Radiation Physics and Chemistry 117 (2015) 191–197

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Isolation and characterization of mold fungi and insects infecting sawmill wood, and their inhibition by gamma radiation

Aparna Kalawate [a,c], Sayaji Mehetre [b,c,*]

[a] Zoological Survey of India, Western Regional Centre, Vidyanagar, Sector-29, P.C.N.T. Post, Rawet Road, Akurdi, Pune 411044, India
[b] Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400085, India
[c] Indian Plywood Industries Research & Training Institute, Bangalore, India

HIGHLIGHTS

- Study found new fungus *Fusarium proliferatum* infecting wood first time from India.
- Chemicals and gamma radiation found to be very effective to inhibit fungi and insect.
- Gamma radiation at 10 kGy dose was suitable for control of insect and fungi.
- There was increase incidence of insect and fungi at higher dose of 50 kGy.
- SEM showed no effect of radiation on wood at 10 kGy dose.

ARTICLE INFO

Article history:
Received 7 December 2014
Received in revised form
26 July 2015
Accepted 21 August 2015
Available online 22 August 2015

Keywords:
Gamma radiation
Powderpost beetles
Mold fungi
Wood preservation

ABSTRACT

This article describes the isolation, identification, and characterization of wood-rotting fungi and insects, and their inhibition was studied using gamma radiation. Products manufactured from plantation timber species are deteriorated by wood-rotting fungi such as *Hypocrea lixii*, *Fusarium proliferatum,* and *Aspergillus flavus*, and insects such as powderpost beetles. Proper preservation methods are necessary for ensuring a long service life of wood products. In this study, wood samples were treated with 2.5% copper ethanolamine boron (CEB) (10% *w*/*v*) and subsequently irradiated with gamma rays (10 kGy). It was observed that CEB-treated and gamma-irradiated samples controlled fungi and powderpost beetles significantly. As wood is a dead organic material, penetration of chemicals into it is very difficult. Gamma rays easily pass through wooden objects with hidden eggs and dormant spores of insects and fungi, respectively. Gamma irradiation was proved very effective in reducing damage caused by both fungi and insects.

1. Introduction

Wood, a natural, organic material, is susceptible to biodegradation by insects, fungi, and bacteria. A wide variety of insects bore through bark, sapwood, and even heartwood, declining the quality of logs. The mold fungi of *Ascomycetes* and *Deuteromycetes* groups grow on wood and wood-containing materials, disfiguring the materials as well as soft rot of lignocelluloses, if allowed to grow for a long time with favorable conditions such as high humidity and moisture. The spores and mycotoxins produced by mold fungi during their growth threaten the environment and human/animal health by means of different allergies and/or infections (Zawisza and Samolinski, 1998; Wiszniewska et al., 2004). *Cladosporium* spp., *Penicillium* spp., and *Alternaria* spp. are the major forms of molds found in homes that are recently known to cause chronic sinus infections, respiratory infections, and asthma (Mann, 1999). A potentially lethal mold, *Stachybotrys atra,* produces airborne toxins that can cause inflammation and injury to gastrointestinal and pulmonary tissues in humans. It also produces volatile organic compounds (VOCs) that can be irritating when present in high concentrations. Wood covered with mold fungi is considered as a material of lower quality and, in consequence, lesser trade value.

Wood/plywood made from plantation hardwood species is susceptible to infestation by insects called powderpost beetles (PPBs) (*Lyctus* spp.). It is a major pest found in sawmills, timber yards, factory premises, and warehouses, where hardwood

* Corresponding author at: Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400085, India.
E-mail addresses: aparna_ent@yahoo.co.in (A. Kalawate), smehetre@gmail.com (S. Mehetre).

http://dx.doi.org/10.1016/j.radphyschem.2015.08.016
0969-806X/

products such as planks, boards, veneers, and plywood are stored for further processing. This pest infestation is easily recognized by the presence of flight holes in timber and panel material together with abundant frass (powder) tightly packed into the tunnels, which forms small heaps beneath or around the flight holes. The flight holes are circular (diameter 1.5–3.0 mm), with no staining on the margins. Initial attack by PPBs is hard to detect, and is generally ignored until it is much advanced.

Protection of wood from fungi and insects has always been a challenge. Only the use of highly durable wood species or pretreated wood with appropriate wood preservatives offers a satisfactory, cost-effective, and long-term protection (Kumar, 1995). Conventional proprietary wood preservatives such as copper chrome arsenic (CCA) and copper chrome boron (CCB) are under scrutiny because of their environmental threat (Onuorah, 2000). Disposal of CCA-treated wood causes serious environmental problems because of its retention of high levels of toxic elements (Sye et al., 2010). Humar et al. (2004) predicted that the volume of the CCA-treated wood waste would be 16 mm^3 by 2020. Therefore, there is an urgent need to develop an environment-friendly method to protect wood from various wood-destroying agents.

Gamma rays, high-energy ionizing electromagnetic rays, easily pass through wooden objects. It is known to be very effective in the disinfection of wooden artifacts (Unger et al., 2001; Katusin-Razem et al., 2009; Fairand and Ražem, 2010) and also for wood sterilization (Sharman and Smith, 1970; Shuler, 1971). Gamma rays completely pass through wooden objects, sterilize microorganisms, and also kill insects. This article describes the isolation, characterization, and identification of wood-rotting fungi and insects, and their inhibition by gamma radiation.

2. Materials and methods

2.1. Isolation, characterization, and molecular identification of fungi

Preliminary surveys to collect wood affected by fungi and insects from sawmills near Bangalore (Karnataka, India) were conducted in 2013. Wood samples with highest infestations (Fig. 1) were brought to the laboratory and fungi in them were isolated.

Isolation of fungi was done by serial dilution technique (Stanghellini and Hancock, 1970). Five isolates of fungus were grown on potato dextrose agar (Himedia, India) by incubation at 30 °C for 3–4 days. The fungi were observed under microscope for their colony characterization and hyphal structure, and further characterized by sequencing 18S rDNA gene. The genomic DNA from these isolates was extracted using a previously reported method (Mehetre et al., 2008). Aliquot from extracted DNA was run on 1% agarose gel to check its quality. The extracted DNA was used as a template to amplify the 18S rDNA gene (~600 bp) by polymerase chain reaction (PCR) with the forward primer internal transcribed spacer (ITS) (1) TCTGTAGGTGAACCTGCGG and reverse primer ITS (4) TCCTCCGCTTATTGATATGC. PCR amplification was performed in an Eppendorf gradient Mastercycler (Eppendorf AG, Germany). Each reaction mixture with a final volume of 25 µl contained 800 µmol of deoxynucleotide triphosphates (dNTPs), 0.8 pmol of primers, 1 × Taq DNA polymerase buffer, and 1U Taq DNA polymerase (Bangalore Genei Pvt. Ltd.). The thermal cycling program included initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min, and primer extension at 72 °C for 1.5 min. This was followed by a final extension at 72 °C for 10 min. The tubes were cooled to 4 °C. The PCR products were resolved by electrophoresis on 0.8% agarose gel, containing 0.5 µg/ ml of ethidium bromide, at 80 V, 25 mA, for 2 h using 0.5 × standard Tris Borate EDTA (TBE) buffer (44.5 mmol /l Tris, 44.5 mmol /l boric acid, and 2 mmol /l EDTA and pH 8.3). Purification of the PCR products was done using QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA, USA). The sequencing PCR was set up with ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit in accordance with manufacturer's instructions. The raw sequences were manually edited for inconsistency. The nucleotide sequences for the 18S rDNA of wood fungi were obtained by aligning the sequences with the aforementioned primers. A similarity search for the nucleotide sequence of 18S rDNA of the test isolate was conducted online at http://www.ncbi.nlm.nih.gov, using the basic local alignment search tool (BLAST) search program for the nucleotide database, and the sequences were subsequently submitted to GenBank.

Fig. 1. Fungi attack as seen in some sawmills of Bangalore.

2.2. Insect rearing and identification

Wood samples infected by insects were brought to the laboratory, mass multiplied on tapioca, and preserved for further studies. The insects were identified using the identification key (Eugene, 1957).

2.3. Chemical treatment of wood samples

The wood was treated with 3% CCB and 2.5% copper ethanolamine boron (CEB) (10% *w*/*v*) by dipping method. The CCB was prepared in accordance with the guidelines of BIS 10013 Part-III (BIS, 1981), and was used as a standard check to compare the efficacies of CEB-treated and gamma-irradiated samples against wood-destroying bio-agents. CCB is the most commonly used wood preservative in India, and was prepared by the previously described procedure (Kalawate, 2013). The stock solution of CEB (10% *w*/*v*) was prepared by mixing monoethanolamine, octanoic acid, copper sulfate, boric acid, and propiconazole in tap water in a molar ratio of 1:0.0838:0.223:0.158:0.0045, and the sequence of the mixing was ethanolamine followed by water, copper sulfate, octanoic acid, and boric acid. Water was added again, followed by propiconazole and the remaining water. The pH of CEB was 10, which remained unchanged up to a month of storage. The samples were conditioned further and passed for gamma-ray treatment.

2.4. Gamma-radiation study

After conditioning, the samples were irradiated with gamma rays from a ^{60}Co source (Gamma Chamber 5000, Board of Radiation and Isotope Technology, Mumbai, India) at a dose rate of 3.5-kGy h^{-1} to obtain total doses of 10, 20, and 50 kGy. The samples were taken in triplicate, wrapped in an aluminum foil, and incubated at room temperature for further observations. Control samples were only gamma irradiated (without any chemical treatment) and chemically treated (without any irradiation).