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FUMIGATION OF IMPORTED *Tectona grandis* AND SOUTHERN YELLOW PINE WITH *Pongamia pinnata* SEED OIL AGAINST SAPSTAIN AND MOULD

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ABSTRACT

An eco-friendly alternative of *Pongamia pinnata s*eed oil was tested for fumigation of imported *Tectona grandis* and *Pinus spp.* (Southern yellow pine) against sap stain and mould fungi at 25°C± 2°C and 75 % ± 5 % relative humidity. Veneeer and block samples of both species, and Petri dishes with agar media were fumigated with three different concentrations (w/v) of *Pongamia pinnata s*eed oil and exhibited significant inhibition of fungal growth in comparison to the control sets, with the highest concentration of 6,4 g/ 8 mL^3 proving to be the most lethal. *Tectona grandis* exhibited no infestation of sapstain and mould in either type of specimens whereas highly perishable Southern yellow pine displayed slight infestation of 4 % and 5 % with 1,6 g/ 2 mL^3 of *Pongamia pinnata s*eed oil for venner and block specimens as compared to 18 % and 20 % infestation observed in respective controls. The infestation on Southern yellow pine reduced to 1 % and 2 % respectively for veneers and blocks with 4 g/ 5 mL^3 of *Pongamia pinnata s*eed oil and was completely inhibited at 6,4 g/ 8 mL^3 for both type of Southern yellow pine specimens. Petri plates returned similar findings where all higher concentrations inhibited mould growth completely and the lowest concentration exhibited meagre mould growth. Sapstain was inhibited successfully by all three concentrations of *Pongamia pinnata s*eed oil on agar media.

Keywords: *Alternaria alternata*, fumigation, mould, *Pongamia pinnata* oil, sap stain, southern yellow pine, *Tectona grandis*.

INTRODUCTION

Microbial activities on wood and wood products result in considerable loss for certain application. Several wood species of commercial importance in the Indian subcontinent are susceptible to attack by fungi like sapstain and mould if not treated well in advance. These fungal contaminations not only affect the aesthetical appearance of timber but on many occasions may also act as a precursor to more severe fungal attack by wood rotting fungi, often inflicting revenue loss (Ganguly *et al.* 2020). This makes sapstain infestation of wood a concern of paramount importance to the wood working sector. The pigmented hyphae of sapstain fungi secrete soluble pigments which diffuse into host cells and impart colour to the wood cell wall (Dhiman *et al.* 2017). The fungal spores of the staining fungi germinate and penetrate the wood surface of freshly felled timber or wood that has favourable condition for fungal attack through ruptured vessels and within two days the fungus colonizes heavily on the surrounding parenchyma cells (Olofinboba 1974). Mould, on the other hand, is mostly considered a surface phenomenon, although it comes under fungi kingdom and is seen as an unsightly surface growth with no significant or very littleimpact on strength or end use. Mould is the common

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name for many types of micro fungi. *Asperigillus spp*. and *Penicilium spp.* reportedly, are common mould fungi found on tropical hardwoods (Cartwright and Flndlay 1958). Wood and wood products in its all forms and shape can be colonized (Kržišnik *et al.* 2016). Appearance and activities of both mould and sapstain are predominantly restricted to the sapwood, with more or less similar impact on wood strength and absorptivity (Scbeffer 1958). Damage done to wood is reportedly minimal (Schmidt 2006) and can be termed as cosmetic (Humar *et al.* 2008).

A study entitled "Trends and Outlook for Forest Products, Consumption and Trade in the Asia Pacific Region" (Zhang *et al.* 1997) quite correctly highlighted the need to import timber in India. Additionally, unavailability of good quality and durable indigenous timber due to ban on natural felling across the country, has resulted in the use of many imported timbers like Southern yellow pine (SYP) and imported *Tectona grandis* (TG) in the Indian wood working sector (Ganguly and Tripathi 2018, Ganguly *et al*. 2021). Incessant demand for wood based products from India, due to low manufacturing and labour cost, has surged globally and to meet this demand, over the past three years India has become a major importer of raw timber, pulpwood and allied products which amounts to an estimated $2 billion in the past decade (Sood 2019). However, their performance for several end uses needs to be assessed as therein lies a probability of these timbers being susceptible to wood decaying microorganisms in the tropical climate of the Indian subcontinent (Ganguly *et al.* 2020) resulting in early and frequent replacement (Samani *et al.* 2019).

Fumigation plays a significant role as a control measure in protecting imported timbers from insects and fungi. Non-fumigants do not reach structurally destructive insects that live in the core of the wood, in such cases; the ability to get the chemical to pest becomes impossible unless fumigation is performed. With increase in import of timber, this practice is gaining more importance. Methyl bromide (MB) is one of the most widely used fumigants used in phytosanitary treatments. However, it has been reported for several environmental concerns including its role in Ozone depletion and other health hazards for relatively longer exposure.

For a change, to mitigate the effect of MB on environment and human health, there is an increasing interest in exploring the bioactivity of plant based fumigants (Pant and Tripathi 2011, Ganguly *et al.* 2020). Fumigants from plant origin can be of immencepotential on the basis of their efficacy, economic value and use in large scale storage in the coming days. Yang and Clausen (2007) investigated the inhibitory effects of natural plant extracts, such as essential oils, on wood with promising results. Pant and Tripathi (2011) and Ganguly *et al.* (2020) through their studies had already established the efficacy of Neem Seed oil (NSo) as an alternative wood fumigant. These findings encourage to explore the use of plant based oils for natural wood protection and to check wood decay fungi or mould infestation by surface-treatment or fumigation of wood products. *Pongamia pinnata s*eed oil (PSo) is also known to have antibacterial and antifungal properties (Baswa *et al.* 2001, Rani *et al.* 2013). The mature seeds contain about 5 % of shell and 95 % of oleaginous kernel. A study on a sample of air-dried kernels yielded its composition as follows: fatty oil - 27,5 %, protein - 17,4 %, crude fibre -7,3 %, starch - 6,6 % and ash - 2,4 %. The seeds also have mucilage - 13,5 %, traces of essential oil, a complex amino acid known as glabrin ($C_{21}H_{42}O_{12}N_3$) along with fourfuranflavones, Karajan ($C_{18}H_{12}O_4$), Pongapin ($C_{19}H_{12}O_6$), Kanjone ($C_{18}H_{12}O_4$), Pongaglabrone ($C_{18}H_{12}O_5$) and a Diketone ($C_{21}H_{14}O_4$) (Kumar 2009)**.** Wood preservation by heat treatment method had also been done effectively by using this oil (Tripathi *et al*. 2012), but no study has been performed on the use of PSo as a fumigant on imported wood species so far. Keeping in mind the same, in the present study, efficacy of PSo was screened against *Alternaria alternata* and mould fungi on imported TG and SYP.

MATERIALS AND METHODS

Experiments were carried out in Wood Preservation Discipline, Forest Products Division, Forest Research Institute, Dehradun, India.

The untreated sawn planks of SYP and TG were imported from South America and Ghana respectively and were procured through local vendors. The planks of *Bombax ceiba* were procured from the Forest Range office of Forest Research Institute, Dehradun (30° 31' 65 North latitude and 7803' 22" East longitude) which served as the virulence control species in the expereminet as per IS 4833 (1993). For the study, samples with mixed sapwood and heartwood were considered.

Seeds of *Pongamia pinnata*, without pods,were collected from the Silviculture Division, Forest Research Institute, Dehradun. The seeds were crushed prior extraction and extrcatedwith petroleum ether and the oil obtained was viscous and yellowish brown in color (Kumar 2009). The yield of the oil was 38,90 % which

was calculated as per Puri (1967). Three different Weight/Volume ratios of the oil were taken for the study. Quantities of 1,6 g/ 2 mL^3 (w/v), 4 g/ 5 mL^3 and 6,4 g/ 8 mL^3 of PSo were taken and the crude oil was diluted in 1 mL - 2 mL of petroleum ether (60°C - 80°C).

Test fungi for the experiment

Pure cultures of fungi *Alternaria alternata* and green mould (*Penicillium* sp.) were isolated from *Rhododendron arboretum* at the Forest Pathology Discipline and cultures of the same were collected for the study. Black mould (*Stachybotrys* sp.) (national type culture collection No.1175), was procured from Forest Protection Division, Forest Research Institute, Dehradun, India for research purpose.

Preparaton of culture media and conoidal suspension

Culture media

The culture media was prepared by dissolving 39 g Potato Dextrose Agar (PDA) powder (Hi-Media make) in 1 liter of distilled water and was cooked till the opaque solution became transparent. The media was then sterilized in an autoclave (Obromax™) at 103421 N/m^2 (15 Psi) and 120°C temperature for about 20 minutes (Datar 1995). After autoclaving, an amount of approximately 30 mL of the media was poured in each Petri plate (of 9 cm diameter) under the aseptic condition of laminar flow**.** The Petri plates were further sterilized with spirit prior being autoclaved again. Afterwards the plates were cooled till the media solidified.

Small fractions of fungal fruiting body/mycelia were inoculated with the help of sterilized inoculation needle in an aseptic condition under laminar flow and were kept in Bio-Oxygen Demand (BOD) incubator at 25°C ± 2°C and 75% ± 5% relative humidity (RH) for 7 days for growth of fungal mycelia.

Conoidal suspension

To prepare the conoidial suspension, first, Petri plates with no visual contamination and significant amount of growth were favoured. 10 mL distilled water was poured into each plate and the surface was scrapped with the help of an inoculation needle and the same was collected in a beaker. The volume was made up to 500 mL by adding sterile distilled water and simultaneously stirred with the help of magnetic stirrer for 10 minutes. Suspension was strained and stored in an aseptic sprayerin cold condition (4°C). The CFU/mL of the suspension was 2 x 10^6.

Efficacy evaluation of PSo through petri plate and block bioassay

Petri plate bioassay

Petri dishes with PDA medium were kept inside a desiccator and the desiccator was connected to the fumigation assembly (Figure 1). Partial vacuum of 60 mm Hg was excercised prior starting the fumigation process so as to replace air, for appoximataly 10 mins. During this initial vacuum process the stop cock 2 of the assembly was kept in “open” mode and stop cock 1 was kept “closed” (Figure 1). PSo was kept in a conical flask and the oil was heated and fumes were allowed to pass into the desiccator through the tube attached. During the process of fumigation, stop cock 1 was kept open whereas stop cock 2 was maintained at “closed” position. Three different concentrations of PSo, as mentioned above, were used for fumigation.For all three concentrations of oil, the PSo was heated for 1h and the fumigated petri plates were kept in the desiccator for 24h (Pant 2010).

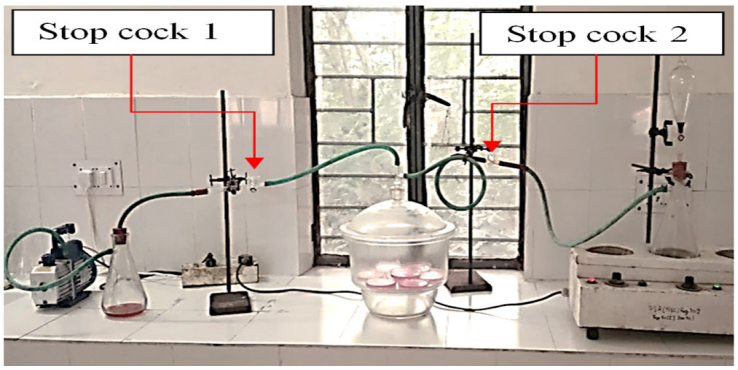

Figure 1: Fumigation assembly.

Under aseptic conditions of laminar flow, 6 fumigated Petri plates were inoculated with *Alternaria altrenata* ,*Stachybotrys sp.* and *Penicillium sp*., as mentioned in 2.2. Control plates (6 nos) did not receive any fumigation and were maintained separately. The plates were incubated at 25 ºC ± 2 ºC and 75 % ± 5 % RH for 14 days. The results were recorded after 15 days in terms of percent surface coverage by the test fungi over the agar media (Pant 2010, Wedge *et al*. 2000). Surface coverage of the test fungi was graded as per the following schedule (Table 1).

Table 1: Degree of surface coverage of the test fungi on PDA medium without treatment following PSo fumigation.

Growth pattern	Coverage of fungal mycelium on the surface of growth medium (%)
None	0
Sporadic	0-5
Little	6-25
Moderate	26-50
Considerable	51-75
Complete	>75

Block and veneer bioassay

The fumigation assembly was set up as depicted in Figure 1. For each concentration and controls, number of replicates were maintained at ten. Test specimens prior fumigation were kept in a desiccator which had connection to a vacuum pump. Initially, prior the fumigation process, a partial vacuum of about 60 mm Hg was excercised for approximately 5-7 minutes to expel the air. Afterewards, PSo of a fixed amount was heated in a conical flask, kept on a hot plate. The flask was connected to the desiccator with the help of a rubber tube to allow flow of fumigant/volatiles from the flask to the desiccator. Both, test blocks and veneer samples, followed the same fumigation treatment (blocks for 1 h and veneers for 0,5 h) and the samples were kept in the chamber for 24 h (blocks) and 0,5 h (veneers).

Preparation of wood specimens

Test blocks

Defect free planks with no visible evidence of mycelium growth or sapstain were cut into test blocks of size 3,5 cm x 3,5 cm x 3,5 cm. Nine holes were drilled on each side of these blocks, 1 cm away from each other and approximately 0,7 cm from the edges. Blocks were kept in a desiccator with calcium chloride salt.

Test veneers

Planks free of knots, cracks and other visually detectable defects, of *Bombax ceiba, Tectona grandis* and SYP were converted into billets of size 100 cm x 2,5 cm x 2 cm. Subsequent conversion of these billets were done into veneers of dimension 10 cm x 2,5 cm x 0,6 cm. (Figure 2).

Moisture Content was determined on oven dry (OD) basis as per IS 4833 (1991). prior the experiment was conducted for all the specimens and all the block samples were conditioned to a moisture content of 10 % -15 %.

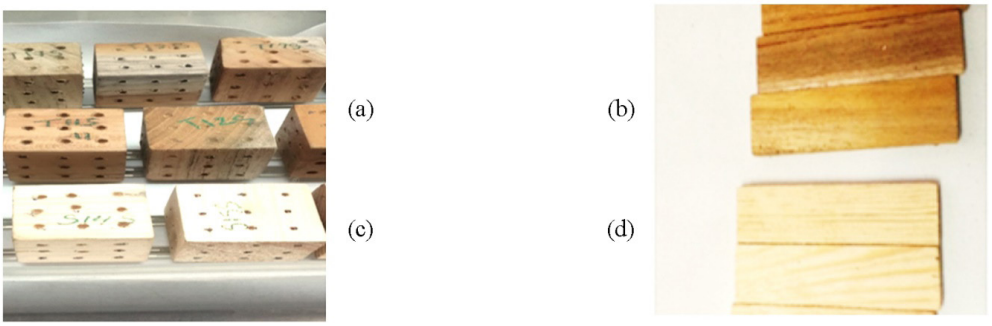

Figure 2: Wood specimens (a) and (b) Wood blocks and Veneers of TG; (c) and (d) Wood blocks and Veneers of SYP.

Details of the treatments

TGT_1, TGT_2, TGT_3, $SYPT_1$,$SYPT_2$, $SYPT_3$, TGC_1 and $SYPC_1$

Where,

TG= *Tectona grandis* , SYP= Southern yellow pine

T_1: PSo 1,6 g/ 2 mL^3,

T_2: PSo 4 g/ 5 mL^3

T_3: PSo 6,4 g/ 8 mL^3

C_1=Control of test species SYP/ TG

C_2= Control of *Bombax ceiba*

Total no. of specimens (veneers + blocks) were 180 (10 replicates per set of treatment).

For Petri plate bioassay 48 Petri plates in total (6 replicates per set of treatment) were considered.

Preparation of test box

Transparent boxes (30 cm x 20 cm x 10 cm) were cleaned by spirit and sterilized after wards. Sterilized pads of moistened Whatmans filter paper was placed at the bottom and it was pressed flat so as to remove air bubbles. The filter paper was kept wet by sprinkling distilled water to maintain the humidity during test. Two sterilised bamboo slivers of length equal to the length of boxes were placed on the sterilized paper in each box as base for the samples (Ganguly *et al.* 2020).

Fumigation of wooden block and veneer specimens

A total number of 10 samples of each species (blocks/veneers) were placed inside a desiccator and werefumigated as described above. The fumigation was carried on for 1 h, for the test blocks and 0,5 h for the veneers respectively. Afterwards the samples were taken out and inoculated with test fungi.

Inoculation of the specimens and grading

The treated blocks and the controls were placed on the sterilized bamboo slivers and sprayed with the spore/ hyphal suspension. The fungal suspension was sprayed every 3 days until the surface of the controls were completely covered with fungal mycelium.

The plastic boxes containing the inoculated wooden specimens were incubated at 25°C ± 2°C and 75% ± 5% RH for 4 weeks. Humidity inside the test boxes was maintained by regular spraying of distilled water.

Visual observation for fungal growth was carried out as per ASTM D4445-09 2009. The sampled were graded further as follows (Table 2).

Table 2: Grade of fungal growth.

Infestation	Grading
No stain or fungal growth	0
Stain or fungal growth covering less than 20 % of upper surface	1
Stain or fungal growth covering 20 %-40 % of upper surface	2
Stain or fungal growth covering 40 %-60 % of upper surface	3
Stain or fungal growth covering 60 %-80 % of upper surface	4
Stain or fungal growth covering more than 80 % of upper surface	5

Qualitative analysis for major functional groups present in the oil and fumigated wood

A few drops of the PSo extracted, was dissolved in 4mL of petroleum ether solvent (60 °C -80 °C) for qualitative analysis in order to determine the presence of major functional groups (Vogel 1978, Stahl 1969).

Tests for phenol, flavanoid, terpenoid and alkaloid

Ferric chloride ($FeCl_3$) test was performed to ascertain the presence of phenol in oil. 3-4 drops of the aqueous solution of AR grade $FeCl_3$were added to PSo. Appearance of green colour indicated the presence of Phenol. For Flavanoids, 3-4 drops of PSo were added in 2 mL ethanol. 5 drops of 2N HCL and Mg turnings (reagent grade) were subsequrntly added. Presence of Flavanoid was indicated by red colour. For the presence of alkaloid, the methodology described by Vogel (1978) and Stahl (1969) were followed. Liberman Burchard test was conducted to assess the presence of Terpenoids. All these analyses were in accordance to the methodology specified by Vogel (1978) and Stahl (1969).

RESULTS AND DISCUSSION

It is well documented that performance of wood above ground primarily depends on the degree of inherent resistance against decaying agents. (Brischke *et al.* 2013, Humar *et al.* 2019). However, this resistance changes between species, climatic conditions and with the presence and absence of various extractives in wood (McDonald and Donaldson 2001) and in general wood in humid and tropical climate is more susceptible to biodeterioration (Thelandersson *et al.* 2011, Brischke *et al.* 2013). The presence of extractives like phenols, terpenoids and alkaloids or any other chemical substrate of similar nature in wood prevents it from rapid degradation and often inhibits primary decay. Such presence in more frequent in heartwood which makes it more durable although in imported species, quantification of heart and sapwood is difficult as it is available mostly in sawn form (Ganguly *et al.* 2020). Thus it becomes important to know about the presence of such chemicals in wood before and after treatment so as to predict its performance when exposed although isolation and quantification of these are extremely difficult.

Analysis of the treating solution revealed the presence of phenols, flavanoids, terpenoids and alkaloids (Table 3). Untreated wood specimens developed intense green colour which ascertains presence of phenol in good quantities. Flavanoid and terpenoid were also found in substantial quantities in untreated samples and it can be fairly concluded that the wood before treatment had inherited natural durability because of their presence. Alkaloid was absent from untreated specimens but was present in trace amounts (Table 3, Figure 3) after the fumigation, in treated specimens which establishes the efficacy of the process overall.

Table 3: Qualitative test of PSo and wood specimens.

Sl. No.	Qualitative analysis	Colour developed in PSo	Colour developed in untreated wood specimens	Colour developed in treated wood specimens
1	Phenol	+++	++++	++++
2	Flavonoid	++	++	+++
3	Alkaloid	++	-	+
4	Terpenoid	++	+++	+++

Color developed by the detecting reagents, absence or presence of functional groups tested is indicated as: absence (-); slight presence (+); considerable (++); appreciable (+++) and intense (++++).

Figure 3: Veneer speciemns exhibiting functional groups in treated and untreated samples (Red indicating Flavanoid, Green color indicating Phenol, Brown colour Alcaloid, Brown colour marking presence of terpenoid in treated and untreated veneers).

Grading of fungal growth was done as described in sections 2.2; 2.7 and Table 2 exhibits the outcome of the studies on PDA medium. On PDA medium sporadic appearance was found for moulds, covering 2 % area of the PDA medium upon receiving T_1 while there were no visual growths observed for T_2 and T_3. However, considerable growth of mycelium was seen in the control plates with about 60 % (Table 4). The three concentrations of PSo likewise performed quite effectively against the sap stain fungi as there was no visual sap stain growth for any of the treatments. However, the control specimens showed complete sapstain infestation of 100% (Figure 4). This ascertains the efficacy of PSo fumigation at minimal concentrations for wood protection specially when the wood is in use. From Table 2 it can be fairly interpreted that PSo with increasing concentrations, was significantly ($p < 0,05$) effective in inhibiting growth of both mould and sapstain.

Table 4: Grading of surface coverage of mould and sapstain on PDA medium.

Treatments	Mould			Sapstain		
	Infestation (approx.) (%)	Grading of Surface Coverage	Growth Type	Infestation (approx.) (%)	Grading of Surface Coverage	Growth Type
Control	60	3	Considerable	100	5	Complete
T_1	2	1	Sporadic	0	0	None
T_2	0	0	None	0	0	None
T_3	0	0	None	0	0	None

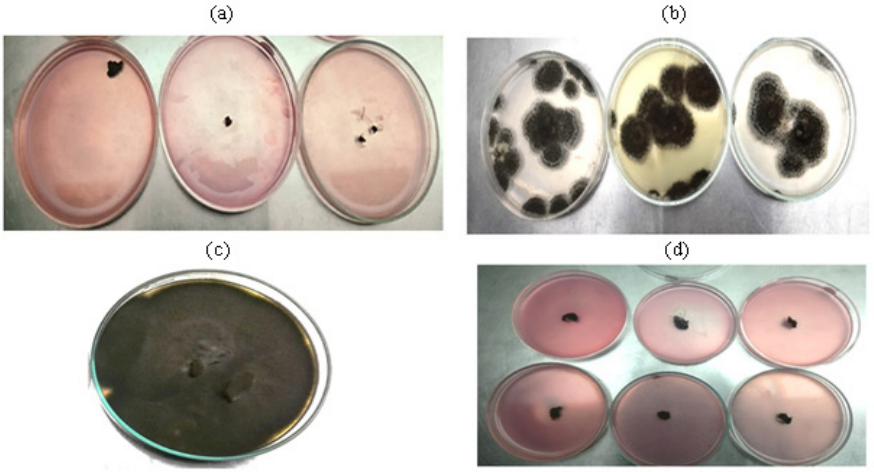

Figure 4: Mould Growth on PDA medium (a) Mould on PDA medium incoulated with T_3; (b) Control Plates Mould; (c) Control Plate sapstain; (d) Sapstain on PDA medium inoculated with T_3).

Neither treated veneers nor the controls of TG were infested by sapstain or mould (Table 5, Figure 5). Whereas, for SYP, 18 % surface infestation was seen in the controls (Figure 5) which was reduced to 4 %, 1 % and 0 % respectively after T_1, T_2 and T_3 of PSo. TG is a naturally durable species which was evident from the results and it can be said that much like its indigenous counterpart, imported TG is not so susceptible to mould and stain fungi. SYP exhibited less natural resistance and was found more susceptible to fungal inhibition which is in accordance to the poor durability of pine wood. The highly perishable *Bombax ceiba* speciemns served as untreated controls for the entire test set up and showed as high as 90 % surface growth (Figure 5) which establishes the virulence of the testing fungi.The results found in Table 5 justifies the potential of PSo as a eco-friendly fumigant. Although, it is to mention that more research on other perishable wood species in line of the present study may provide some more valuable inputs and establish PSo for commercial acceptance.

Table 5: Infestation (%) (approx.) on TG and SYP veneers by mould and sapstain.

Treatment	Infestation (%) on total surface and Grading (Veneer)					
	TG	Grading	SYP	Grading	*Bombax ceiba* (C2)	Grading
C_1	0	0	18	3	90	5
T_1	0	0	4	1		
T_2	0	0	1	1		
T_3	0	0	0	0		

Similar performance was exhibited by the block specimens and the data is summarized in Table 6. SYP showed infestations of 5 %, 2 % and 0 % respectively for the three treatments T_1, T_2 and T_3. Neither treated nor control TG, had any visual infestation while control specimens of SYP showed fungal infestation of 20 % . It is interesting to report that highly perishable and susceptible to biodeterioration *Bombax ceiba* C_2 exhibited infestation of 97 %. This finding is in accordance with the findings with the veneer specimens and based on these studies, inherent resistance or natural durability of imported SYP and TG can be established. For TG, this can be attributed to the presence tectoquinones (Rudman *et al*. 1958). Findings with PSo for both block and veneers are somewhat comparable inspite of the specimens having different surface area and volume. This ensures the fact that wood in any shape and volume can be equally protected by fumigation with PSo. PSo in all concentrations proved to be highly effective for inhibiting fungal growth in wood of different durability class and shape.

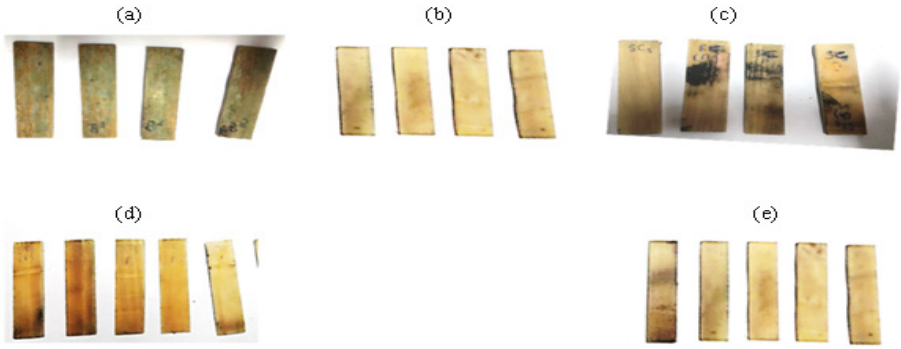

Figure 5: Veneer Samples Treated and Untreated. (a) Control *Bombax ceiba*, (b) Untreated TG, (c) Untreated SYP, (d) Treated SYP (T_3) (e) Treated TG (T_3).

Table 6: Infestation (%) (approx.) on TG and SYP blocks by mould and sapstain.

Treatment	Infestation (%) on total surface (Block)					
	Tectona grandis	Grading	SYP	Grading	*Bombax ceiba*	Grading
C_1	0	0	20	1	-	-
C_2	-	-	-	-	97	5
T_1	0	0	5	1	-	-
T_2	0	0	2	1	-	-
T_3	0	0	0	0	-	-

Figure 6: Treated and Control Blocks (a) Control TG, (b) Control SYP, (c) TG after T3, (d) SYP after T3) (a) and (b) exhibits fungal growth where as (c) and (d) remains unaffected.

CONCLUSIONS

Awareness of the side effects of traditional synthetic fumigants has now propelled the quest for non synthetic, environmental friendly alternatives. Laboratory trial of PSO as a fumigant, which was conducted to evaluate its potential effect on sap stain and mold fungi in imported *Tectona grandis* and SYP wood, established its efficacy in alleviating the reduction in activity of these organism. The study showed that PSo was a potent fungul infestation inhibitor in both wood species which makes it a likely-looking option that can replace toxic synthetic chemicals.

A large amount of seeds of the plant are wasted in forest as collection of seeds are a cumbursome process. This study would lead reserchers to further investigate its properties and successful outcomes like this may generate rural livelihood. An extensive large scale research exploring PSo's role as a natural fumigant would

be instrumental in exploiting this resource for more practical and feasible uses.

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