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2 **VIABILITY OF WOOD DECAYING FUNGAL MYCELIUM AFTER**
3 **MICROWAVE RADIATION OF BAMBOO CULM**

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13 **ABSTRACT**

14 The present study was carried out to evaluate the effects of microwave (MW) radiation on
15 viability of wood decaying fungi. The white rot (*Trametes versicolor*) and brown rot (*Rhodon-*
16 *placenta*) fungi were grown on bamboo culm-samples. The mycelium growths were observed
17 in controlled as well as microwave treated samples. The results showed that the viability of
18 fungi decreased according to the applied MW time. This study proved the ability of the
19 microwaves and exposure time MW3 (180 seconds) to kill the fungal colonies and do not allow
20 for the growth of fungal spores, means the rate of growth of fungal colonies is inversely
21 proportional to time of microwave exposure.

22 **Keywords:** Bamboo culm wood, *Bambusa vulgaris*, decay, fungi, microwave treatment,
23 viability.

1 INTRODUCTION

2 The chief economic product of forest is wood i.e., utilized for various purposes such as
3 construction, furniture, door, window etc. Failure of wood in service through biodeterioration
4 is a prime cause of user dissatisfaction. Although many types of organisms deteriorate wood,
5 the greatest damage is from fungi and insect, and to a minor extent- from bacteria (Highley *et*
6 *al.* 1994; Schmidt 2006).

7 The most important and potent wood-decay fungi are white and brown-rot. For decay to
8 occur, the minimum moisture content is slightly below the fibre saturation range (average 25-
9 30 percent) and the temperature between about 2 °C and 45 °C (Pasanen *et al.* 1992; Highley
10 *et al.* 1994; Morris and Winandy 2002; Schmidt 2006). Stienen *et al.* (2014) showed mycelia
11 growth on wood with 17,4 % moisture content and mass loss over 2 % at 24,6 % moisture
12 content. Oxygen must also be available because decay fungi are aerobic organisms. Finally, a
13 suitable substrate, such as wood, must be present to nourish the fungus. Interference with any
14 of these requirements stops the decay process.

15 Bamboo has a low natural durability (Liese and Kumar 2003). As a biological material like
16 wood, it is susceptible to degrade by different organism such as insect and fungi (Schmidt *et*
17 *al.* 2011). Bamboo deterioration can be controlled by impregnation with chemicals, but due to
18 toxic behaviours of these chemicals, namely, pentachlorophenol, copper chrome arsenate,
19 creosote and several others many countries have banned their use for wood preservation
20 (Poonia and Tripathi 2016). Public concern about the use of synthetic chemicals concomitantly
21 with significantly tightened environmental regulations have created demands for the
22 development of alternative methods for the chemical wood protection that we can go for
23 substitute like microwave treatment.

24 Microwave (MW) radiation is an innovative method for improving wood permeability,
25 treatability and the drying rate of wood. This method can also be capable of inactivating

1 microbial contaminations not only on exposed surfaces but inside them as well (Poonia and
2 Tripathi 2018). It can result in reducing or stopping colonization of microbiologically
3 contaminated surfaces and thus can decrease the number of agents contributing to the adverse
4 effects on growing environment of fungi (Chipley 1980).

5 The majority of the experiments i.e., effects of microwave radiation were conducted on
6 bacteria and yeasts. There are several studies however some studies on basidiomycetes. For
7 example, Baumann-Ebert and Körner (2013) used portable MW devices to kill the most
8 important European indoor rot fungus *Serpula lacrymans* (brown rot) within thick wooden
9 beams. Plarre *et al.* (2013) performed corresponding experiments with *S. lacrymans* and larvae
10 of the beetles *Anobium punctatum* and *Hylotrupes bajulus*. There are already national standards
11 for the use of MW treatment against wood-destroying organisms. For example, the German
12 standard DIN 68800-4 (DIN 2012) allows the use of MW treatment against insects in buildings,
13 however not against fungi due to less research results.

14 The present work was carried out to evaluate the effect of MW on viability and decay of
15 future wood decaying fungi.

16

17 **MATERIALS AND METHODS**

18 **Preparation of bamboo culm specimens**

19 Bamboo (*Bambusa vulgaris* Schrad.) culms were procured from Sirsi (Lat.14° 61' N,
20 Long.74° 85' E), Karnataka, India and converted into small clear specimens of size 2 cm × 2
21 cm. The defect free specimens were randomly selected and further smoothen by sand paper to
22 remove the silica layer.

23 **Preparation of Culture media**

24 A nutrient medium (i.e., PDA) consists of 4 g/l potato starch, 20 g/l glucose and 15 g/l
25 agar was prepared in an Erlenmeyer bottle. The Erlenmeyer was then plugged with cotton,
26 wrapped with foil and autoclaved at 2,1 bar and 121 °C for 20 minutes.

1 **Inoculation of fungi**

2 The effect of microwave radiation was tested against the white rot fungus (*Trametes*
3 *versicolor* (L.: Fr.) Pilat) and the brown rot species *Rhodonia placenta* (Fr.) Niemelä, K.H.
4 Larss. & Schigel. The fungi were inoculated on potato dextrose agar (PDA) in Petri dishes
5 under a laminar air flow. Inoculated plates were then kept at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and $75\% \pm 5\% \text{ RH}$
6 for 7 days for growth. After the growths of mycelial, spore suspension was prepared according
7 to IS: 4873 (2008).

8 Bamboo culm samples were exposed to fungal attack by placing them in the petri dishes
9 in which there are actively growing cultures of *T. versicolor* and *R. placenta*. The spore
10 suspension was sprayed over the bamboo samples for aerial contact with fungal mycelium.
11 Then, the petri dishes were further kept at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and $75\% \pm 5\% \text{ RH}$ for growth of fungi
12 on bamboo samples. After initiation of growth, the petri dishes were kept in MW oven for
13 different times.

14 **Microwave treatment**

15 MW oven with maximum output power 900 W at frequency 2450 MHz was used.
16 Treatments were done at different times i.e., 60 s (T-1), 120 s (T-2) and 180 s (T-3). Six
17 replicates in each treatment. The controls were maintained simultaneously.

18 After MW treatment, inoculated bamboo samples were further kept at $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and
19 $70\% \pm 4\% \text{ RH}$. The results were recorded after 21 days in terms of per cent surface covered
20 by the test fungi over the bamboo samples (Tripathi *et al.* 2009). The percent inhibition in
21 growth of fungi was calculated by using visual scores and by weight loss (Poonia *et al.* 2015).

22 **Weight loss and visual scores assessment of fungal growth**

23 The bamboo blocks were weighed before (W1) and after 21 days of inoculation of fungi
24 (W2). In MW treated specimens the weight after MW treatment were considered as initial
25 weight. The efficacy of treatment was categories on basis of weight loss and surface coverage
26 by test fungi. Weight loss was calculated as follows (Eq. 1):

1 Weight loss method (%) = $\frac{W_1 - W_2}{W_1} \times 100$ (1)

2 Where, W1= Weight of Petri dish containing bamboo blocks before inoculation of fungi (g)

3 W2= Weight of Petri dish containing bamboo blocks after 21days inoculation of fungi (g)

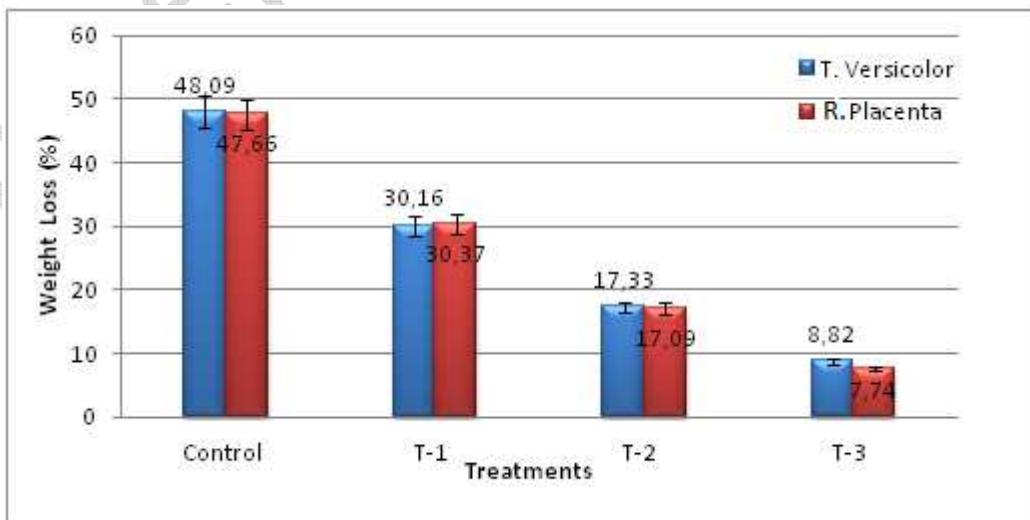
4 **Statistical analysis**

5 Statistical analysis was conducted using SPSS 16 version (IBM 2007). Effect of MW
6 treatment time on fungi growth/viability were compared using variance analysis (ANOVA)
7 and the Duncan homogeneity test at 95 % confidence interval.

8

9 **RESULTS AND DISCUSSIONS**

10 The results revealed that the control samples exhibited higher weight loss i.e., 48,09
11 and 47,66 percentage in white (*T. versicolor*) and brown rot (*R. placenta*) fungi respectively.
12 Further, it was observed that the 60 s showed less effect on growth with weight loss of 30,16
13 and 30,37 % (*T. versicolor* and *R. placenta*, respectively) as shown in Fig. 1. Whereas, the
14 weight loss percentage were significantly decreased as increased in MW treatment duration in
15 both the fungi at ($P \leq 0,05$) level. The MW treatment time with specific frequency plays an
16 important role in the effect of the microwaves which is time-dependent, where the intensity of
17 the weight loss carried by fungal infection is inversely proportional to the total absorbed
18 microwave energy.



19

20 **Figure 1:** Effect of microwave treatment on weight loss of Bamboo blocks/samples.

1 The results in term of surface coverage by test fungi i.e., *T. versicolor* and *R. placenta*
 2 over bamboo samples containing PDA medium are shown in Table 1 and Fig. 2.

3 **Table 1:** Effect of microwave treatment on bamboo sample surface coverage of wood decaying
 4 fungi.

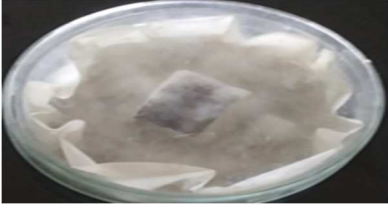

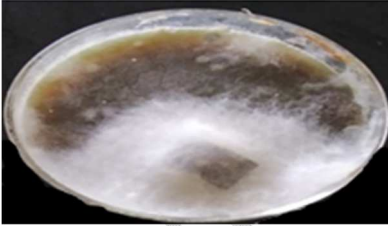




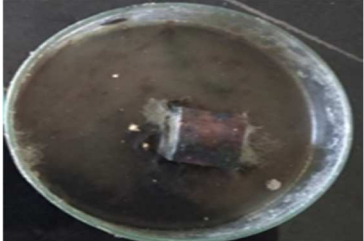
Sample No.	Treatment	Surface coverage (%)		Efficacy of treatment
		<i>T.versicolor</i> (Tv)	<i>R.placenta</i> (Rp)	
1	Control	100 ^a	100 ^a	Growth complete (> 75 %)
2	T-1	32,50 ^b	40,50 ^b	Growth moderate (25 % to 50 %)
3	T-2	10,70 ^c	8,75 ^c	Growth Little (5 % to 25 %)
4	T-3	0,00 ^d	0,00 ^d	Growth none (0 %)

5 Mean square error of Tv and Rp is 6,34 and 6,74 respectively at ($P \leq 0,05$) level. Different
 6 letters denote significantly different groups

7 It was observed that T-1 treatment (60 s of exposure) showed less effect on growth of both
 8 fungi, the inhibition was 67,50 and 59,50 % (*T. versicolor* and *R. placenta*) respectively. While
 9 the results shown that after T-3 treatment (180 s of exposure) the fungal growth were
 10 completely inhibited and the percentage of inhibition were 100 % for both fungi as compare to
 11 controls. The viability of mycelium of both fungi were significantly decreased ($P \leq 0,05$) after
 12 MW exposure. The reason behind inhibition of fungal growth may be due to heat generated by
 13 microwaves create steam pressure within the wood/micro-organism due to presence of
 14 moisture exerted the killing effect on fungi (Gorny *et al.* 2007).

15 The microwaves treatment at frequency 2450 MHz, with high energy and for a sufficiently
 16 long period of time, their thermal effect is most likely dominant to kill fungal mycelium (Al-
 17ayah and Ali 2010). However, at lower duration treatment, the killing effect of microwave
 18 radiation was significantly decreased and happened only after a prolonged period of irradiation,
 19 because of a lower transformation of microwave energy to heat. Some other studies showed
 20 that the extent of killing of microorganisms was correlated with the moisture content of the
 21 experimental specimens. In contrast, when microorganisms were irradiated with microwaves
 22 at temperatures lower than the thermal destruction level; various effects were observed, from

1 killing to enhanced growth (Kuchma *et al.* 1992; Ahmed *et al.* 2015). Hence, the study
 2 concluded that microwave radiation method has better possibilities of application using wood
 3 of building constructions (Gorny *et al.* 2007). Covering a larger space with microwave field
 4 create the possibility to obtain a more homogeneous temperature distribution on the wood
 5 subjected to reduce the growth of fungus. So that using of microwave radiation treatment we
 6 can reduce the wood deterioration.

S. NO	Treatment	Surface coverage (%)	
		<i>T.versicolor</i> (Tv)	<i>O.placenta</i> (Op)
1	Control		
2	T-1		
3	T-2		
4	T-3		

7 **Figure 2:** Surface coverage by wood decaying fungi after MW treatment.

8

9 However, it should be considered that surface coverage of wood or bamboo culm

10 samples are no sure indicator for degradation of the respective wood tissues. There are several

1 wood-decay fungi which produce severe wood degradation with no or only meager surface
2 mycelium, for example, the brown cellar fungus *Coniophora puteana* in buildings (Schmidt
3 2006).

4

5 CONCLUSIONS

6 The present study was carried out to evaluate the viability of wood decaying fungi
7 growing on bamboo culm samples against microwave treatment. The study revealed that the
8 microwave play a significant role in controlling the fungal growth/viability. Hence, this study
9 suggests that to apply this technology at pilot scale can control fungal growth in building
10 materials.

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