Pectolytic enzyme activities of some foliar fungi isolated from mangrove plants and their response to tannin

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ABSTRACT: Fifteen foliar fungi isolated from mangrove plants of Sundarbans were tested for their pectolytic enzyme activities and growth responses to tannin. Among the 15 fungi tested, *Colletotrichum gloeosporioides* and *Coniella musaiaensis* showed maximum and minimum enzyme activities respectively under identical conditions. Tannin (0.2%) inactivated pectolytic enzymes of all fungi but most significantly of *Phoma nebulosa*. *Pestalotiopsis* sp. and *Myrothecium cinctum* were the least and the most sensitive to tannin respectively. There was no correlation either between growth and enzyme activity or between tannin sensitivity and enzyme activity. However, an apparent correlation was noted between high tannin sensitivity and low enzyme activity of 5 fungi.

Keywords: Mangrove plants, pectolytic enzyme, tannin

A large number of fungi have been found to grow on living leaves of mangrove plants of Sundarbans and in most cases these pathogens cause restricted lesions on leaf surfaces. Mangrove plants usually contain a significant amount of tannin (Lakshmanan and Dhanalakshmi, 1989) which is believed to be associated with disease resistance of several host species. It is probable that tannin can inactivate pectolytic enzymes (Bateman and Millar, 1966) of parasitic fungi during plant pathogenesis. The purpose of this investigation is to determine the effect of tannin on growth and pectolytic enzyme activities of different foliar fungi of mangrove plants.

MATERIALS AND METHODS

Fungal cultures and assessment of mycelial growth

Cultures of fungi were obtained from stock cultures of the Department of Botany, University of Calcutta. Fungi were grown in a medium containing pectin (NH\textsubscript{4}NO\textsubscript{3}, 1.0g; KH\textsubscript{2}PO\textsubscript{4}, 2.5g; MgSO\textsubscript{4}•7H\textsubscript{2}O, 2.5g; pectin 10g; distilled water - 1 litre). The inoculated flasks (50 ml/250 ml flasks) were incubated at 28-30°C for 8 days and the mycelia were collected, dried at 60°C for 96h, cooled in desiccator and weighed.

Assay of pectolytic enzyme activity

After harvesting of mycelia, the culture filtrates were taken for enzyme assay. Pectolytic enzyme activities were assayed by viscometric method at 30°C (Trione, 1960). The reaction mixture contained 10 ml buffered substrate (1% pectin in 0.05M acetate buffer at pH 5.5) and 1 ml of culture filtrate. The percentage reduction in viscosity was calculated as follows:

\[
\frac{T_0 - T}{T_0 - T_w} \times 100,
\]

where \(T_0\) = Flow time of reaction mixture at 0 time, \(T_w\) = Flow time of
Fig. 1 (a-f):

- Curvularia pallescens, b - C. clavata, c - C. verruculosa, d - C. tuberculata, e - Myrothecium roridum, 
  f - Fusarium sp.

CF-T = Culture filtrate without tannin
CF+T = Culture filtrate with tannin
reaction mixture after each desired incubation period, $T_w =$ Flow time of water. The enzyme activity has been expressed as percentage reduction in viscosity after each desired time (0 to 120 min at 15 minutes interval).

RESULTS AND DISCUSSION

Comparison of pectolytic enzyme activities of different foliar fungi isolated from mangrove plants

Fifteen foliar fungi were grown in a medium containing pectin and culture filtrates were collected after 8 days following inoculation. Pectolytic enzyme activities were assayed as described earlier and the results are shown in Figs. 1-3.

Colletotrichum gloeosporioides (Str. F720), Curvularia clavata, C. pallescens exhibited maximum reduction (91-98%) in viscosity of buffered pectin. Similar findings were also reported by Trione (1960). He observed that the viscosity of sodium polypectate (1.2%) dropped rapidly up to about 100% within 10 minutes indicating the presence of polygalacturonase in the culture filtrate of Fusarium oxysporum f. lini. Minimum reduction was noted for Coniella musaiaensis. These results reveal the range of pectolytic enzyme activities of test fungi.

C. pallescens, C. clavata, Colletotrichum gloeosporioides (Str. F720) showed rapid decrease in viscosity of pectin substrate. This could be due to the presence of endo-type of pectolytic enzymes as suggested by Bateman and Millar (1966). On the contrary, C. verruculosa, C. tuberculata, M. roridum, M. cinctum, Pestalotiopsis sp., C. gloeosporioides (Str. F744) and P. pinophilum showed gradual decrease in viscosity of pectin which indicated the existence of exo-type.

Effect of tannin on enzyme activities

To study the effect of tannin on enzymes, 9.9 ml of culture filtrate was taken and 0.1 ml of 20% tannin (tannic acid) solution was added to it so that the ultimate concentration of the tannin solution became 0.2%. It was incubated for 30 min at 30°C. One ml of this mixture was added to 10 ml of buffered substrate (mentioned previously) and enzyme activity was measured as stated earlier. Results are given in Figs. 1-3. It appears from the results that tannin has an inhibitory effect on pectolytic enzymes but the inhibitory action of tannin varies with fungal species. The inactivation of enzymes by phenolics including tannin of the host plant was recorded earlier by Bateman and Millar (1966). Degree of inactivation of pectolytic enzymes by tannin depends upon the type of pectic enzymes produced by the organisms and the concentration of tannin. Species or strains of a species may produce different types of pectolytic enzymes. Senaratna et al. (1991) observed that two isolates of C. gloeosporioides produced two types of pectolytic enzymes, namely, PG and PL in the culture filtrate of the fungus but the activity of both PG and PL was greater in isolate 5 than in isolate 1. Since the quality and quantity of pectolytic enzymes differ with the fungal species, tannin may not be equally effective in inactivating the types of pectolytic enzymes present in the culture filtrates. Therefore, the variation in percentage reduction in viscosity of pectin after addition of tannin is not unnatural.

Response of foliar fungi to different concentrations of tannin

Fungi were grown in glucose-asparagine medium (50 ml/250 ml flask) containing different concentrations (0.05%, 0.1%, 0.2%) of tannin. Medium without tannin served as control. Flasks were incubated for 8 days at 30°C. Mycelia were collected after 8 days of incubation, dried at 60°C for 96 h, cooled and weighed. Results are summarized in Table 1.

Although higher concentration (0.2%) of tannin inhibited growth of all test fungi, lower concentration (0.05%) stimulated growth of 6 fungi (Pestalotiopsis sp., C. gloeosporioides Str. F720, C. gloeosporioides Str. F744, Curvularia lunata, C. clavata, P. pinophilum). There is evidence that
Fig. 2 (a-f):

a - *F. solani*, b - *Pestalotiopsis* sp., c - *Colletotrichum gloeosporioides* Str. F744, d - *Curvularia lunata*,
e - *Penicillium pinophilum*, f - *Coniella musaiaensis*
Table 1. Effect of different concentrations of tannin on mycelial growth of fungi

<table>
<thead>
<tr>
<th>Fungus</th>
<th>*Mycelial dry wt. (mg)</th>
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<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Curvularia pa'lescens Boedijn.</td>
<td>218.33±9.8</td>
</tr>
<tr>
<td>C. clavata B.L. Jain</td>
<td>194.00±2.3</td>
</tr>
<tr>
<td>C. verruculosa Tandon and Bilgrami ex M.B. Ellis</td>
<td>276.75±6.9</td>
</tr>
<tr>
<td>C. tuberculata Jain</td>
<td>136.33±6.9</td>
</tr>
<tr>
<td>Myrothecium roridum Tode</td>
<td>284.50±1.4</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>57.50±2.6</td>
</tr>
<tr>
<td>F. solani (Martius) Sacc.</td>
<td>116.16±3.3</td>
</tr>
<tr>
<td>Pestalotiopsis sp.</td>
<td>160.25±7.6</td>
</tr>
<tr>
<td>Curvularia lunata (Wakker) Boedijn</td>
<td>157.66±4.4</td>
</tr>
<tr>
<td>Penicillium pinophilum Hedge</td>
<td>325.00±0.5</td>
</tr>
<tr>
<td>Coniella musaiaensis var. hibisci B. Sutton</td>
<td>155.33±7.8</td>
</tr>
<tr>
<td>Colletotrichum gloeosporioides (Str. F720) Penzig.</td>
<td>198.50±3.7</td>
</tr>
<tr>
<td>Phoma nebulosa (Pers. Fr.) Berk.</td>
<td>149.70±2.5</td>
</tr>
<tr>
<td>Colletotrichum gloeosporioides (Str. F744) Penzig.</td>
<td>289.00±1.0</td>
</tr>
<tr>
<td>Myrothecium cinctum (Corda) Sacc.</td>
<td>142.00±4.0</td>
</tr>
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</table>

*Average of 3 replicates/treatments.

tannin enhanced the growth of fungi like Aspergillus flavus, A. nidulans, A. niger and Penicillium sp. (Sivaswamy, 1982). Increase in mycelial dry weight of Curvularia lunata in presence of wattle tannin was also reported by Sambandam (1983). The increase in growth of some fungi at low concentration of tannin may be due to utilization of tannin as carbon source (Grant, 1976; Collett, 1992).

There is no correlation between fungal growth and pectolytic enzyme activity or between tannin sensitivity and enzyme producing ability of the organism. However, 5 fungi like C. musaiaensis,
Curvularia verruculosa, M. roridum, M. cinctum and P. nebulosa showed very high tannin sensitivity and low pectolytic enzyme activity in vitro.

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REFERENCES


