

Original Research Article

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## Interception and Characterization of *Trichoderma koningiopsis* Reported in Imported Wooden Logs to India

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### ABSTRACT

#### Keywords

*Trichoderma koningiopsis*, Plant Quarantine, Molecular characterization, Imported wooden logs, Discolouration

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Throughout the import inspection of wooden logs from Ghana and Panama a profuse amount of white cottony mycelium was observed numerous times in consignments. The fungal pathogen was identified as *Trichoderma koningiopsis* by morphological and molecular characterization. Though it's not a tree pathogen but has been reported to cause wood discolouration and disfiguration. Inspection of imported wooden logs encumbers major challenge for detection of pathogen in timber logs owing to lack of available information for species identification of intercepted pathogens. The article aims at providing valuable information towards taxonomic identification.

### Introduction

International trade and travel have opened new frontiers for countries to increase economy and as well to enjoy the products and services of those from distant lands. It has also led to increased risk of introduction of new pest and pathogens (Raju *et al.*, 2019). India has emerged as a major importer of

tropical woods/timber in recent years (Rao and Remadevi 2006). These woods are usually dry or in semidry condition for uses ranging from structural material for construction to beautiful custom-made furniture. These woods may be poorly packed, exposed and under treated providing amply opportunity for bio -invasion. As of now very few reports are available on the

entry of plant pathogens in imported wooden logs in India (<http://164.100.163.200/PQISMain/Default.aspx#>). This is because the plant pathogen encountered during import of wooden logs draws minimum attention since: they may not be recognized as harmful in their native ranges; timber not being an essential commodity, there are very few pathogens reported infecting the wooden logs. Recently, during the import of teak wooden logs, it was observed that most of the consignments were fully covered with white mycelial growth covering the entire wooden logs resembling that of pure cotton. It may be due to improper treatment at the point of loading or due to congenial humid conditions prevailing in the container during the transit. There is no proper information whether this mycelial growth will affect the quality of wooden logs or whether they are tree pathogens. Though some species of *Trichoderma* i.e. *Trichoderma koningiopsis*, *T. dorotheae* and *T. gamsii* were reported to cause wood discoloration in South Korea (Huh *et al.*, 2011).

Samples of decayed wood collected from forest of Poland reported many *Trichoderma* species along with *Trichoderma koningiopsis* (Błaszczuk *et al.*, 2015). Probably, the little attention focused on wood-colonizing *Trichoderma* species can be explained by the fact that they cause little or no structural damage except for the disfigurement of the wood surface. Nevertheless, the economic damage is high as discoloured wood losses in value (Huh *et al.*, 2011). In this context an attempt was made to identify the fungal pathogen from imported woods/timbers.

## Materials and Methods

Inspection of imported wooden logs were done as per the Plant Quarantine Order (Regulation of Import into India) 2003 and Standard Operating Procedures for Import

inspection at Plant Quarantine Station, Mangalore. Teak wooden logs (*Tectona grandis*) were imported from Panama and Ghana countries. During the inspection whitish mycelia mat type growth of fungus was observed on the bark as well as core part of wooden logs. Beneath this superficial growth of mycelium, patches of light to dark brownish discoloration of wood was observed. Hence the infected parts of wooden logs as well as mycelial growth were collected for lab analysis.

The Intercepted fungal pathogen was collected in vials containing sterile distilled water, along with chips of wood logs covered with fungal growth and were sent to ICAR-Indian Institute of Horticultural Research, Division of Plant Pathology, Bengaluru for Morphological and molecular characterization.

The samples received from Plant Quarantine Station; Mangalore were subjected to isolation. The samples were subjected to standard surface sterilization procedures: small cut pieces were washed repeatedly in sterilized distilled water and one time in 4 percent sodium hypochlorite solution for 1 min, followed by 3 to 4 wash in sterilized distilled water.

The wood pieces were transferred to autoclaved petriplates containing Potato dextrose agar (PDA) and incubated at room temperature for further observations. After 10 days of incubation the mycelial growth was subcultured and utilized for morphological and cultural characterisation.

To standardise growth media, the pathogen was sub-cultured on different media i.e. Potato Dextrose Agar, Carrot Dextrose Agar, Potato Carrot Agar, Richards Synthetic Agar, Rye Dextrose Agar and Corn Meal Agar.

### Morphological identification

The infected wood sample and mycelium were examined under the light microscope for morphological studies. The mycelia of cultured fungus was mounted on glass slide and observed under microscope at different magnification. Cultural characters on different media were recorded.

### Fungal genomic DNA purification

The cultured fungus was inoculated in Potato Dextrose Broth at room temperature for 10 days. The fungal mycelium was collected by filtration through Whatman No.1 filter paper and dried. Total genomic DNA isolation was carried out by following CTAB method (Doyle and Doyle 1990). The isolated DNA was dried, re suspended in 50 ml of TE buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA, pH 8.0). The genomic DNA was checked on 0.8% agarose gel and stored at -20°C for further use.

### PCR amplification

Furthermore, to confirm the exact identity of the pathogen, total genomic DNA was used for PCR amplification by primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTA TTGATATGC-3') (White *et al.*, 1990). The DNA amplification was run with 35 cycles with denaturation for 1 min at 94°C, primer annealing for 45 seconds at 55°C, and primer extension for 90 seconds at 72°C, with an initial denaturation for 3 min at 94°C and a final extension for 15 min at 72°C. The final volume of 25 µL PCR mix containing 1.5 U *Taq* polymerase, 2 µL DNA template, 2 mM dNTPs, 25 mM MgCl<sub>2</sub> and 25 pmole of each primer. Gels were stained with ethidium bromide (10 mg/mL) and were visualized and documented by Alpha digidoc1000 system (Alpha Innotech Corporation, USA). PCR products were

loaded (1 h at 80 volts) in 1.2% agarose gel in Tris-borate-EDTA buffer, pH 8.0. The amplified PCR products (550bp) were purified by gel extraction kit (Qiagen) and sequencing was done by Eurofins Genomics India Pvt. Ltd (Karnataka, India).

### Sequence analysis

Sequence similarity searches were performed by comparing sequence to sequences available in the database using BlastN1. The sequences showing highest scores with the present isolates were obtained from database and aligned using SEAVIEW program (Galtier, *et al.*, 1996).

### Results and Discussion

The consignment of wooden logs (*Tectona grandis*) from Panama and Ghana countries were examined by following plant quarantine procedures. During the inspection whitish mycelia mat type growth of fungus was observed on the bark as well as core part of wooden logs. Beneath this superficial growth of mycelium, patches of light to dark brownish discoloration of wood was observed. Hence the infected parts of wooden logs as well as mycelial growth were collected for lab analysis. In Laboratory the infected wood samples were subjected to isolation procedure. For isolation Potato Dextrose Agar media was used. After isolation the petriplates were incubated at 25±2°C for 10 days. the wood samples and mycelium were also examined under light microscope for morphological studies. After 10 days of incubation on PDA plates, dense mass of white compact mycelium was observed. The mycelial was scraped and placed on a glass slide containing a drop of sterile water. The specimen was observed at 100X magnification under microscope. In microscopic study there was no sporulation on the PDA media. So, the fungal culture was

further sub-cultured on different media and observations were recorded after 10 days of incubation at  $25\pm 2^\circ$ . Though profuse mycelial growth was recorded in all the media, abundant sporulation was recorded in Rye agar media whereas, perfect concentric rings were recorded in Potato Carrot agar sometimes forming cottony pustules 15 days after inoculation. Mycelial growth in culture plates produced conidia moderately in

concentric rings. There was no pigment diffusion through agar. It was observed that a faint coconut odour was emitted from the pure culture. Conidial masses were light to yellowish green in colour. Under microscopic conidiophores and conidia were observed. Conidiophores were  $2.5\text{--}3.5\text{ }\mu\text{m}$  wide and conidia broadly ellipsoidal in shape, smooth,  $2.8\text{--}3.6 \times 2.6\text{--}3.0\text{ }\mu\text{m}$  in size.

**Fig.1a** Fungal growth on timber logs covering entire part the wood inside the container



**Fig.2a** Fungal growth on wooden logs showing discoloration inside the container



**Fig.3b** Luxurious fungal growth on timber logs covering core part of the wood logs

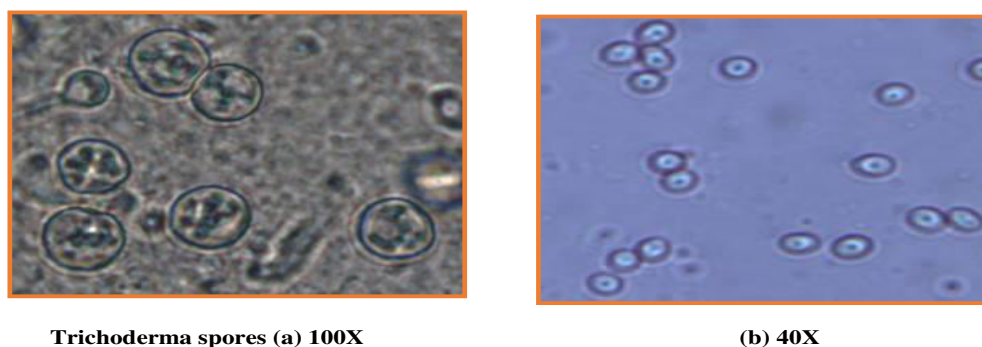




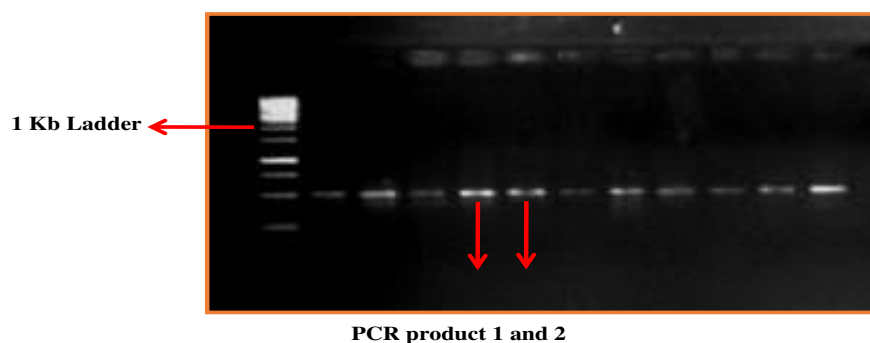
**Fig.2** Growth of *Trichoderma koningiopsis* on different media exhibiting colony characters and sporulation



**Fig.3** Microscopic view of spores of *Trichoderma koningiopsis* under 100 X and 40 X magnifications



**Fig.4** Agarose gel electrophoresis of the PCR products amplified by using ITS 1 and ITS 2 primers



The total genomic DNA was amplified using ITS universal primers. The sequence is available in GenBank (NCBI) with the accession number, KU933355. The ITS sequence of *Trichoderma* isolate was blasted with other *Trichoderma* species available in the NCBI database (Hassan, *et al.*, 2019). The blasted results showed 100% similarity with *Trichoderma koningiopsis* infecting *Lupines albus* and *Fragaria* sp. Therefore, the fungus that caused wood decay and discoloration on Teak wood was identified as *Trichoderma koningiopsis* based on morphological and Molecular characterization.

As saprotrophs, different species of *Trichoderma* contribute to the degradation of plant debris, wood and bark (Klein and Eveleigh 1998). Degradation of plant cell wall biopolymers is a complex process, which requires role of a large number of cellulase and hemicellulase enzymes. Several *Trichoderma* species, has the ability to produce extracellular enzymes *i.e.* *T. harzianum* complex, *T. koningii*, *T. longibrachiatum*, *T. viride*, *T. virens* and *T. citrinoviride* (Strakowska *et al.*, 2014). Though several *Trichoderma* sp. are reported from soil and wood decay *T. koningiopsis* and *T. longibrachiatum* are preferably isolated from tropical and sub-tropical areas (Kubicek *et al.*, 2008). In a study conducted to identify various woods inhabiting *Trichoderma* sp. *T. dorotheae*, *T. gamsii*, and *T. koningiopsis* were reported for the first time in Korea (Huh *et al.*, 2011). The ability of *T. koningiopsis* to cause wood decay and wood discoloration was also recorded in a study conducted in Central Europe to identify *Trichoderma* sp. causing wood decay symptoms in mountain forest of Central Europe (Błaszczuk *et al.*, 2015).

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### Conflicts of interest

The authors declare no conflicts of interest.

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