Antifungal activity of some botanicals extracts against *Fusarium oxysporum f. sp. pisi* a causal agent of wilt of pea (*Pisum sativum* L.)

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**Abstract**

Pea (*Pisum sativum* L.) is an important cash as well as pulse crop in India. Wilt diseases caused by *Fusarium oxysporum f. sp. pisi* has reported as one of serious threat to the crop production affects plant growth and yield. In the present study antifungal activity of four botanical extracts i.e., Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), Lemongrass (*Cymbopogon citratus*) and Ginger (*Zingiber officinale*) were tested in *in-vitro* condition against wilt *F. oxysporum* isolated from diseased plants of pea. Three replicates of different concentrations of the botanical extract viz 5, 15 and 25 per cent were tested against the pathogenic fungus, respectively, using Poison food technique. Result showed that growth of fungal mycelia was inhibited with increase in concentration of botanical extract. Lemongrass extract (25%) inhibited the maximum (35.93%) growth of fungal mycelia followed by Neem extract (32.59) and Ginger extract (27.78%) with Eucalyptus extract showed minimum inhibition (25.19%).

**Keywords:** botanical extracts, *fusarium oxysporum f. sp. pisi*, poison food technique, growth inhibition

**Introduction**

Pea (*Pisum sativum* L.) is a legume crop and is native to South Europe and grown a garden or field crop throughout the temperate regions of the world and was originally cultivated in the Mediterranean countries. In India, pea is one of the most important *Rabi* pulse crop, in which the crop is grown on a field scale for its dry seeds and smaller scale for green peas. It is being highly rich in protein contents and is consumed as both i.e., pulse as well as vegetable food. It is also a good source of carbohydrates, vitamins A and C, calcium, phosphorus and has a small quantity of iron (Kripalini et al. 2018) [3].

India is the second largest producer of pea in the world. The crop occupies 459 thousand hectares in India and shares 21 per cent production of the world. The production of pea in Uttarakahnd is 93.40 tonnes with 1.72 per share (Anon 2018) [1]. In this state people prefer pea in fresh state as well as in processed form like frozen, canned or dehydrated.

The climate of Uttarakmand is known for high humidity which favours several diseases of pea caused by fungi, bacteria and viruses. Among these diseases, wilt disease caused by *Fusarium oxysporum f. sp. pisi* is the most destructive disease of pea and occurs almost every year. Wilt is a soil-inhabiting fungus surviving from year to year in the soil as thick-walled, very hardy spores that can sit in the soil surviving all kinds of conditions for more than 10 years. When the plant is in its early growth stage, the fungus kills it outright but the attack at the later stage results in shrivelled grains and heavy yield losses. A lot of work has been done on the management of fusarium disease through plant extracts.

Verma and Dohroo (2003) [11] evaluated twelve different botanicals against *F. oxysporum f. sp. pisi* causing wilt of pea in Himachal Pradesh. Garlic clove extract was completely inhibitory to the wilt pathogen and the growth rate of the fungus was recorded to be zero at 24 h of incubation. The antifungal activity of ethanol extracts of medicinal plants was evaluated against *F. oxysporum f. sp. pisi* by modified disc technique by Sahani and Saxena (2009) [9] various plant extracts resulted in inhibition on the growth of mycelium however bark of *Euphorbia nerifolia* exhibited absolute toxicity against the test fungus (98.0%).

Minz et al., (2012) [6] tested antifungal potential of aqueous extracts of forty plants of different families against *F. oxysporum f. sp. ciceri*, causal agent of wilting of chick pea. Among forty plant species tested aqueous extract of *Chenopodium ambrosioides* recorded significant antifungal activity against the test fungi.
In-vitro Evaluation of Treatments

Poisoned food technique was used to check the efficacy of treatments. Purified plate of 7-10 days old culture taken and 8mm pieces of culture was cut out with the help of Cork borer. Media was poisoned with treatments at different concentrations of plant extracts (5%, 15%, and 25%) respectively and placed inside incubator at 25±2 °C. Completely Randomized Design was followed with three replications of each treatment and plate having no treatments served as control. Growth was checked after 3, 6 and 9 days intervals.

Zone of Inhibition calculated by using formula (Vincent, 1947):
\[ I = \left(\frac{T}{C}\right) \times 100 \]
Where, \( I \) = Per cent growth inhibition, 
\( C \) = Growth in control plates, 
\( T \) = Treated plates growth

Experimental Results

Efficacy of different botanical extract on radial mycelia growth and per cent inhibition of *Fusarium oxysporum* f. *sp. pisi*

Radial mycelia growth

Data given in Table 2 show that the effectiveness of the extracts increased with the increase in time of DAI and concentration of the extracts. After 3 DAI, at 5 per cent concentration eucalyptus showed minimum radial growth diameter (23.00 mm) as compared to 30.00 mm in control. At 15 and 25 per cent concentration, lemon grass extract proved better as compared to other treatments with 20.00 and 15.67 mm diameter respectively which was significantly lower as compared to other treatments. The trend remained almost same after 6 and 9 DAI with lemon grass extract proving better treatment as compared to other treatments. After 6 DAI, the radial diameter was 52.00 mm in case of lemon extract which decreased to 45.00 and 41.67 mm at 15 and 25 per cent concentration respectively. After 9 DAI also lemon grass proved best with 67.33, 60.00 and 57.67 mm radical diameter as compared 90.00 mm in control at 5, 15 and 25 per cent concentration respectively. Lemon grass was followed by neem in effectiveness at 3, 6 and 9 DAI at all the concentrations. Among all the treatments ginger proved least effective throughout the experimentation.

Table 2: Effect of Botanical extract against *Fusarium oxysporum* f. spp. *Pisi* (Myelia Growth Colony Diameter in mm)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 DAI</th>
<th>6 DAI</th>
<th>9 DAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radial growth (mm)</td>
<td>Radial growth (mm)</td>
<td>Radial growth (mm)</td>
</tr>
<tr>
<td>T1</td>
<td>5%</td>
<td>15%</td>
<td>25%</td>
</tr>
<tr>
<td>24.00</td>
<td>22.33</td>
<td>20.00</td>
<td>53.33</td>
</tr>
<tr>
<td>T2</td>
<td>23.00</td>
<td>22.33</td>
<td>21.33</td>
</tr>
<tr>
<td>T3</td>
<td>24.00</td>
<td>23.33</td>
<td>23.00</td>
</tr>
<tr>
<td>T4</td>
<td>53.33</td>
<td>20.00</td>
<td>15.67</td>
</tr>
<tr>
<td>T5(control)</td>
<td>30.00</td>
<td>30.00</td>
<td>30.00</td>
</tr>
<tr>
<td>C.D. at 5%</td>
<td>0.02</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>SE(m)</td>
<td>0.15</td>
<td>0.82</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*= Mean Of Three Replications. 
C.D = Colony Diameter Mean 
S.E (m) = Standard Error Mean

Myelia inhibition

The data given in (Table 3 and Plate 1) show that maximum myelia inhibition of fungus was recorded in case of lemon grass extract almost throughout the experimentation. The inhibition increased with the increase in period of DAI and concentration of the extracts. At 3 DAI though at 5 per cent concentration eucalyptus gave better results as compared to other treatments, lemon grass was significantly superior then other treatments till the termination of the experiment. The inhibition was 33.33 and 47.78 per cent at 15 and 25 per cent concentration at 3 DAI, 13.34, 25.00 and 30.55 per cent at 5, 15 and 25 per cent concentration at 6 DAI and 25.18, 33.33 and 35.93 per cent at 5, 15 and 25 per cent concentration at 9 DAI respectively in case of lemon grass. In this case Lemon grass followed Neem in effectiveness and minimum inhibition was recorded in case of ginger.
Table 3: Mycelia per cent inhibition of Fusarium oxysporum f. spp. pisi.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 DAI inhibition%</th>
<th>6 DAI inhibition%</th>
<th>9DAI inhibition%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
<td>15%</td>
<td>25%</td>
</tr>
<tr>
<td>T1</td>
<td>20.00</td>
<td>25.56</td>
<td>33.33</td>
</tr>
<tr>
<td>T2</td>
<td>23.33</td>
<td>25.56</td>
<td>28.89</td>
</tr>
<tr>
<td>T3</td>
<td>20.00</td>
<td>22.22</td>
<td>23.33</td>
</tr>
<tr>
<td>T4</td>
<td>22.22</td>
<td>33.33</td>
<td>47.78</td>
</tr>
<tr>
<td>T5(control)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Plate 1: Inhibitory effect on radial growth of Fusarium oxysporum f. spp. Pisi

Discussion

The effects of neem (A. indica) and willow (Salix babylonica) aqueous extracts on fusarium wilt disease in tomato seedlings were investigated by Hanan et al., (2011) [4]. Treatment of tomato plants with neem aqueous extract reduced the percentage of disease incidence to the level of 25.5 per cent after 6 weeks of infection.


Dwivedi and Sangeeta (2015) [2] analyzed the antifungal potentiality of aqueous extract of five medicinal plants against F. oxysporum f. sp. ciceri by using poisoned food technique. Among all medicinal plant extracts, Tinospora cordifolia, C citratus and Moringa oleifera showed the promising antifungal potentiality against F. oxysporum f. sp. ciceri with maximum inhibition of 100 per cent at 75 per cent concentration followed by Z. officinale and Trachyspermum ammi respectively.

Rawal and Adhikari (2016) [8] conducted study to determine the antimicrobial activity of dried ginger powder using paper disc diffusion assay against F. oxysporum f. sp. lycopersici and found the potent antimicrobial activity of the ginger extract against the pathogen. Zafar et al., (2020) [12] four Plant Extracts applied and overall, all the Plant Extracts performed well while comparing to control. Different concentrations of plant extracts used with different days interval. In-vitro neem found to be most effective against mycelial growth followed by ginger and eucalyptus. In-vivo conditions four plant extracts used at different concentration of neem found to be most effective followed by garlic, ginger and Eucalyptus at all concentrations against Fusarium wilt disease.

In present study, it was shows that the all botanical extract against Fusarium oxysporum f. sp. pisi and inhibited its mycelia growth over untreated control. Botanical extract found most effective in order to T4-Lemongrass (Cymbopogon citratus), T1-Neem (Azadirachta indica), T4-Rhizome of Ginger (Zingiber officinale) and T2-Eucalyptus (Eucalyptus globulus).

Conclusion

In our present investigation, the results are quite encouraging because almost of the plant extracts showed antifungal efficacy against test fungus. However, the extracts of Lemongrass (Cymbopogon citratus), Neem (Azadirachta indica), Eucalyptus (Eucalyptus globulus), and Ginger (Zingiber officinale) can be used as an alternative drug for controlling diseases and Disease Management in pea crop.

References


