africa and West Africa. There, it was first encountered by Europeans, so it obtained the name Congo Pea. By means of the slave trade, it came to the American continent, probably in the 17th century. Pigeonpea occupies a prominent place in Indian rainfed agriculture. It is the second most important pulse crop next to chickpea, covering an area of around 4.42 m ha (occupying about 14.5% of area under pulses), production of 2.86 MT (contributing to 16% of total pulse production) and productivity of about 707 kg/ha. Deep roots improve physical properties of the soil and pulpize the soil. The plants shed large amount of leaves, this biomass adds organic matter to soil. Besides, it also leaves 30-50 kg ‘N’ to the succeeding crop and also benefiting the inter-cropped cereals through increased ‘N’ supply. Pigeonpea in some areas is an important crop for green manure, providing up to 90 kg nitrogen per hectare. India is producing 14.76 million tonnes of pulses from an area of 23.63 million hectares, which is one of the largest pulses producing countries in the world. However, about 2-3 million tons of pulses are imported annually to meet the domestic consumption requirement. Thus, there is a need to increase production and productivity of pulses in the country by more intensive interventions. Maharashtra state (32.37%) ranks first in area under pigeonpea crop followed by Karnataka (18.76%), Andhra Pradesh (12.75%), Uttar Pradesh (10.14%), Madhya Pradesh (9.64%) and Gujarat (6.69%), while in case of production of pigeonpea, again Maharashtra state (39.24%) ranks first and followed by Karnataka (17.57%), Uttar Pradesh (11.85%), Andhra Pradesh (10.94%), Gujarat (10.65%) and Madhya Pradesh (7.86%).
The crop is sensitive to water logging, even for a very short period. Injurious irrigations may make the crop prone to Fusarium wilt. It is the most important disease of pigeonpea in India resulting in yield losses up to 67 per cent at maturity and 100 per cent in case of infection at pre-pod stage (Kannaiyan and Nene, 1981) [4]. The Fusarium wilt in pigeonpea was first reported from Bihar by Butler (1910) [2]. Surveys conducted for the disease by Kannaiyan et al. (1984) [5] have indicated it to be a major problem in the states of Bihar and Maharashtra (Reddy et al., 1990) [10]. Fusarium wilt characterized by wilting of the affected plants and characteristic internal browning or blackening of the xylem vessels extending from root system to stems. Partial wilting of the plants (Upadhayay and Rai, 1992) [14] and patches of dead plants (Reddy et al., 1993) [11] were reported to be common in the fields during advanced stages of plant growth. Fusarium udum is soil borne and is capable of saprophytic survival on crop residues in the soil for up to eight years (Nene, 1980). Chemical control of the disease is therefore difficult, impractical and uneconomical, as the large scale soil application of chemicals required is expensive, hazardous and disturbs the biological balance (Songa, 1990). Hence, investigation was carried out with in vitro evaluation of phyto-extracts for control of Fusarium oxysporum f. sp. udum causing wilt diseases of Pigeonpea.

Materials and Methods

The experiment was conducted at Department of plant pathology, College of Agriculture Parbhani, VNMKV, Parbhani (M.S.). The pathogen was isolated from diseased root of Pigeonpea on PDA incubated at 27±2°C. Aqueous extracts of twelve botanicals were evaluated in vitro (each @ 10 and 20%) against F. udum. Leaf / bulb / rhizome extract of the test botanicals were prepared by grinding with mixture-cum-grinder. Washed 100 g each leaves / Turmeric rhizome / Onion bulb / Garlic cloves were macerated separately in 100 ml distilled water (w/v) and the macerates obtained were filtered separately through double layered muslin cloth. Each of the filtrate obtained was further filtered through Whatman No. 1 filter paper using funnel and volumetric flasks (100 ml cap). The final clear extracts obtained formed the standard plant extracts of 100 per cent concentration. These were evaluated (each @ 10% and 20%) in vitro against F. udum, by applying Poisoned Food Technique (Nene and Thapliyal, 1993) [8] and using Potato dextrose agar (PDA) as basal culture medium.

Quantified 20 ml of potato dextrose agar media was poured in sterilized petri plates and to be solidified. Fungal disks of 10 mm diameter were placed in the center of the petri dish containing medium under aseptic condition, incubated at 27±2 °C for 7 days. Three replicated plates were used for each concentration of every phyto-extracts. Three replicated PDA plates received no phyto-extracts served as control. Diameter of the colonies on PDA with and without phyto-extracts was measured from the bottom side of the petri dishes. The colony diameter of the fungus pathogens on medium was recorded and per cent inhibition was calculated by using following formula (Vincent, 1927) [15]:

\[
\text{Per cent inhibition} = \frac{\text{T} - \text{C}}{\text{C}} \times 100
\]

Where,

\[
\text{T} = \text{growth of the test fungus in treated plates}
\]

\[
\text{C} = \text{growth of the test fungus in untreated control plates}
\]

Results and Discussion

Results revealed (Table 1 and Fig. 1) that at 10 per cent, mycelial growth inhibition was ranged from 25.19 (Bougainveillia spectabilis) to 68.52 per cent (Azardirachta indica). However, it was significantly highest in Azardirachta indica (68.52%), followed by Allium sativum (64.08%), O. sanctum (59.26%), Eucalyptus globulus (55.19%), Allium cepa (52.59%), Lactana camara (50.74%), Pongamia pinnata (48.15%), Vinca rosea (46.67%), Asparagus racemosus (35.93%), Glyceridia maculate (35.19%), Moringa oleifera (31.48%) and Bouganveillia spectabilis (25.19%). At 20 per cent, mycelial growth inhibition was ranged from 32.59 (Bougainveillia spectabilis) to 85.93 per cent (Azardirachta indica). However it was significantly highest in Azardirachta indica (85.93%) followed by Allium sativum (84.44%), O. sanctum (69.26%), Allium cepa (65.93%), Eucalyptus globulus (65.19%), Lactana camara (64.07%), Pongamia pinnata (61.85%), Vinca rosea (59.26%), Asparagus racemosus (44.07%), Glyceridia maculate (44.07%), Moringa oleifera (41.48%) and Bouganveillia spectabilis (32.59%).

Average mycelial growth inhibition recorded in the test botanicals was ranged from 28.89 per cent (Bougainveillia spectabilis) to 77.23 per cent (Azardirachta indica). However it was significantly highest in Azardirachta indica (77.23%) followed by Allium sativum (76.11%), O. sanctum (64.08%), Allium cepa (59.26%), Eucalyptus globulus (60.19%), Lactana camara (57.41%), Pongamia pinnata (56.30%), Vinca rosea (53.71%), Asparagus racemosus (40.00%), Glyceridia maculate (39.63%), Moringa oleifera (36.48%) and Bouganveillia spectabilis (28.89%).

These results are in harmony with the findings of those workers who reported plant extracts viz., Allium cepa, A. sativum, Azardirachta indica, O. sanctum, etc. at various concentrations had significantly inhibited mycelial growth of F. udum causing wilt disease in pigeonpea (Kanherkar et al., 2007; Sahani and Saxena 2008; Singh et al., 2010; Mehta et al., 2010; Gawande et al. 2015 and Mishra and Kumar, 2015) [12, 13, 6, 3, 7].

### Table 1: In vitro efficacy of phyto-extracts against F. udum.

<table>
<thead>
<tr>
<th>T. No.</th>
<th>Treatments</th>
<th>Col. dia.*(mm) at conc.</th>
<th>Av. (mm)</th>
<th>% Inhibition</th>
<th>Av. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10% (v/v)</td>
<td>20% (v/v)</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>T₁</td>
<td>Allium cepa (Onion)</td>
<td>42.67</td>
<td>30.67</td>
<td>36.67</td>
<td>52.59</td>
</tr>
<tr>
<td>T₂</td>
<td>Lantana camara (Ghineri)</td>
<td>44.33</td>
<td>32.33</td>
<td>38.33</td>
<td>50.74</td>
</tr>
<tr>
<td>T₃</td>
<td>Oscinium sanctum (Tulsi)</td>
<td>37.00</td>
<td>27.67</td>
<td>32.34</td>
<td>58.89</td>
</tr>
<tr>
<td>T₄</td>
<td>Glyceridia maculate (Glycer)</td>
<td>58.33</td>
<td>50.33</td>
<td>54.33</td>
<td>35.19</td>
</tr>
<tr>
<td>T₅</td>
<td>Azadirachta indica (Neem)</td>
<td>28.33</td>
<td>12.67</td>
<td>20.5</td>
<td>68.52</td>
</tr>
<tr>
<td>T₆</td>
<td>Allium sativum (Garlic)</td>
<td>29.00</td>
<td>14.00</td>
<td>21.5</td>
<td>67.78</td>
</tr>
<tr>
<td>T₇</td>
<td>B. spectabilis (Bouganveillia)</td>
<td>67.33</td>
<td>60.67</td>
<td>64</td>
<td>25.19</td>
</tr>
<tr>
<td>T₈</td>
<td>Moringa oleifera (Drumstick)</td>
<td>61.67</td>
<td>52.67</td>
<td>57.17</td>
<td>31.48</td>
</tr>
</tbody>
</table>

*) ~ 20 ~
Table 9: Eucalyptus globulus (Eucalyptus) 40.33 31.33 35.83 55.19 (47.96) 65.19 (53.83) 60.19 (50.90)
Table 10: Pongamia pinnata (Karanj) 44.33 34.33 39.33 50.74 (45.41) 61.85 (51.87) 56.30 (48.62)
Table 11: Asparagus racemosus (Shatavari) 57.67 50.33 54 35.93 (36.81) 44.07 (41.58) 40.00 (39.20)
Table 12: Control (Untreated) 90.00 90.00 90 0.00 (0.00) 0.00 (0.00) 0.00 (0.00)
S.E. + 2.05 2.04 5.13 1.35 1.40 3.56
C.D. (P=0.01) 5.98 5.97 15.84 3.95 4.09 10.98
*: Mean of three replications, Dia.: Diameter, Av.: Average, Conc.: Concentration
Figures in parentheses are angular transformed values

Fig 1: In vitro efficacy of phyto-extracts against F. udum

References

"21"