Efficacy of some Medicinal Plant Extract Against *Fusarium oxysporum* f. sp. *ciceri* Causing Chickpea Wilt

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ABSTRACT

In the present study, *in vitro* test was carried out to analyze the antifungal potentiality of aqueous extract of five medicinal plants against *Fusarium oxysporum* f. sp. *ciceri* by using poisoned food technique at four different concentrations i.e., 10, 25, 50 and 75%. Among all medicinal plant extracts *Tinospora cordifolia*, *Cymbopogon citratus* and *Moringa oleifera* showed the promising antifungal potentiality against *F. oxysporum* f. sp. *ciceri* with maximum inhibition i.e., 100% at 75% concentration followed by *Zingiber officinale* and *Trachyspermum ammi*, respectively.

Key words: Antifungal activity, fungal diseases, *Fusarium* wilt, management, phytopathogens

INTRODUCTION

Plants are known to be susceptible to different type of plant pathogens which are responsible for the economic loss (Gupta and Sharma, 2008). Plant diseases are caused by many pathogens such as fungi, bacteria, nematodes and viruses (Strange and Scott, 2005). Among all plant pathogens, fungi are the major disease causing agents and can be responsible for about 90% of agricultural yield loss (Maninegalai *et al*., 2011). Pulses are cheap source of protein. Beside protein they are rich source of carbohydrate. Nutrients like phosphorus, vitamin C, riboflavin and amino acids are also major constituent. A variety of pulse crops are grown in India and world. Among all the pulses the major pulses are gram, pigeonpea, lentil, field peas etc. India is the largest producer, consumer and importer of the pulses in the world. It accounts for about 22% of the world pulse production (FAOSTAT., 2009). Chickpea is the world’s second-largest cultivated food grains. However, inspite of abiotic stresses, fungal diseases are major impediments to chickpea production. Among fungal diseases, chickpea wilt caused by *F. oxysporum* f. sp. *ciceri* is one of the most important yield limiting factor in the production of chickpea (*Cicer arietinum* L.). It is seed and soil-borne disease and therefore, it is difficult to eradicate, as fungal spores can survive in soil upto 6 years even in the absence of host plant (Haware *et al*., 1996). For the management of this pathogen, number of chemical fungicides were used but frequent and indiscriminant use of synthetic fungicides posed a serious threat to the environment. Due to the aforementioned problems, the people are now moving towards the use of natural plant extracts as fungicides. Plants contain hundreds or thousands of metabolites. Many herbal plants are the gift of nature to human beings as they have some medicinal property and can be used to control various infections and diseases (Khalil *et al*., 2007). The use of natural products in disease prevention and control has received attention in recent years (Sati and Joshi, 2011; Kilani-Jaziri *et al*., 2011). The medicinal...
plants have been recognized for their antimicrobial activity for many years. *Trachyspermum ammi* is one of the aromatic seed spices which is generally contains thymol, the major phenolic compound, has been reported to be a germicide, antispasmodic and antifungal agent (Shankaracharya et al., 2000). *Tinospora cordifolia* commonly known as ‘Guduchi’ and its dry barks has antispasmodic, anti-pyretic and anti-allergic properties. *Moringa oleifera* seed extracts have been known for their antimicrobial properties (Jamil et al., 2007).

The present study was undertaken to test whether the commonly available natural plant extracts are efficient in inhibiting the growth of the pathogen i.e., *F. oxysporum* f. sp. *ciceri* under *in vitro* condition or not and if so, at which concentration it completely inhibit the mycelial growth of the pathogen.

**MATERIALS AND METHODS**

The present study was undertaken from the month of March-September, 2013 in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow.

**Isolation of *Fusarium oxysporum* f. sp. *ciceri***: Fungal plant pathogen i.e., *F. oxysporum* f. sp. *ciceri* was isolated from the infected plant part of chickpea collected from the area in the vicinity of Sitapur and Lucknow (Uttar Pradesh). Infected plant parts i.e. roots and stems were washed thoroughly under running tap water and then cut into small pieces. Its surface was sterilized with 2% sodium hypochlorite solution. Then plant parts were again washed with distilled water for many times for removal of sodium hypochlorite. These infected plant parts were dried on sterilized filter paper before inoculation on sterilized and cooled Czapek’s-Dox agar medium. The inoculated plates were incubated at 25±2°C and were observed daily for the fungal growth. The identified fungal pathogen was purified by single spore method and preserved for the further analysis.

**Plant collection**: The plants or plant parts were collected in polythene bags from different parts of Lucknow. *Moringa oleifera* bark was collected from the area near South city, *Tinospora cordifolia* leaves were collected from the Babasaheb Bhimrao Ambedkar University, Lucknow campus area, *Zingiber officinale* (rhizome) and *Trachyspermum ammi* (seeds) were purchased from the South city market whereas, *Cymbopogon citratus* leaves were collected from the CIMAP, Lucknow.

**Preparation of aqueous plant extracts and evaluation of antifungal property**: The plant parts were washed under running tap water and then surface sterilized with 1% sodium hypochlorite solution for 2 min and then were washed thoroughly with several changes of distilled water to remove the residual of sodium hypochlorite.

The washed plant parts were air dried under shade. About 200 g of collected plant parts were crushed separately using pestle and mortar by adding equal amount of sterilized distilled water i.e., 200 mL (1:1, w/v). The crushed mass was squeezed through the cheese cloth and then through Vacuum Seitz filter. The necessary amount of filtrate was added in Czapek’s-Dox agar medium separately just before pouring in the petri plates to get the desired concentration of 10, 25, 50 and 75% and gently shaken for thoroughly mixing of the extract. After solidification of agar plates containing the plant extract were inoculated aseptically with the pathogen block of 5 mm diam cut actively growing margin of the culture under aseptic condition. Three replicates were maintained for each treatment. The basal medium (Czapek’s-Dox agar) without any plant extract served as
control. All the inoculated Petri plates were incubated at 25±2°C. The radial growth of the test fungus was observed daily and was measured on 2nd, 4, 6 and 8th day of inoculation and compared with the control.

The percent inhibition of fungal mycelial growth was calculated by using the formula given below:

\[ I = \frac{C - T}{C} \times 100 \]

where, \( I \) is the percent growth inhibition, \( C \) is the colony diameter/radial growth of pathogen in control and \( T \) is the colony diameter/radial growth of pathogen in treatment.

**Data analysis:** The data were expressed as Mean±SD, \( n = 3 \) and were analyzed statistically in completely randomized design (factorial) by using analysis of variance technique. The probability of 0.05 or less was considered as significant. Least significant test were applied to compare the means.

**RESULTS**

The antifungal activity of selected plant extract of the *Trachyspermum ammi* (seeds), *Zingiber officinale* (rhizome), *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark) were tested against *Fusarium oxysporum* f. sp. *ciceri* and were compared with control (Table 1-4). The result showed that the mean radial growth of the test fungus at four different concentrations i.e., 10, 25, 50 and 75% of the plant extract were significantly \((p<0.01)\) different from control at all the four days i.e., 2nd, 4, 6 and 8th day of inoculation.

**On second day of inoculation (Table 1):** The antifungal activity of the aqueous plant extract against the mycelial growth of the *F. oxysporum* f. sp. *ciceri* is presented in the Table 1. It was observed that the mycelial growth at all the four concentrations (10, 25, 50 and 75%) in the presence of five aqueous plant extracts separately were significantly \((p<0.01)\) different from control.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration mean radial growth (mm)</th>
<th>Control</th>
<th>10 (%)</th>
<th>25 (%)</th>
<th>50 (%)</th>
<th>75 (%)</th>
<th>( F^2 )-value</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td></td>
<td>23.63±0.42</td>
<td>17.03±0.15**</td>
<td>13.17±0.21***</td>
<td>8.77±0.25**</td>
<td>5.40±0.20**</td>
<td>**</td>
<td>1.93</td>
</tr>
<tr>
<td>ZO</td>
<td></td>
<td>23.63±0.42</td>
<td>13.20±0.20***</td>
<td>7.23±0.12***</td>
<td>5.63±0.06***</td>
<td>5.03±0.06***</td>
<td>**</td>
<td>1.97</td>
</tr>
<tr>
<td>TC</td>
<td></td>
<td>32.80±0.26</td>
<td>9.33±0.15***</td>
<td>7.54±0.03***</td>
<td>5.40±0.10***</td>
<td>4.00±0.00***</td>
<td>**</td>
<td>1.31</td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>34.73±0.31</td>
<td>7.47±0.42***</td>
<td>6.33±0.06***</td>
<td>5.10±0.10***</td>
<td>0.00±0.00***</td>
<td>**</td>
<td>2.21</td>
</tr>
<tr>
<td>MO</td>
<td></td>
<td>21.57±0.55</td>
<td>10.37±0.72***</td>
<td>8.80±0.26***</td>
<td>6.77±0.25***</td>
<td>0.00±0.00***</td>
<td>**</td>
<td>4.61</td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.48</td>
<td>3.44</td>
</tr>
</tbody>
</table>

Mean±SD \((n = 3)\), Values in parentheses correspond to percent inhibition of *Fusarium oxysporum* f. sp. *ciceri* growth; \( F^{**} \): Significantly different from control at 1% level using LSD test; \( F^{***} \): Significant at 1% level, CV(%): Coefficient of variation. In a column, means followed by a common letter are not significantly different at 1% level using LSD test, TA: *Trachyspermum ammi* (seeds), ZO: *Zingiber officinale* (rhizome), TC: *Tinospora cordifolia* (leaves), CC: *Cymbopogon citratus* (leaves), MO: *Moringa oleifera* (bark)
The mycelial growth (Table 1) of *F. oxysporum* f. sp. *ciceri* at 75% concentration was completely inhibited in the presence of *T. cordifolia* (leaves), *C. citratus* (leaves) and *M. oleifera* (bark) whereas in presence of *T. ammi* (seeds) the mycelial growth was 5.40 mm followed by *Z. officinale* (rhizome) (5.03 mm). It was observed that all the five aqueous plant extracts significantly (p<0.01) inhibited the growth of the test pathogen. It was noticed that out of five only three aqueous plant extracts i.e., *T. cordifolia* (leaves), *C. citratus* (leaves) and *M. oleifera* (bark) completely inhibited (100%) the growth of the test pathogen at 75% concentration followed by *Z. officinale* (rhizome) (78.70%) and *T. ammi* (seeds) (79.41%), respectively. It was observed that at 50% concentration the maximum growth inhibition was by *C. citratus* (leaves) (85.32%) which was significantly (p<0.05) similar to *T. cordifolia* (leaves) (83.53%) followed by *Z. officinale* (rhizome) (76.16%), *M. oleifera* (bark) (68.60%) and *T. ammi* (seeds) (62.89%) which were significantly (p<0.05) different from each other.

The radial growth inhibition of *F. oxysporum* f. sp. *ciceri* at 10 and 25% concentration showed that growth inhibition caused by all the five aqueous plants extracts were significant effective (p<0.01) and significantly (p<0.05) different from each other.

Table 2: Radial growth of *Fusarium oxysporum* f. sp. *ciceri* on 4th day after inoculation in the presence of aqueous plant extract at different concentrations i.e., 10, 25, 50 and 75%

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration mean radial growth (mm)</th>
<th>Control</th>
<th>10 (%)</th>
<th>25 (%)</th>
<th>50 (%)</th>
<th>75 (%)</th>
<th>F-value</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td><strong>33.73±0.38</strong></td>
<td>24.93±0.50**</td>
<td>18.73±0.50**</td>
<td>9.67±0.21**</td>
<td>6.27±0.25**</td>
<td><strong>2.08</strong></td>
<td>(0.00)</td>
<td>(26.09)</td>
</tr>
<tr>
<td>ZO</td>
<td><strong>33.73±0.38</strong></td>
<td>17.20±0.20**</td>
<td>9.53±0.06**</td>
<td>6.43±0.06**</td>
<td>5.10±0.10**</td>
<td><strong>1.39</strong></td>
<td>(0.00)</td>
<td>(49.01)</td>
</tr>
<tr>
<td>TC</td>
<td><strong>41.60±0.46</strong></td>
<td>13.17±0.35**</td>
<td>10.27±0.64**</td>
<td>6.40±0.10**</td>
<td>0.00±0.00**</td>
<td><strong>2.72</strong></td>
<td>(0.00)</td>
<td>(68.34)</td>
</tr>
<tr>
<td>CC</td>
<td><strong>42.20±0.72</strong></td>
<td>10.57±0.51**</td>
<td>7.17±0.15**</td>
<td>5.43±0.12**</td>
<td>0.00±0.00**</td>
<td><strong>3.10</strong></td>
<td>(0.00)</td>
<td>(74.94)</td>
</tr>
<tr>
<td>MO</td>
<td><strong>33.33±0.31</strong></td>
<td>13.23±0.25**</td>
<td>9.57±0.40**</td>
<td>7.43±0.40**</td>
<td>0.00±0.00**</td>
<td><strong>2.45</strong></td>
<td>(0.00)</td>
<td>(60.29)</td>
</tr>
<tr>
<td>F-value</td>
<td><strong>2.08</strong></td>
<td><strong>1.39</strong></td>
<td><strong>2.72</strong></td>
<td><strong>3.10</strong></td>
<td><strong>2.45</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.28</td>
<td>2.44</td>
<td>3.75</td>
<td>3.06</td>
<td>5.33</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±SD (n = 3), Values in parentheses correspond to percent inhibition of *Fusarium oxysporum* f. sp. *ciceri* growth; F**: Significantly different from control at 1% level using LSD test; F***: Significant at 1% level, CV (%): Coefficient of variation. In a column, means followed by a common letter are not significantly different at 1% level using LDB test, TA: *Trachyspermum ammi* (seeds), ZO: *Zingiber officinale* (rhizome), TC: *Tinospora cordifolia* (leaves), CC: *Cymbopogon citratus* (leaves), MO: *Moringa oleifera* (bark).
The percent inhibition in the presence of *C. citratus* (leaves), *T. cordifolia* (leaves), *Z. officinale* (rhizome) and *T. ammi* were significantly (p<0.05) different from each other. At 10% concentration all the treatments were significantly (p<0.05) different from each other. The highest percent of inhibition was recorded in presence of *C. citratus* (leaves) (74.94%) followed by *T. cordifolia* (leaves) (68.34%), *M. oleifera* (bark) (60.29%), *Z. officinale* (rhizome) (49.01%) and *T. ammi* (seeds) (26.09%), respectively.

On sixth day of inoculation (Table 3): The mycelial growth of *F. oxysporum* f. sp. *ciceri* in the presence of all aqueous plant extracts was significantly (p<0.01) different from control. At 75% concentration, *T. cordifolia* (leaves), *C. citratus* (leaves) and *M. oleifera* (bark) showed 100% inhibition of the radial growth of *F. oxysporum* f. sp. *ciceri*. At 50% concentration all the treatments were significantly (p<0.05) different from each other. The percent inhibition ranges between 72.50-89.03%. The minimum inhibition was recorded in presence of *T. ammi* (seeds). At 25% concentration the maximum inhibition was by *C. citratus* (leaves) (84.32%) followed by *T. cordifolia* (leaves) (75.56%), *Z. officinale* (rhizome) (75.16%), *M. oleifera* (bark) (69.60%) and *T. ammi* (seeds) (49.33%), respectively. At this concentration, *T. cordifolia* (leaves) and *Z. officinale* (rhizome) are significantly (p<0.05) similar. At 10% concentration the highest inhibition was observed by *C. citratus* (leaves) (73.94%). All the treatments were significantly (p<0.05) different from each other.

On eighth day of inoculation (Table 4): At 75% concentration all the five aqueous plant extracts were significantly effective against *F. oxysporum* f.sp. *ciceri*. The minimum inhibition was recorded by *T. ammi* (seeds) (88.89%) which is significantly (p<0.05) different from other treatments. At 50% concentration all the treatments were significantly (p<0.05) different from each other. The percent inhibition ranges between 77.48-91.50%. At 25% concentration the highest percent inhibition was recorded by *C. citratus* (leaves) (83.99%) followed by *Z. officinale* (rhizome) (78.92%), *T. cordifolia* (leaves) (77.93%), *M. oleifera* (bark) (73.71%) and *T. ammi* (seeds) (56.76%), respectively. At 10% concentration the percent inhibition of the test pathogen in presence of all aqueous plant extracts were significantly (p<0.05) different. All the treatments were highly effective against *F. oxysporum* f.sp. *ciceri*.

Table 3: Radial growth of *Fusarium oxysporum* f.sp. *ciceri* on 6th day after inoculation in the presence of aqueous plant extract at different concentrations i.e., 10, 25, 50 and 75%

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>10 (%)</th>
<th>25 (%)</th>
<th>50 (%)</th>
<th>75 (%)</th>
<th>F-value</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>47.77±0.45a</td>
<td>33.70±0.20d**</td>
<td>24.20±0.20e**</td>
<td>13.13±0.15f**</td>
<td>6.37±0.06g**</td>
<td>**</td>
<td>0.99</td>
</tr>
<tr>
<td>ZO</td>
<td>47.77±0.45a</td>
<td>22.07±1.01c**</td>
<td>11.87±0.15b**</td>
<td>7.27±0.06a**</td>
<td>5.13±0.06d**</td>
<td>**</td>
<td>2.65</td>
</tr>
<tr>
<td>TC</td>
<td>50.87±0.76c</td>
<td>17.27±0.25b**</td>
<td>12.43±0.21c**</td>
<td>7.10±0.10a**</td>
<td>0.00±0.00e**</td>
<td>**</td>
<td>2.12</td>
</tr>
<tr>
<td>CC</td>
<td>51.87±0.31c</td>
<td>13.47±0.15d**</td>
<td>8.10±0.10a**</td>
<td>5.67±0.06b**</td>
<td>0.00±0.00f**</td>
<td>**</td>
<td>1.02</td>
</tr>
<tr>
<td>MO</td>
<td>45.07±0.12a</td>
<td>18.20±0.20b**</td>
<td>13.70±0.26d**</td>
<td>7.80±0.20c**</td>
<td>0.00±0.00d**</td>
<td>**</td>
<td>1.07</td>
</tr>
<tr>
<td>F-value</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.96</td>
<td>2.32</td>
<td>1.37</td>
<td>1.54</td>
<td>1.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±SD (n = 3), Values in parentheses correspond to percent inhibition of *Fusarium oxysporum* f.sp. *ciceri* growth; F**: Significantly different from control at 1% level using LSD test; F**: Significant at 1% level; CV (%): Coefficient of variation. In a column, means followed by a common letter are not significantly different at 1% level using LSD test, TA: *Trachyspermum ammi* (seeds), ZO: *Zingiber officinale* (rhizome), TC: *Tinospora cordifolia* (leaves), CC: *Cymbopogon citratus* (leaves), MO: *Moringa oleifera* (bark)
**DISCUSSION**

The crude extract of *T. ammi*, *T. cordifolia*, *C. citratus*, *Z. officinale* and *M. oleifera* have antagonistic activity against *F. oxysporum* f.sp. *ciceri*. It was observed that all the five aqueous plant extracts of locally available plants showed the strong antifungal activity. The similar results were reported by many other scientists. Previous study reported that *Tinospora cordifolia* (leaves), *Cymbopogon citratus* (leaves) and *Moringa oleifera* (bark) completely inhibited the growth of *Fusarium oxysporum* f. sp. *lini* at 75% concentration.

The essential oil of *Trachyspermum ammi* have fungi toxic behaviour against *Aspergillus niger*, *A. flavus*, *A. ochraceus*, *Fusarium moniliforme*, *F. graminearum*, *Penicillium citrinum*, *P. viridicatum*, *P. madriti* and *Curvularia lunata* (Singh et al., 2004). *T. ammi* have also been reported to have antibacterial activity against *Salmonella typhi*, *Escherichia coli*, *Lactobacillus* and *Bacillus licheniformis* (Aggarwal and Goyal, 2012). The *T. ammi* essential oil exhibits strong activity against both bacteria and fungi, with greater inhibition of bacterial growth compared with fungi (Gandomi et al., 2014). It contains about 40% (v/v) thymol (Shankaracharya et al., 2000) and is strong germicide, antispasmodic agent and antifungal agent (Mahmoud, 1994). Saxena and Vyas (1986) reported that carvacol and thymol are responsible for the antimicrobial property of *Trachyspermum ammi*. The thymol has ability to kill the bacteria even those which are resistant to third generation antibiotics or multi drugs (Khanuja, 2004). Thymol and carvacol can be bactericidal or bacteriostatic agents depending on their concentration used (Caccioni et al., 2000).

Singh et al. (1980) observed the antifungal activity of essential oil of rhizomes and leaves of *Zinger chrysanthum* against *Alternaria* sp. and *Fusarium*. Weil (2005), White (2007) and Shovan et al. (2008) also studied the antagonistic activity of *Zingiber officinale*. *Tinospora cordifolia* showed the antifungal activity against *Aspergillus niger*, *A. fumigatus*, *Mucor* and *Penicillium* species (Nagaprasanthi et al., 2012). Islam et al. (2014) reported the antibacterial activity of *Zingiber officinale* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella* spp. and *Salmonella* spp. Aquatic extract of fresh Ginger exhibited excellent and best antibacterial activity against both gram positive and gram negative bacteria (Hindi et al., 2014). Mahesh and Satish (2008) studied the antifungal activity of *Tinospora cordifolia* extract against *Aspergillus flavus*, *Dreschlera turcica* and *Fusarium verticillioides* and observed that these were highly antagonistic against the pathogens.
T. cordifolia exhibited a higher inhibitory activity against methicillin-resistant Staphylococcus aureus and Klebsiella pneumonia (Bonvicini et al., 2014). Singh et al. (2013) reported that aqueous extract of Cymbopogon citratus possess a high degree of antifungal activities against the Saprolegnia parasitica. Aqueous extract of Cymbopogon citratus inhibit the growth of Colletotrichum graminicola by 100%. It also prevents the development of Colletotrichum graminicola. Similarly, Trivedi and Singh (2014) reported the antifungal property of C. citratus against Aspergillus flavus and Mucor sp. The Indian lemon grass (Cymbopogon citratus) consists of many organic compounds such as terpenoids but the major component is citral. Other terpenoids in this species include nerol, limonene, linaloale, b-caryophyllene and myrcene (Kasumov and Babaev, 1983). Lemon grass extract prevented the growth of Aspergillus flavus (SGS-421) infestation and Aflatoxin B1 formation in maize grains (Atanda and Olopade, 2013). Lemongrass extract (aqueous) showed the antifungal activity against rot fungus Sclerotium rolfsii Sacc. (Osemweigie et al., 2004).

CONCLUSION

From the result obtained in our present study we can conclude that all the five aqueous plant extract have strong antifungal property. Among these, three medicinal plant extracts (Tinospora cordifolia (leaves), Cymbopogon citratus (leaves) and Moringa oleifera (bark)) showed greater antifungal activities against test pathogen. Therefore, these can be used as natural biocontrol agent.

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REFERENCES


