Antifungal activity of some plant extracts against guava wilt pathogen
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ABSTRACT

Guava wilt pathogen was isolated from soil of guava orchards and identified as Fusarium solani on the basis of its morphological and cultural characteristics. The resistance of this pathogen to the wide variety of chemicals has stimulated the search of new alternatives for control measures. Use of plants extract is one of the most promising, effective, safer and eco-friendly method to eradicate the pathogens from soil. The present paper aims at the study on efficacy of four plant extracts viz., Eugenia caryophyllata (Clove), Moringa oleifera (Sehjan), Trachyspermum captivum (Ajwain) and Zingiber officinale (Zinger) against Fusarium solani at 10%, 25% and 50% concentrations. Among all four plant extracts tested, Eugenia caryophyllata was found to be most effective against Fusarium solani on 3rd, 5th and 7th day at 50% concentration and conquered the colony growth by 100% compared to control followed by Trachyspermum captivum and Moringa oleifera, 3rd day (91.70% and 67.42%), 5th day (92.74% and 72.35%) and 7th day (94.35% and 78.04%) respectively. Zingiber officinale has shown least efficiency to suppress the colony growth of Fusarium solani on 3rd day (60.65%), 5th day (63.55%) and 7th day (73.50%) after inoculation at 10%, 25% and 50% concentration over control.

Keywords: Guava, Fusarium solani, plant extract, wilt, management.

1. Introduction

Fusarium solani is a widely distributed soil inhibiting fungus that causes diseases in several economically important crops including guava. Guava is grown almost in all the states of India. Guava crop is severely affected by wilt disease leading to substantial loss in the crop production. Fusarium solani is found as the most predominant pathogen causing wilt of guava. Since long the workers are using different chemicals and synthetic compounds against fungal pathogens to control it. Unfortunately, these chemicals are not readily biodegradable; tend to persist for years in the environment and developed new physiological races of the pathogens (Ocamb et al., 2007; Siripornvisal, 2010). This led to the urgent need for development of novel fungicides that are more effective, economically feasible and eco-friendly than the conventional fungicides. Therefore, there is need of alternative methods to control phyto-pathogens which are safer and environment friendly.

Antifungal compounds from plants origin are most suitable being less toxic and more environmentally compatible by nature. From past many years number of plants have been extracted and screened for their antifungal activities and valuable results have been achieved (El.Shami et al., 1986; Bansal and Gupta, 2000; Bajwa et al., 2004; Abdulrahman, 2005; Gupta et al., 2009; Zaker and Mosallanejad, 2010; Shukla and Dwivedi, 2012). In this respect, the present investigation was undertaken to evaluate the antifungal activity of locally
available plant extracts viz., Eugenia caryophyllata, Moringa oleifera, Trachyspermum captivum and Zingiber officinale against Fusarium solani causing guava wilt.

The bioactivity of Eugenia caryophyllata attributed to eugenol, oleic acid and lipids (Hammer et al., 1999), whereas Trachyspermum captivum extracts contains thymol (39.1%), oleic acid (10.4%), linoleic acid (9.6%), α-terpinene (2.6%), palmitic acid (1.6%) and xylene (0.1%) (Nagalakshmi and Shankaracharya, 2000; Singh et al., 2004). Zingiber officinale has been shown to have a range of medicinal benefits and contains gingerols, gingerdiol and shagelol as the main antifungal compound. (Ficker et al., 2003) while Moringa oleifera attributed two main phytochemicals viz., pterygospermin and benzyl isocyanate which have strong antifungal and antimicrobial activity (Jed and Fahey, 2005).

2. Materials and methods

2.1 Evaluation of plant extracts

Antifungal activity of four plant extracts (Table 1) viz., Eugenia caryophyllata (Flower bud), Zingiber officinale (Stem), Trachyspermum captivum (Dried fruits), Moringa oleifera (Fresh leaves) were evaluated by “Poisoned food technique” (Thapliyal, 1993) and (Bansal and Gupta, 2000) for their efficacy against Fusarium solani which was isolated from wilt affected soil of guava orchards. The collected plant parts of Eugenia caryophyllata, Zingiber officinale, Trachyspermum captivum and Moringa oleifera were washed under tap water followed by distilled water. Washed samples were soaked with sterilized blotting paper to remove adhered water and were crushed (100g) manually with the help of sterilized pestle and mortar by adding 100 ml of distilled water (1:1 W/V). The extract obtained was subjected to the vacuum filtration followed by shaking. The processed extracts were poured in the Erlenmeyer flasks, plugged with cotton separately and heated at 50 ºC for 15 minutes to avoid contamination (Madavi et al., 2005). Different concentrations (10%, 25%, and 50 %) of plant extracts were incorporated to Czapek- Dox agar medium for inoculation of test pathogen in sterilized Petri dish. Each Petri dish was inoculated with 2mm disc of the pathogen in the center of the plate separately and incubated at 28±2ºC for 7 days. The radial growth of the colony was recorded on 3rd, 5th and 7th day of intervals and % inhibition of mycelial growth was calculated over control.

3. Statistical analysis

The data were calculated as Mean±SE and analyzed using analysis of variance technique (ANOVA). Probability of 0.05 or less was considered significant according to Duncan’s Multiple Range.

4. Results and discussion

All plant extracts used had shown antifungal activity against Fusarium solani. An increased inhibitory effect of the mycelial growth was observed with the increase in concentration from 10 to 50%. Among four plant extracts, E. caryophyllata completely inhibited the growth of Fusarium solani on 3rd, 5th and 7th day after inoculation at 50% concentration compared to control followed by T. captivum and M. oleifera by (91.70% and 67.42%)3rd day, (92.74% and 72.35%) 5th day and 94.35% and 78.04% respectively on 7th day (Table 2, 3, and 4). All the values were found significant at level (p<0.05). Minimum inhibition was found with Z. officinale on all observations i.e. 3rd day (60.65%), 5th day (63.55%) and 7th day (73.50%)
(Table 2 to 4). The value of T1, T2 and T3 were found significantly different from T0 at level p<0.05 on 3rd, 5th and 7th day (Table 2, 3 and 4).

**Table 1:** Plants used in present work with their medicinal use

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Local name</th>
<th>Family</th>
<th>Plant part used</th>
<th>Medicinal use</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eugenia caryophyllata</em></td>
<td>Clove</td>
<td>Myrtaceae</td>
<td>Flower buds</td>
<td>Antimicrobial</td>
<td>(El Shami et al., 1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Hammer et al., 1999)</td>
</tr>
<tr>
<td><em>Trachyspermum captivum</em></td>
<td>Ajwain</td>
<td>Umbelliferae</td>
<td>Dried fruits</td>
<td>Antispasmodic throat infection, bronchitis, antibacterial, antifungal</td>
<td>(Park et al., 2007); (Siripornvisal, 2010)</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td>Zinger</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>Antimicrobial, carminative</td>
<td>(Weil, 2005); White B (2007); (Shovan et al., 2008)</td>
</tr>
<tr>
<td><em>Moringa oleifera.</em></td>
<td>Sehjan</td>
<td>Moringaceae</td>
<td>leaves</td>
<td>used to combat malnutrition, medicinal use of plants</td>
<td>(Dahot, 1998); (Anwar et al., 2007)</td>
</tr>
</tbody>
</table>

Overall a general trend was found that as the concentration of extract and incubation period was increased the % inhibition of pathogen also increased. The correlation between the mycelial growth of *Fusarium solani* and concentration of plant extracts after 7th day of inoculation is presented in Figures1-4. The higher value of *E. caryophyllata* [B] (R² =0.669) showed that this extract has highest potential to inhibit the mycelial growth of *Fusarium solani* followed by *T. captivum* [A] (R² =0.651) and [C] *M. oleifera* (R² =0.640) (Fig 3). Lower value was observed in case of extract of *Z. officinale* [D] (R² =0.636) which indicates that it has least potential to inhibit the growth among all studied plant extracts.

Godara and Pathak (1995) reported that *Ocimum sanctum* leaf extract was highly effective against conidial germination of *Botryodiplodia theobromae* causing fruit rot of sweet orange. Patil et al., (1995) also observed that water extract of *Ocimum sanctum* inhibited conidial germination of *B. theobromae* causing fruit rot of mango. Wilson et al., (1997) tested 49 essential oils from various plants and found that the oil and clove buds of *Eugenia caryophyllata* was effective to control of *Botrytis cinerea*. Singh et al., (1980) observed inhibitory effects of essential oils of *C. martini*, *C. oliveri*, and *Trachyspermum ammi* on *Helminthosporium oryzae*, as well as inhibitory effects of the essential oils from rhizomes and leaves of *Zingiber chrysanthum* on plant pathogens such as *Alternaria* sp. and *Fusarium* sp.
Yadav and Majumdar (2004) found that at higher concentration i.e. 80 and 100% Ocimum baccicum cause significant inhibition against the mycelial growth of Lasiodiplodia theobromae causing die-back in guava. Jamil et al., (2007) tested the efficacy of plant extract including M. oleifera and reported that selected plant extracts showed antifungal activity against Macar mucedo and Aspergillus niger more strongly than Aspergillus tamari and Rhizoctonia solani. Siva et al., (2008) reported that Ocimum sanctum (water extract method) showed 96% inhibition against Fusarium oxysporum causing wilt disease of Solanum melogena L. whereas Joseph et al., (2008) studied that Ocimum sanctum extracts showed antifungal activity against Mucor mucedo and Aspergillus niger more strongly than Aspergillus tamari and Rhizoctonia solani.

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Table 2: Efficacy of plant extracts against Fusarium solani on 3rd day after inoculation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations (%)</th>
<th>T. captivum</th>
<th>E. caryophyllata</th>
<th>M. oleifera</th>
<th>Z. officinale</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>Control</td>
<td>41.0±0.57ᵃ</td>
<td>41.0±0.57ᵃ</td>
<td>41.0±0.57ᵃ</td>
<td>41.0±0.57ᵃ</td>
</tr>
<tr>
<td>T₁</td>
<td>10%</td>
<td>15.9±0.95ᵇ</td>
<td>14.0±3.51ᵇ</td>
<td>25.3±0.63ᵇ</td>
<td>27.4±0.23ᵇ</td>
</tr>
<tr>
<td>T₂</td>
<td>25%</td>
<td>7.3±0.33ᶜ</td>
<td>1.3±0.33ᶜ</td>
<td>18.3±1.20ᶜ</td>
<td>23.7±0.65ᶜ</td>
</tr>
<tr>
<td>T₃</td>
<td>50%</td>
<td>3.4±0.30ᵈ</td>
<td>0.0±0.00ᵈ</td>
<td>13.3±0.66ᵈ</td>
<td>16.1±0.35ᵈ</td>
</tr>
<tr>
<td>CD at</td>
<td>p&lt;0.05</td>
<td>1.99</td>
<td>5.91</td>
<td>2.68</td>
<td>1.60</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.60</td>
<td>1.78</td>
<td>0.81</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Table 3: Efficacy of plant extracts against Fusarium solani on 5th day after inoculation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations (%)</th>
<th>T. captivum</th>
<th>E. caryophyllata</th>
<th>M. oleifera</th>
<th>Z. officinale</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>Control</td>
<td>52.3±0.33ᵃ</td>
<td>52.3±0.33ᵃ</td>
<td>52.3±0.33ᵃ</td>
<td>52.3±0.33ᵃ</td>
</tr>
<tr>
<td>T₁</td>
<td>10%</td>
<td>16.7±1.12ᵇ</td>
<td>13.1±0.63ᵇ</td>
<td>25.6±0.40ᵇ</td>
<td>28.4±0.28ᵇ</td>
</tr>
<tr>
<td>T₂</td>
<td>25%</td>
<td>7.4±0.31ᶜ</td>
<td>1.5±0.24ᶜ</td>
<td>19.0±1.46ᶜ</td>
<td>24.3±0.40ᶜ</td>
</tr>
<tr>
<td>T₃</td>
<td>50%</td>
<td>3.8±0.25ᵈ</td>
<td>0.0±0.00ᵈ</td>
<td>14.4±2.03ᵈ</td>
<td>19.0±0.41ᵈ</td>
</tr>
<tr>
<td>CD at</td>
<td>p&lt;0.05</td>
<td>2.04</td>
<td>1.25</td>
<td>2.59</td>
<td>1.19</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.61</td>
<td>0.38</td>
<td>0.78</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Table 4: Efficacy of plant extracts against *Fusarium solani* on 7th day after inoculation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations (%)</th>
<th><em>T. captivum</em></th>
<th><em>E. caryophyllata</em></th>
<th><em>M. oleifera</em></th>
<th><em>Z. officinale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>Control</td>
<td>66.6±0.17a</td>
<td>66.6±0.17a</td>
<td>66.6±0.17a</td>
<td>66.6±0.17a</td>
</tr>
<tr>
<td>T1</td>
<td>10%</td>
<td>17.0±1.52b</td>
<td>22.0±1.52b</td>
<td>26.9±1.15b</td>
<td>28.9±0.08b</td>
</tr>
<tr>
<td>T2</td>
<td>25%</td>
<td>7.50±0.35c</td>
<td>1.60±0.37c</td>
<td>22.0±0.57c</td>
<td>24.6±0.58c</td>
</tr>
<tr>
<td>T3</td>
<td>50%</td>
<td>3.76±0.23d</td>
<td>0.00±0.00d</td>
<td>14.6±0.18d</td>
<td>17.6±0.26d</td>
</tr>
<tr>
<td>CD at p&lt;0.05</td>
<td></td>
<td>2.64</td>
<td>2.62</td>
<td>2.18</td>
<td>1.10</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.79</td>
<td>0.79</td>
<td>0.65</td>
<td>0.33</td>
</tr>
</tbody>
</table>

SEM: Standard error mean, Mean of radial growth in (mm). Data are expressed as mean±SE (n=3). Means within the same column and followed by the different letter are significantly different from each other according to Duncan’s Multiple Range Test at p<0.05 level of significance.

Figure 1: Correlation between mycelial growth of *Fusarium solani* and concentration of plant extract of *Trachyspermum captivum*.

Figure 2: Correlation between mycelial growth of *Fusarium solani* and concentration of plant extract of *Eugenia caryophyllata*.
Antifungal activity of some plant extracts against guava wilt pathogen

Figure 3: Correlation between mycelial growth of *Fusarium solani* and concentration of plant extract of *Moringa oleifera*

![Figure 3](image)

Figure 4: Correlation between mycelial growth of *Fusarium solani* and concentration of plant extract of *Zingiber officinale*

![Figure 4](image)

A commercial product based on the formulation of plant extracts and essential oils from pepper, mustard, cassia, and clove extracts was effective in reducing the population density of *F. oxysporum f. sp. chrysanthemi*, (Bowers and Locke, 2000). Extracts of numerous plant species contain antimicrobial compounds that can be used as alternatives to synthetic fungicides, including fumigants and contact pesticides (Cutler and Hill, 1994).

5. Conclusion

The main purpose of using plant extracts was to study their antifungal activity against the pathogen used in the present study as eco-friendly means as most of the plant extracts and plant essential oils are readily available, environmentally safe, less risky for developing resistance in pests, and pest resurgence, has less adverse effect on plant growth, less harmful to seed viability and quality, and above all, less expensive. The results of the present study
may be exploited for formulating integrated disease management for guava wilt and eradicate the pernicious pathogens from soil.

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6. References


34. Siva, N. S., Ganesan, N., Banumathy., and Muthuchelian., (2008), Antifungal effect of leaf extract of some medicinal plants against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L. Ethnobotanical leaflets, 12, pp 156-163.


