Assessment of antifungal potentials of some plant extracts against chickpea wilt

Jyoti Srivastava, SK Dwivedi

Abstract
Chickpea enjoys a major share in the pulse cultivation across the globe because of its multifarious uses and its pivotal utility in human diet. Fusarium wilt caused by *Fusarium oxysporum* f.sp. *Ciceri* is amongst one of the major constraints limiting its yield. The present study addresses the efficacy of four plant extracts in controlling the growth of wilt pathogen. For the present study aqueous and alcoholic extracts of *Callistemon lanceolatus*, *indicus*, *Terminalia Arjuna* and *Zizyphus jujuba* were prepared at three different concentrations viz. 100, 200 and 500ppm concentrations. Alcoholic extracts of all the tested plants were superior as compared to their water counterparts at all concentrations. At 500ppm alcoholic extract of *Callistemon lanceolatus* was found to be the most effective among the tested plant varieties inhibiting the growth to (97.55%) followed by *Zizyphus jujuba* (70.62%), *Tamarindus indicus* (61.68%) and *Terminalia arjuna* (58.89%).

Keywords: Chickpea, wilt, plant extracts.

1. Introduction
Grain legumes play a pivotal role in improving the livelihood, nutritional security of farmers and populations in less developed countries as well as in sustainable agriculture of the arid and semi-arid regions worldwide. Chickpea (*Cicer arietinum* L. diploid, 2n=16) is considered to be one of the founder crops of modern agriculture [1]. It is an important source of human food and animal feed and a significant factor in improving soil fertility. Of the many biotic stresses limiting its production in the Mediterranean basin and Indian subcontinent Fusarium wilt caused by *Fusarium Oxysporum* f. sp. *ciceri* is the most notable [2]. Heavy annual losses are encountered due to Fusarium wilt epidemic which may reach 100% under conditions favorable for disease [3-4]. Conventional approach of disease management through chemical pesticides is under public scrutiny due to the potential harmful effects on environment, their undesirable effects on non-target organism and possible carcinogenicity [5]. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market [6]. Other practices like crop rotation soil solarization, pathogen free seed have been employed but with limited success.

Hence persistent effort to seek better alternative is inevitable. An interesting vista is the use of natural plant products in controlling the fungal disease due to their being ecofriendly [7] and positive role in sustainable agriculture. Phytofungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants and could be applied in the form of extract, powders and cakes or as plant exudates [8].Vast fields in developing countries are blessed with abundant plants with fungicidal potential with preparation and application attracting lower capital investment than synthetic fungicides [9]. Plant extract have been tested against *Fusarium oxysporum* for their inhibitory effect and their control efficacy under greenhouse conditions [10-12]. The present study was undertaken to evaluate the potential of some local plant leaves extracts in reducing the population of *Fusarium oxysporum* f.sp. *ciceri*.

2. Material Method
2.1 Isolation and identification of the test fungal strain
The pathogen *Fusarium oxysporum* f. sp. *ciceri* (FOC) used in the present study was isolated from roots of wilt infected chickpea plants collected from the farm fields of Kanpur and
leaves extract as in food poison technique [14]. The medium in and 500ppm were prepared in PDA medium amended with bottles to avoid contamination. Concentrations of 100, 200 instead of water. The extrac ts were poured in screw cap bottles. For the preparation of alcoholic extract alcohol was used for 3 minutes. The filtrate was then taken as 100% stock solution.

sterile water. The leaves were air dried and then grinded with the help of pestle and mortar. It was then crushed with equal amount of water (w/w). Centrifuged at 5000rpm for 15 minutes. The filtrate was then taken as 100% stock solution.

In order to study the effectiv eness of some local trees as botanical toxicant , leaves of 4 medicinal plants species viz. Callistemon lanceolatus, Tamarindus indicus, Terminalia arjuna and Zizyphus jujuba were collected from nearby areas of Kanpur and Unnao district. Fresh plant material was collected in resealable plastic bags.

2.3 Determination of mycellal inhibition by poisoned food technique
In order to study the effectiveness of some local trees as botanical toxicant, leaves of 4 medicinal plants species viz. Callistemon lanceolatus, Tamarindus indicus, Terminalia arjuna and Zizyphus jujuba were collected and washed with sterile water. The leaves were air dried and then grinded with the help of pestle and mortar. It was then crushed with equal amount of water (w/w), Centrifuged at 5000rpm for 15 minutes. The filtrate was then taken as 100% stock solution. For the preparation of alcoholic extract alcohol was used instead of water. The extracts were poured in screw cap bottles to avoid contamination. Concentrations of 100, 200 and 500ppm were prepared in PDA medium amended with leaves extract as in food poison technique [14]. The medium in petriplates was then inoculated from 7 day old culture of the pathogen. The Petri dishes containing media devoid of the pathogen were inoculated from 7 day old culture of the pathogen. The Petri dishes containing media devoid of the pathogen in control plate and dt is growth of the pathogen in treatment set plate.

Table 1: Percent inhibition in the radial growth of Fusarium oxysporum f. sp. ciceri at different concentrations of water and alcohol extracts:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of plants</th>
<th>Concentration in ppm</th>
<th>Radial growth in mm</th>
<th>Percent Inhibition</th>
<th>Radial growth in mm</th>
<th>Alcohol extract</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Callistemon lanceolatus</td>
<td>100</td>
<td>40.43±0.75</td>
<td>40.36±1.11</td>
<td>33.1±1.15</td>
<td>30.69±1.71</td>
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<td></td>
<td></td>
<td>200</td>
<td>29.06±0.90</td>
<td>57.71±1.31</td>
<td>25.26±1.10</td>
<td>63.11±1.00</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>5.4±0.52</td>
<td>92.20±0.76</td>
<td>1.66±0.6</td>
<td>97.55±0.84</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Tamarindus indicus</td>
<td>100</td>
<td>53.13±1.02</td>
<td>21.62±1.51</td>
<td>47.96±1.26</td>
<td>28.54±1.88</td>
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<tr>
<td></td>
<td></td>
<td>200</td>
<td>50.1±1.01</td>
<td>27.11±1.47</td>
<td>42.1±0.85</td>
<td>38.54±1.24</td>
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<tr>
<td></td>
<td></td>
<td>500</td>
<td>37.72±0.64</td>
<td>45.51±0.92</td>
<td>26.13±1.02</td>
<td>61.68±1.50</td>
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<td>3</td>
<td>Terminalia arjuna</td>
<td>100</td>
<td>55.2±0.64</td>
<td>18.48±0.94</td>
<td>50.4±1.25</td>
<td>24.87±1.86</td>
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<td></td>
<td></td>
<td>200</td>
<td>51.76±0.68</td>
<td>24.69±0.99</td>
<td>46.13±1.10</td>
<td>32.65±1.61</td>
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<td></td>
<td></td>
<td>500</td>
<td>43.00±1.00</td>
<td>37.91±1.44</td>
<td>28.1±1.01</td>
<td>58.79±1.48</td>
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<tr>
<td>4</td>
<td>Zizyphus jujuba</td>
<td>100</td>
<td>51.06±1.00</td>
<td>24.68±1.48</td>
<td>46.4±0.52</td>
<td>30.88±0.78</td>
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<td></td>
<td></td>
<td>200</td>
<td>44.06±1.10</td>
<td>35.89±1.60</td>
<td>41.13±0.80</td>
<td>39.95±1.17</td>
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<td></td>
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<td>500</td>
<td>29.61±1.21</td>
<td>57.26±1.75</td>
<td>20.03±1.05</td>
<td>70.62±1.54</td>
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<tr>
<td></td>
<td>Control</td>
<td>100</td>
<td>67.8±1.31</td>
<td>Control</td>
<td>67.13±1.80</td>
<td>68.5±1.32</td>
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<td></td>
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<td>200</td>
<td>68.74±1.09</td>
<td>Control</td>
<td>69.26±0.64</td>
<td>68.2±0.9</td>
<td></td>
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</table>

Values shown are the mean ± SD of 3 replicates, significant at p<0.05.
Fig 1: Radial colony growth inhibition (%) of FOC at 100ppm

Fig 2: Radial colony growth inhibition (%) of FOC at 200ppm

Fig 3: Radial growth inhibition (%) of FOC at 500ppm
4. Discussion
In the recent years, the need to develop fungal disease control measures using phytochemicals as alternative to synthetic chemicals has become a priority of scientists worldwide [13]. Ark and Thompson [16] showed that garlic extracts contain a potent fungicide. They were able to effectively protect peaches against brown rot (Monilinia fructicola) with deodorized garlic extract preparations. Singh et al. [17] found that essential oils from Cymbopogon martini, C. oliveri, and Trachyspermum ammi exhibited strong antifungal activity against Helminthosporium oryzae. Plant fungicides have been reported to be safe to beneficial organisms such as pollinating insects, earthworms and to humans [18]. Khalid et al. [19] reported that their toxic effect is normally of an ephemeral nature disappearing within 14-21 days. Antifungal action of plant extracts has great potential as they are easy to prepare and apply. Several authors have confirmed the antifungal properties of several plant parts and phytochemicals [20-22]. The active constituents which are considered responsible for the antifungal properties of various phytochemicals are generally low molecular weight phenolics (hydroxybenzoic acid, flavanoids, hydroxycinnamic acid, acetophenone, stilbenes and lignans) as well as oligo or polymeric forms such as hydrolysable and condensed tannins and lignins [23-24]. Antimicrobial properties of numerous plant extracts, polar and non-polar fractions, their pure compounds, and essential oils have been investigated by many researchers against different strains of Fusarium [25-26].

5. Conclusion
A large number of earlier workers have reported anti-fungal properties of several plant species. Anjorin [32] reported that combination of two or more plant extracts proved more effective and could reduce the risk of resistance developing by the target fungi. The present study indicates that plant extracts of Callistemon lanceolatus, Tamarindus indicus, Terminalia arjuna and Zizyphus jujuba can serve as a cheap, easily available, cost effective, and a holistic option of managing the wilt of chickpea.

6. Acknowledgement
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7. References
6. Pal KK, Gardener BS. Biological control of plant pathogens. The Plant Health Instructor. 2006; 10(1094), 1117 - 02.


