Biological control of white rot on wood by *Trichoderma viridae*

Aparna Kalawate*

Biology Division, Indian Plywood Industries Research and Training Institute, Bangalore-560022, Karnataka (India).

*Corresponding Author’s Email: aparna_ent@yahoo.co.in

**ARTICLE INFO**

**Article history:**
Received 21 Nov. 2012
Accepted 06 Dec. 2012
Available online 20 Dec. 2012

**Keywords:**
Biological control;
Basidiomycetes;
White rot;
*Trichoderma viridae*;
Poplar wood

**ABSTRACT**
The objective of the present study was to evaluate the bioefficacy of *Trichoderma viridae* against white rot on wood. A laboratory trail has been conducted to assess the biological control potential of *Trichoderma viridae*. The method used for the treatment of solid wood was dipping in the solution of *Trichoderma viridae*. Decay test was performed by agar block or Kolle flask method. The different concentrations of *Trichoderma viridae* viz., 0.125, 0.25 and 0.5% was tested against white rot (*Polyporous versicolor*). Results of the study showed that the *Trichoderma viridae* at 0.5% provided excellent control of white rot on poplar wood. The present study is the preliminary work to assess the bioefficacy of *Trichoderma viridae* against white rot on wood.

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**Introduction**

Wood is a major forest product which is being used widely for many purposes such as building construction, furniture, pulp and paper products etc. It has been well established that wood rotting fungi particularly basidiomycetes damage forest wood even more than insects. These basidiomycetes are categorised as either white rot fungi (WRF) or brown rot fungi (BRF) (Rauel and Barnoud, 1985). Among these two, WRF degrade all major components of wood i.e. cellulose, hemicellulose and lignin by secretion of cellulytic and lignolytic enzymes. The fungi that grow on wood and decay it are called as lignicolous fungi.

At present, use of chemical preservatives is the only way to make the wood and wood products free from fungal decay. The chemical preservatives which are being used to control the wood decay are Copper Chrome Arsenic (CCA), Sodium pentachlorophenate, Creosote, Zinc, Boric acid and Borax as an active ingredient. Among these Creosote and CCA can cause activated T cell autoimmunity, B cell dysregulation and functional immunosuppression in workers of wood industries (McConnachie and Zahalsky 1991; Lippmann 2000). Increased concern over the environmental effects of chemical biocides and the legislative constraints on the use of some chemicals has made the wood industry people and scientists to search alternative to replace these preservatives. One possible alternative is the use of microbial insecticides that contain micro-organisms or their by-products. The popularity of microbial insecticides is increasing because of their extremely low toxicity to non-target animals and human beings. Bio-pesticides are safe for both the pesticide user and user of treated products, compared to commonly use synthetic chemical pesticides. The basic concept is to use the natural ecological antagonisms of selected organisms (most often micro fungi) against target wood decay fungi.

Most of the research work has been made on the use of *Trichoderma* spp. as possible control agents for wood decay fungi (Nelson and Theis 1985, Siefert et al. 1988). *Trichoderma* has received more attention because of the promising results obtained in earlier studies (Ricard et al. 1969). It is one of the widely studied potential control agents for a wide range of plant pathogens in agricultural systems (Papavizas 1985) but not in the field of wood preservation to control wood decay fungus.
The potential of using antagonistic fungi as biocontrol agents to protect wood against decay has long been recognized. The pioneering work of Weindling (1934) revealed antagonistic activities of *Trichoderma* against other fungal species, since then *Trichoderma* and *Gliocladium* species have received much attention as biocontrol agents, particularly against soilborne pathogens. Lindgren (1958) specifically advocated the study of antibiosis and competitive effects of *Trichoderma* spp. against destructive fungi in pulp, plywood and logs. Such biological control still remains a relatively unexplored in the field of wood protection. Schoeman et al. (1994) investigated the application of *Trichoderma* spores in chainsaw oil as a means of protecting freshly harvested wood against sapstain and basidiomycete decay organisms.

*Trichoderma* Species have effectively been used in agriculture to control plant pathogens. It is a soil borne, green-spored ascomycetes and is ubiquitous in nature. The reason behind the successful utilisation of this fungus is its ability to produce diffusible/volatile antibodies, hydrolytic enzymes like chitinase and β-1, 3-glucanase and also competition for nutrients and space. The hydrolytic enzymes produced by the *Trichoderma* partially degrade the pathogen cell wall and eventually parasitized it (Kubicek et al. 2001). By keeping in view the hazardous nature of the wood preservative chemicals, an attempt has been made to find out a biocontrol agent against white rot on wood. Hence, the present study was carried out with an objective to assess the biological control properties of *Trichoderma viridae* against white rot in laboratory condition.

**Material & Methods**

**Species of wood**

Poplar (*Populus deltoides*) wood was utilized for the present study. It comes under durability class III and the average life is less than 60 months as per IS: 401 (Anonymous 2001). It is a very susceptible timber species and gets easily attack by the decay fungus. Hence, in the present study it was chosen to evaluate the bioefficacy of *Trichoderma viridae* against white rot.

**Preparation of test sample**

The test procedure was followed according to IS-4873 (2008) (Anonymous 2008). The wood blocks of Poplar were taken from the sapwood portion, free from knots, mould and stain. The size of the block was 50mm x25mm x15mm along the length of the grain. The moisture content of the blocks was in the range of 20-25%. The samples were then dipped in the preservative solution and kept for 4 hours. The beakers in which the blocks were dipped, was covered with lid to prevent the evaporation of the preservative solution. The blocks were then taken out and weighed ($W_2$) immediately after wiping the excess of preservative and retention of the preservative was calculated. The amounts of preservative solution absorbed (retention) by the samples was calculated according to IS: 4873 (Anonymous 2008) and result were shown in table 1.

**Decay test by Kolle-flask method**

White rot cultures were grown in 2% malt agar at 27˚C in Kolle-flasks until they covered the entire malt agar surface. Test blocks were then planted on the fungus mat side by side in the Kolle flask. Control blocks were also introduced in Kolle-flasks on fungal cultures. The whole set of flasks were then incubated for 12 weeks at 27˚C. The samples kept in Kolle flask is shown in Fig. 1. After completion of twelve weeks the samples were removed from the Kolle flask carefully and the mycelium present on it was removed by brush. After removing the mycelium the blocks were then kept in oven till the constant weight achieved. Percentage weight loss was calculated as per IS 4873 (Anonymous 2008).
Results

The data on the average retention/absorption of the preservative chemical was calculated (Table 1) and subjected to ANOVA (Table 2). The average retention of 0.158 kg/m$^3$ has been recorded in *Trichoderma viridae* at 0.5% concentration level. *Trichoderma viridae* at 0.25% concentration level recorded 0.078 kg/m$^3$ of absorption. The least absorption of 0.049 kg/m$^3$ was found in *Trichoderma viridae* at 0.125% concentration. Results indicated that there is a significant difference in the absorption of *Trichoderma viridae* in poplar wood in different concentration.

Table 1. Average Retention/Absorption of *Trichoderma viridae* in poplar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (%)</th>
<th>Average* Retention/Absorption (kg/m$^3$)</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma viridae</em></td>
<td>0.5</td>
<td>0.158</td>
<td>0.000085</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.078</td>
<td>0.00025</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>0.049</td>
<td>0.00025</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Average of three samples

Table 2. ANOVA for absorption of *Trichoderma viridae* in poplar

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>*P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.018871</td>
<td>2</td>
<td>0.009435</td>
<td>47.65376</td>
<td>0.00020</td>
<td>5.143253</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.001188</td>
<td>6</td>
<td>0.000198</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.020059</td>
<td>8</td>
<td>0.00198</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 0.05% level

Table 3. Bio efficacy of *Trichoderma viridae* against white rot

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (%)</th>
<th>Average* % weight Loss</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma viridae</em></td>
<td>0.5</td>
<td>10.96</td>
<td>0.973333</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>12.83</td>
<td>0.023333</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>17.41</td>
<td>0.000833</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>25.07</td>
<td>2.893333</td>
</tr>
</tbody>
</table>

* Average of three samples

Table 4. ANOVA for Bio efficacy study

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>*P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>354.8106</td>
<td>3</td>
<td>118.2702</td>
<td>121.5886</td>
<td>5.18E-07</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>7.781667</td>
<td>8</td>
<td>0.972708</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>362.5923</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 0.05% level

At the end of 12 weeks the test blocks were removed carefully from Kolle-flasks, cleaned and oven-dried. Average percent weight loss was calculated based on oven dry weight (Table 3). Test results against white rot showed that all the tested concentration of *Trichoderma viridae* were effective in controlling the decay. Whereas, the untreated control failed to arrest the growth of fungus, recording the maximum average percent weight loss of 25.07. *Trichoderma viridae* at 0.5% concentration was emerged as the best treatment. It has resisted more than 50% of the white rot attack. The second best treatment was *Trichoderma viridae* at 0.25% (12.83 Average percent attack). 17.41 of average percent...
attack was recorded in *Trichoderma viridae* at 0.125%. The ANOVA result has been presented in Table 4. From the table 4 it can be inferred that there is a significant difference among the treatment concentration and the white rot attack. As the concentration increases the white rot attack decreases.

Discussion

The biological control of fungi has to be considered as a viable option in wood protection and hence, it is necessary to evaluate the efficacy of such viable parasitic organism against the decay causing bioagents in wood. The observed biocontrol in the *Trichoderma viridae* treated poplar wood may be a result of competition for nutrients between the *Trichoderma viridae* and *Polyporous versicolor*. Competition for nutrients is also one of a possible mechanism, in which *Trichoderma viridae* might have inhibited the growth of *Polyporous versicolor*.

From the results it was found that the tested pathogen has provided good protection against white rot in the present study. *Trichoderma viridae* at 0.5% resulted as the best antimycotic agent. Similar results were also reported by several workers (Brown and Bruce 1997; Schubert et al. 2008a: Schubert et al. 2008b). The antagonistic activity of *Trichoderma* spp may be due the fact that it has been reported to produce sidephores (iron chelating compounds) and this may contribute to the biological control of wood decay fungi (Anke et al. 1991, Dutta et al. 2006). From the result of the present study it is clear that *Trichoderma viridae* has potential to control white rot on wood.

Conclusion

The high moisture content in the wood leads to the attack by wood destroying fungus. This fungal attack can be control by treating the wood with proper wood preservatives. Some of the conventional wood preservatives are on the screening list of Central Insecticide Board in India. Hence, in the present study an alternate wood preservative viz., *Trichoderma viridae* has been evaluated against white rot. In the present investigation *Trichoderma viridae* at 0.5 percent resulted as the best treatment to control the white rot on poplar wood.

References

[7] Lindgren R., 1958, Problems in products pathology - an analysis of basic and applied problems, with priorities assigned to them. Red Cover Report., USDA, Forest Products Laboratory, Madison, Wisconsin.


