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Effect of Neem Extract on Fungus Inhibition in *Toona ciliata* M. Roem.

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Abstract

The increasing human population and continuous demands of timber and its derived products like composite wood, has created a significant pressure on the demand for high quality timber from forests. This, ultimately has resulted in pressurising the natural forests and increase in the unvailibility of the production of high quality or durable timber from the forests. The one way to neutralize this pressure on the forests is to modify the other non durable wood species having undesirable properties like hygroscopicity, anisotropy, dimensional instability and biodegradability. Treatment with neem extracts as a biopreservative proves to be very efficient to protect the wood from bacterial or fungal rots. In the present study, wood specimens of *Toona ciliata* M. Roem. were treated with neem leaf and seed extracts for 72 hours in concentrations of 0.25, 0.5, 1, 1.5 and 2% (w/v), and analyzed for fungal growth and inhibition on dry weight basis using *Polyporous rubidus*. The fungal growth was maximum in untreated (control) samples whereas, minimum growth was observed in the neem leaf and seed extracts with 2.00% concentration. This method comes to be one of the most eco-friendly processes of wood preservation and helps in utilization of Toon wood in more effective and sustainable way. Also, the minimal release of harmful chemicals in the environment, helps to widen the industrial applications of *Toona ciliata* wood and make its acceptance to the society with ease.

Keywords: Biopreservative, dimensional instability, biodegradability, hygroscopicity, extract, environment

1. Introduction

Wood is one of the most versatile renewable natural resource obtained from forest, assisting various nations in achieving sustainable development and improving economic and technological aspects (Daly-Hassen et al., 2014, Verhaegen et al., 2014). However, due to increasing human population and decline in raw material supply, especially traditional/ primary timber species, is now impeding the production activities of wood-based industries, resulting in limited output and growth globally (Purnomo et al., 2011, Zhou et al., 2015, AntwiBoasiako and Boadu, 2016). Wood is chemically composed of cellulose, hemicelluloses, lignin and extractives. Tthe strong mechanical properties of wood and its ease to use and processing to meet the aesthetic appearance have not only improved its applications in furniture,decorations etc. (Qiu et al., 2018) but also helped in the emerging fields of transparent woody materials, energy storage, electronic devices (Yang et al., 2018, Li et al., 2018, Chen et al., 2018, Zhu et al., 2016, Yu et al., 2017) and, water clean-up and extraction (Liu et al., 2017, Zhu et al., 2017, Wang et al., 2019). Being a biological material, it can be readily degraded by bacteria, fungi and termites (Schultz and Nicholas, 2002, Brocco et al., 2017,Vanam, 2019). Although hemicelluloses are mainly responsible for degradation but the accessible cellulose, non-crystalline cellulose and lignin are also partially responsible for the same. Digestion of wood by enzymes which are found in different fungi like white rot, soft rot and brown rot etc. at the free hydroxyl sites, is one of the principal reasons that makes wood prone to decay (Rowell et al., 2008). However, it must have high natural durability for these applications (Gupta et al., 2021). *Toona ciliata* belonging to family Meliaceae, is the best known Indian timber species, popularly known as toon and red cedar (Singh and Gupta, 2017). However, the wood is prone to insect and fungal decay, limiting its end use applications. Thus, need to be protected during manufacturing, storage, transportation, and when in service (Uzunovic et al., 2008, Teaca et al., 2019).It is necessary to treat the wood with preservative which not only increase their durability but also do not alter its important properties. Chemical preservatives control wood degradation but have adverse effect on environment and human health because of their high chemical toxicity, low viability, high cost and high viscosity (Ouyang et al., 2018). Thus, biopreservation has evolved as best alternative. The plant extracts (biopreservatives) act as a reservoir for inexhaustible source of harmless fungicides/pesticides, which are non-toxic and easily biodegradable than synthetic

chemicals (Pramod et al., 2017). The adoption of the new plant protection products regulation (EC) No 1107/2009 made the approval of plant extracts possible as "basic substances". Basic substances are active substances which can be useful for the protection of plants whose economic value for the approval of such substances may be limited (Deniau et al., 2019). Plant extracts of *Azadirachta indica* A. Juss., commonly known as neem belonging to the family Meliaceaeact as a potential biopreservative. . Neem extracts have strong inherent antifungal, anti-microbial, insecticidal, fungicidal and nematicidal properties which act as holistic mode (Sonalkar et al., 2014). Neem contains at least 35 biologically active principles of which Nimbin and Azadirachtin are the most insecticidal ingredients and are mostly present in the seeds, leaves and other parts of thetree. Using neem extract not only improves the overall economic feasibility of the process but may also solve the problems associated with extreme ecological impacts that result in the loss of biodiversity and ecosystem services by altering native biodiversity, community structure, composition, and functions (Negi et al., 2019, Broda, 2020).

2. Materials and Methods

2.1. Experimental location

The experiment was conducted in the Department of Forest Products and Department of Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) (30.8613' N, 77.1708' E) located at 1,275 masl.

2.2. Wood material

Wood samples of *Toona ciliate* M. Roem. were taken from a tree aged 35 years having diameter at breast height (DBH) 55 cm and bole height of 16 m, and wood samples of size 5 cm×2.5 cm×2.5 cm±0.25 cm (longitudinally, radially, tangentially) were prepared in the Wood Workshop of the Department of Forest Products, UHF, Solan. These samples were planed and sanded to remove loosely adhering fibres on their surfaces so as to give smoothness and good finish. The experiment was laid out in Completely Randomized Block Design (CRD factorial). In total three replicates were taken and six samples were used in each replication. Three replications with five treatments of preservative of different concentration along with control were confined to each given extract during April–February, 2020–2021.

2.3. Preparation of extract solution for dip treatments

Different concentrations for dip treatment were prepared out of 10% stock solution already prepared. The wood specimens were dipped in different concentrations viz. 0.25 % (T_1), 0.50% (T_2), 1.00% (T_3), 1.50% (T_4), 2.00% (T_5), % (w/v) and control (T_6) extract solution of seed and leaf extract of neem for 72 hours. The samples meant for control were dipped in methanol. After dipping treatment specimens were first dried in air and then oven dried at 105 ±2 °C till constant weight.

2.4. Effect of wood rotting fungus on Neem seed and leaf extract treated wood samples

Treated samples were exposed to a particular wood rotting fugus species *in vitro* to determine the effect of extract on fungus growth and also to study the efficiency of extract inprotecting the wood against the wood decay caused by fungus.

2.5. Isolation and identification

An isolated pure culture of fungus *Polyporous rubidus* Berk. procured from Division of Forest Pathology, Forest Research Institute, Dehradun was revived in Plant Pathology Lab (Figure 1). The isolated fungus was transferred to agar slants and purified by hyphal tip technique.The purified fungal culture was again transferred to petri plates containing potato dextrose agar medium and kept for incubation at 25±1°C (Figure 2).

Fungus growth inhibition (%): Growth Inhibition was

Figure 1: Fungus sample *Polyporous rubidus* Berk. procured from FRI and Fungus growth observed after 7 days

Figure 2: Storage of treated samples in incubator to observe fungus growth

calculated as:

Inhibition (I)%=(C-T)/T ×100 .....(1)

Where,

I= percent growth inhibition

C = percent fungus colonization in control

T = percent fungus colonization in treated wood

2.6. Statistical analysis

• The data collected from treated wood samples was collated to estimate the results in standard terms.

• The collated data was put to standard statistical procedures to validate the results.

• Statistical analysis was done in R Stat Software using CRD Factorial Data Analysis as per the

• Procedure suggested by Gomez and Gomez (1984).

ANOVA for characters under study for factorial completely Randomized Design

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Variance ratio
Replication	(r-1)	Sr	Sr/(r-1) = Mr	Mr/MSe
Treatment combination (N)	(n-1)	Sn	Sn/(n-1) = Mn	Mn/MSe
Extract (E)	(e-1)	Se	Se/(e-1) = Me	Me/MSe
Treatment (T)	(t-1)	St	St/(t-1) = Mt	Mt/MSe
E×T	(e-1) (t-1)	Set	Set/(e-1) (t-1)= Met	Met / MSe
Error	(r-1) (et-1)	Snr	Snr/(et-1)(r-1)= MSe	

3. Results and Discussion

The data on fungus growth index (*Polyporous rubidus*) and fungus growth inhibition, for the extracts and treatments has been showed in Table 1, which reveals that fungus growth index on wood with both leaf and seed extract treatments were statistically significant. Among the extracts, maximum growth index (100%) was recorded in control and minimum growth index (18.75%) was observed in Leaf Extract Treatment 5. Among different concentrations of extract, it was observed that maximum growth index (100%) was observed in T6 (control) (Figure 4) and minimum growth index (23.44%) was observed in T5 (2.00% concentration) (Figure 3). The interaction between extracts and concentrations was also found to be significant, maximum growth (100%) occurred

Figure 3: Minimum fungus colonization observed (2.00% extract concentration)

Figure 4:Maximum fungus colonization (control)

Table 1: Effect of Neem extract on fungus colonization and fungus inhibition on treated and untreated wood

Treatments	Fungus growth index			Fungus growth inhibition	
	Seed (SET)	Leaf (LET)	Mean	Seed extract	Leaf extract
T_1: 0.25%	81.21(9.07)	72.92 (8.60)	77.07(8.83)	18.79	27.08
T_2: 0.50%	78.13 (8.89)	53.13(7.36)	65.63(8.13)	21.87	46.87
T_3: 1.00%	43.75 (6.69)	42.71(6.61)	43.23(6.65)	56.25	57.29
T_4: 1.50%	38.54 (6.28)	33.33(5.85)	35.94(6.07)	61.46	66.67
T_5: 2.00%	28.13 (5.39)	18.75(4.44)	23.44(4.91)	71.87	81.25
T_6: Control	100 (10.05)	100(10.05)	100(10.05)	-	-
Mean	61.63 (7.73)	53.47(7.15)	-	46.05	55.83
CD (*p*=0.05)	T 3.47 (0.275) E 2.00 (0.159) T×E 4.905 (0.389)				

*Values in parenthesis are square root transformed values

in control in both extracts, while minimum growth (18.75%) was observed in Leaf Extract Treatment 5 (LET5) 2.00% concentration. Wood is prone to deterioration when it comes in contact with water, temperature and microbial agents such as fungi, termite and wood boring insects which render it improper for further use. Maximum fungus colonization (100%) was observed in samples which were not treated with extracts, whereas, minimum fungus colonization with 18.75% growth index was recorded for 2.00% samples inleaf extract treated wood. This may be attributed to the antifungal properties of *Azadirachta indica* extract which is mainly composed of 35 chemical constituents such as nimbidin, azadirachtin, gedunim, mehmoodin, gallic acid, catechin, cyclic trisulphide and tetrasulphide and these constituents most probably present in leaf and seed extract which helps in inhibiting the growth of fungus. The fungus inhibition occurs due to the chemical constituents such as nimbidin, azadirachtin, gedunim, mehmoodin, gallic acid, catechin, cyclic trisulphide and tetrasulphide present in the seeds, leaves and other parts of the tree in Neem, which exhibit antifungal and anti-microbial, properties.(Sonalkar et al., 2014). Similarly, Kabir et al.(2008) also have reported that the neem ethanol extract concentration of 3.00% demonstrated best results against the test fungus. Gupta (2016) and Devi (2013) have also evaluated that at 2.00% concentrations of *Lantana camara* L. and *Ageratum conyzoides* L. showed minimal colonization of the fungus inwood. Natural plant-derived oils should therefore provide a wide variety of compounds as alternatives to synthetic fungicides with added advantages of cost effectiveness and environment friendly (Daferera et al.,2003). Neem also act as a biopesticide for several plant diseases (Kak et al., 2000). It is also stated that poisonous plant extract from *Nerium oleander* L. has antimicrobial properties and could make it effective in protecting the wood against the fungal attack and reduces the deterioration of wood (Goktas et al., 2007). Natural products from plants such as *Ageratum conyzoides*, *Ficus retusa*, *Lavandula pubescens*, *Lawsonia alba* etc. have been reported to have fungicidal properties and have huge potential as a new source of pathogenic fungal control (Bazaid et al.,2010). Therefore, the present study justifies the fact that the use of herbal extract can be a better alternative to the use of synthetic fungicides.

4. Conclusion

The studies on *Toona ciliata* M. Roem. with neem extracts as biopreservative showed that fungal colonization decreased with increase in seed and leaf extract concentration of neem and minimum fungus colonization was found at 2.00% concentration. The effect of treatments in all extracts studied under present work was found to be inhibitory against the fungus "*Polyporus rubidus"*.

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