RESEARCH ARTICLE

Identification and substrate utilisation of fungi associated with low country live wood termite, *Glyptotermes dilatatus* Bugnion & Popoff and the host plant, *Camellia sinensis* L.O. Kuntze

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Abstract: The low country live wood termite (*Glyptotermes dilatatus*) is known to be associated with wood decaying fungi on decayed tea (*Camellia sinensis var* Assamica) stems. The diversity and prevalence of wood decaying fungi in decayed tea stems however have not been studied extensively. In this study, wood decaying fungi were isolated from decayed tea stems of TRI 4042 cultivar, living termites (*G. dilatatus*) and termite galleries collected from St. Joachim Tea Estate, Ratnapura, Sri Lanka. Of the fifteen fungi isolated, thirteen were identified using molecular taxonomy. This is the first report of isolation and identification of wood decaying fungi associated with decayed tea stumps. Among the identified fungi species, seven, six, and twelve fungal strains were recovered from the living termites, termite occupied galleries and decayed tea stems, respectively. *Fusarium solani, Fusarium* sp.1, *Fusarium* sp. 2, and *Rhizomucor variabilis* were the common fungi isolated. Results on substrate utilisation patterns of seven fungal species revealed that all the fungi were able to produce at least two enzymes to utilise the test substrates. Further, except with *R. variabilis* and *Neosartorya fischeri*, these fungi could digest cellulose and pectin either by producing polygalactouronase or pectate lyase. In utilisation of lignin, none of the test fungi produced laccase although all were able to produce peroxidase.

Keywords: *Camellia sinensis, Glyptotermes dilatatus*, molecular identification of fungi, substrate utilisation, termites, wood decaying fungi.

INTRODUCTION

The nature of the relationship between insects and fungi is still a subject of debate although a complex relationship between insects and fungi has been studied extensively.

Fungal association of termites has been studied by several scientists (Zoberi & Grace, 1990; Jayasimha, 2006) and it has been shown that termites prefer consuming wood decayed by certain fungi than wood that is not decayed (Esenther *et al*., 1961). Smythe *et al.* (1967) have reported that termites are attracted to decayed wood by wood decaying fungi, and many termite species also ingest excessive amounts of decayed wood than wood that is not decayed. Fungi associated with cellulolytic decay play an important role in termite nutrition and orientation to food materials (Cornelious *et al*., 2002), as the fungi can modify the texture of the wood to favour termite feeding and detoxify the toxic compounds present in plants (Beard, 1974; Jayasimha, 2006). Zoberi and Grace (1990) have reported the isolation of forty species of cellulolytic and pathogenic fungi associated with *Reticulitermes flavifes* (Koller). It has also been shown that the environment of live termites is favourable for the growth of fungi as termites change the moisture content of the wood of the host plant (Beard, 1974). Interactions between termites and fungi range from agonistic to mutualistic and many examples of symbiotic associations between fungi and termites have been reported (Jayasimha, 2006). Termite species of the sub family Macrotermitinae cultivate the fungus, *Termitomyces* sp. for food. The termites cannot live without this fungus and the fungus cannot survive without the termites (Henrik *et al*., 2005). In 2005, Wright *et al*. have reported that *Metarhizium anisopliae* isolated from dead alates of *Coptotermes formosanus* was detrimental to termites. It was also shown that these fungi are present in both interior and exterior surfaces of the termites. The ability of the termites to act as a vehicle

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for fungi was demonstrated by the isolation of fungi from the exterior surfaces and gut tissues of termites. The observation of hyphae, conidiophores, and conidia clinging to the bodies and appendages of termites further proved the presence of fungi in interior surfaces of termites (Jayasimha, 2006)

The low country live wood termite, *Glyptotermes dilatatus* is a major insect pest of low grown tea in Sri Lanka. It has been reported that the alates of *G. dilatatus* are attracted to decayed stems and first colonise inside the decayed stem, and continue their feeding to undecayed wood of the tea plant (Vitarana & Mohotti, 2008). A tea plant infested by *G. dilatatus* predisposes the gradual deterioration and leads to bush debilitation and ultimately decease of tea bushes (Vitarana & Mohotti, 2008). High yielding tea varieties planted in low grown areas contain soft wooded skeletons that suffer massive die back and they are highly vulnerable to wood decaying fungi (Sivapalan & Senaratna, 1981). In the laboratory, we have been able to observe that the hexane fraction of wood rot extract obtained from decayed stem of TRI 4042 is more attractive to alates of *G. dilatatus* when compared with the hexane fraction of decayed stems obtained from tea cultivars TRI 2023, TRI 2027 and TRI 4049 (Senanayke *et al.*, 2014). A hypothesis formulated in the current investigation was that the biodiversity observed within and among the fungi assemblages could be correlated with the attraction of *G. dilatatus* to decayed stem. Hence the present study was initiated as a survey of fungi associated with termite infested decayed stems of the tea cultivar TRI 4042, living termites and termite galleries, as fungi play a positive role in wood decay and orientation of termites (Cornelious *et al.*, 2002). The study was further extended to utilisation of carbon sources by selected fungal species, to investigate the ability of substrate utilisation and evaluate the ability to decompose structural and storage components *in vitro* using pure substrates. It has been reported that enzymes, esterases, lipases, pectinases and polygalacturonases are responsible for the degradation of plant tissues (Petrini *et al.*, 1993). In our ongoing research programme on investigation of the potential of integrated pest management method to control *G. dilatatus*, it was confirmed that the termites are attracted to decayed tea stems (Senanayake *et al.*, 2014). Therefore, the work reported in the present study was aimed at demonstrating that the fungi living in live termites/termite galleries/decayed stumps play a major role in facilitating the decomposition of wood. To our knowledge, this is the first investigation providing data on the biodiversity and substrate utilisation of wood decaying fungi isolated from decayed tea stems.

METHODOLOGY

Experimental site and sample collection

A thirteen year old tea field was selected for this study and 2 1/2 year old pruned stems from TRI 4042 were collected. Field no. 1 of St. Joachim Tea Estate in Ratnapura, Low Country Regional Centre of the Tea Research Institute (latitude 6° 40'58"N and longitude 80° 23'57" E and elevation 128 m amsl), which was infested with the low country live wood termite was chosen. Three study plots consisting of 100 tea bushes per plot were demarcated.

Collection of termites and tea stems

Samples were collected from the three study plots. Six termite infested bushes were selected randomly, and six decayed stumps intact with undecayed wood containing termite colonies were collected from each plot. The samples were brought to the laboratory in sealed clean polythene bags and kept in covered plastic boxes until they were used in the experiments. The samples were processed within 48 hrs for development of cultures. Fungi were isolated from the collected decayed tea stems of TRI 4042 cultivar, termite galleries in tea stems and living termites.

Isolation of fungi from decayed stumps, termite galleries and living termites

Isolation of fungi from decayed stumps of tea plant

The decayed stumps were cleaned using tap water to remove soil particles and kept for a few minutes to drain off excess water. The stumps were dissected into small segments (approximately $1 \times 1 \times 1$ cm) and composite samples were made. Twenty pieces were selected randomly and surface sterilised by sequential immersion for 10 s in 95 % ethanol, 3 min in 0.5 % sodium hypochlorite and 30 s in 75 % ethanol and kept on a sterilised filter paper to drain excess moisture (Balasooriya, 1998; Guo *et al.*, 2003). Surface sterilised segments from each stem sample were inoculated into sterilised potato dextrose agar (PDA) plates supplemented with 1 % streptomycin (4 segments/ plate). The cultures were incubated at 25 °C for 7 – 14 days. Developing fungal colonies from each segment were sub-cultured in 2 % malt extract agar (MEA) for further investigation (Balasooriya, 1998; Kannangara *et al.*, 2009). The fungi were photographed and deposited as voucher specimens at the Department of Chemistry, University of Kelaniya, Sri Lanka and the Tea Research Institute of

Sri Lanka under the accession numbers PS/WR/1, PS/WR/2, PS/WR/3,PS/WR/4, PS/WR/5, PS/WR/6, PS/WR/7, PS/WR/8, PS/WR/ 9, PS/WR/10, PS/WR/11, PS/WR/12 and PS/WR/13 (Figure 1).

Isolation of fungi from termite galleries

Six termite infested bushes from the tea cultivar, TRI 4042 were sampled and a composite sample was made using ten pieces (approximately 1 cm length) of termite galleries from a tea bush, accommodating a total of 60 pieces. Twenty pieces were randomly selected from the composite sample and surface sterilised using the method described by Guo *et al.* (2003). Surface sterilised samples were placed on PDA and incubated at 25 ℃ for 7 – 14 days. Developing fungal colonies from each piece were sub-cultured in 2 % MEA for further investigation (Balasooriya, 1998; Kannangara *et al.*, 2009). They were photographed and deposited as voucher specimens at the Department of Chemistry, University of Kelaniya, Sri Lanka and the Tea Research institute of Sri Lanka under the accession numbers PS/TG/1, PS/TG/2, PS/TG/3, PS/TG/4, PS/TG/5 and PS/TG/6 (Figure 1).

Isolation of fungi from living termites

Samples from six termite infested bushes were collected from TRI 4042 cultivar and a composite sample of sixty living termites was obtained by collecting 10 living termites from each infested bush. Randomly selected twenty living termites from the composite sample were surface sterilised by using the 1 % sodium hypochlorite and then rinsed three times with sterile distilled water (Jayasimha, 2006). These termites were inoculated on to sterilised PDA and incubated for 4 – 7 days. Three replicates were prepared and the developing fungal colonies from each termite were sub-cultured into 2 % MEA for further investigation (Balasooriya, 1998; Kannangara *et al.*, 2009). They were photographed and deposited as voucher specimens at the Department of Chemistry, University of Kelaniya, Sri Lanka and the Tea Research Institute of Sri Lanka under the accession numbers PS/TM/1, PS/TM/2, PS/TM/3, PS/TM/4, PS/TM5, PS/TM/6 and PS/TM/7 (Figure 1).

Morphological identification of fungi isolated from living termites, termite galleries and decayed tea stems

Morphological identification of each fungus was carried out using the sticky tape method (Felgel, 1980) and identification keys (Barnett, 1960; Domsch *et al.,* 1993). The fungal cultures were examined periodically and identified when they sporulated. The cultures were

separated into groups based on their morphological characteristics including growth pattern, colony texture, pigmentation, and growth rate of the colonies on PDA. When the fungal colonies sporulated on PDA, small plaques from the edge and the centre of each growing colony were transferred onto glass slides, and examined using a compound light microscope (Leica DMLS, USA) for characteristics of their vegetative and reproductive structures such as hyphal colour and structures, shape and size of conidia, conidiophores, and microsclerotia (Kumar *et al.*, 1998; Kannangara *et al.*, 2009). The percentage frequency of occurrence of each fungus was calculated using the following formula;

$$\% \text{ Frequency of occurrence} = \frac{\text{No. of pieces colonised by the fungus}}{\text{Total number of pieces plated}} \times 100$$

Molecular identification of isolated fungi

The identification of isolated pure strains of the fungi was done using a molecular biological protocol by genomic DNA extraction, amplification and sequencing.

DNA extraction

The fungal isolates were grown on PDA plates for 4 – 7 days and the mycelia were harvested and ground in liquid nitrogen. Total DNA was extracted from the ground mycelium of each fungus using a DNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer's protocol.

Polymerase chain reaction (PCR) amplification

PCR was carried out using 25 µL reaction volumes, each containing 2 µM ITS primers (ITS1-F, ITS4-B) (1 µL each), 2 mM $MgCl_2$ (2 µL), 2 µM dNTPs (2.5 µL) and Taq polymerase (0.5 µL), buffer (5 µL) H_2O (12 µL). Taq polymerase was added to the mixture just before the start of PCR. Initial denaturation was done at 94 ℃ for 4 mins, and 45 s for other following denaturation steps. Primer annealing was done at 55 ℃ for one min and extension was done at 72 ℃ for 1 1/2 mins. Seven mins were given for the final extension. The PCR was carried out for about 30 cycles to obtain maximum PCR products. Success of PCR was confirmed by running the product in agarose gel. ITS1-F (5' – CCT GGT CAT TTA GAG GAA GTA A – 3') and ITS4-B (5' – CAG GAG ACT TGT ACA CGG TCC AG – 3') primers were used for amplification (Nicolotti *et al.*,

2009). Amplified and purified DNA was subjected to DNA sequencing and the obtained sequences were submitted to the GenBank as a nucleotide query and compared with already existing DNA sequences using NCBI BLAST® (*http://www.ncbi.nlm.nih.gov/blast/*). The most similar reference sequences with the query sequences were obtained and used to identify the genus or species of the fungus. The isolates were assigned to a species if the sequence was ≥ 99 % similar to a valid species sequence deposited with NCBI GenBank.

Utilisation of carbon sources by selected fungi associated with decayed tea stems, termite galleries and living termites

The ability of seven frequently isolated fungi species (*F. solani*, *Fusarium* sp. 1, *Fusarium* sp. 2, *Aspergillus tamari*, *Aspergillus aculeatus*, *R. variabilis* and *N. fischeri*) to produce extracellular enzymes that degrade plant cell wall components and storage materials was tested using pure substrates; starch, pectin, cellulose and lignin through extracellular enzymes. Each sample was replicated three times.

Cellulose

Cellulose agar medium was prepared with the addition of 1 % cellulose powder to the basal medium at pH 6.2 (Eggins & Pugh, 1961). The agar slants containing cellulose medium (6 mL) were autoclaved at 121 ºC for 20 mins. After sterilisation the solution in each test tube was mixed thoroughly by hand shaking. Immediately, the test tubes were placed as slants and cooled in an ice bath to prevent the sedimentation of cellulose. Then the agar slants were inoculated with the test fungi and incubated at room temperature (28 ± 2 ºC) for a month. Tubes were observed weekly. Cellulose utilisation was indicated by clearing of the cellulose agar medium beneath and around the actively growing colonies (Kannangara *et al.*, 2009).

Starch

The ability to degrade starch by each fungus was assessed through amylolytic enzymes of the test fungi. The medium was prepared using Difco nutrient agar with 5 % starch (Hankin & Anagnostakis, 1975; Kannangara *et al.*, 2009). The starch agar plates were inoculated separately with the test fungi and incubated at room temperature (28 ± 2 ºC) for 3 – 5 days. Test plates were flooded with 1 % KI/I$_2$ solution and the production of a clear zone around the active fungal colony indicated the amylolytic activity.

Pectin

The ability of the test fungal species to utilise pectin was assessed by the pectolytic activity of the two enzymes polygalacturonase and pectate lyase. Plates were prepared using the basal medium amended with 0.5 % of liquid citrus pectin (Sigma Aldrich Canada Ltd.) (Eggins & Pugh, 1961; Kannangara *et al.*, 2009). The medium at pH 5 was used to detect polygalacturonase activity and at pH 7 it was used to detect pectate lyase activity for optimal activity of enzymes. After incubation of fungal colonies, the plates were flooded with a 1 % aqueous solution of cetavlon (cetyle trimethyl ammonium bromide), which precipitated intact pectin in the medium. Thus a clear zone around the active colonies indicates polygalactouranase activity and the pectate lyase activity of each fungus tested (Hankin & Anagnostakis, 1975; Abdel-Raheem & Shearer, 2002; Kannangara *et al.*, 2009).

Lignin

Lignin degradation was assessed indirectly by the production of three enzymes, laccase, peroxidase and tyrosinase, which are involved in depolymerisation of lignin. Each fungal strain was grown on MEA at pH 7. After incubation of fungal colonies at 22 ºC for 4 – 5 days, the production of each of the three enzymes was observed using the drop tests (Stalpers, 1978; Kannangara *et al.*, 2009). The production of laccase was observed using a solution of naphthol (0.1 M) in 96 % ethanol. Drops from this solution were tested on the active marginal hypae of each of the fungal species using a dropper. Diffusion of purplish colouration into the medium indicated the presence of laccase (Stalpers, 1978; Kananagra *et al.*, 2009). The production of peroxidase was observed using a solution of 0.4 % H$_2$O$_2$ and 1 % pyrogallol in water [25 mL H$_2$O$_2$ (0.4 %): 25 mL pyrogallol (1 %)]. Drops from this solution (freshly prepared) were tested on the hypae of each of the test fungi. Yellow brownish colouration indicated the production of peroxidase (Amirita *et al.*, 2012). The production of tyrosinase was tested using a test solution of *p*-cresol (0.1 M) prepared in 96 % ethanol. Drops from this solution were tested on the marginal hypae of each test fungal species. Appearance of an orange brown colour diffusing into the medium indicates production of tyrosinase (Kaarik, 1965; Amirita *et al.*, 2012).

Data analysis

Analysis of variance was carried out for comparison of frequency of occurrence of isolated fungi from the decayed wood, termite galleries and living termites using SAS.

Percentages were transformed to arcsine values prior to analysis. Mean separation was carried out using Tukey's studentised test (Aukema *et al*., 2005).

RESULTS AND DISCUSSION

Isolation of fungi from decayed stumps, termite galleries and living termites

Senanayake *et al*. (2014) have confirmed that *G. dilatatus* is attracted to decayed tea stems. In this study molecular taxonomy of wood decaying fungi was done in order to identify the fungal species present in decayed stumps, living termites and termite galleries. The total number of fungal species isolated from the three locations was fourteen. Using DNA sequencing studies the identity of twelve of them was confirmed (Figure 1). The number of fungi identified from decayed stumps, which were two and half years old (decayed) pruned stems, termite galleries and living termites were 13, 6 and 7, respectively.

The frequencies of occurrence of *F. solani*, *Fusarium* sp. 1, *Fusarium* sp. 2, *T. corrugata* and *Lasiodiplodia* sp. in living termites, termite galleries and decayed stumps of TRI 4042 were between 25 – 60 % and it was less than 25 % in *R. variabilis*, *A. tamari, A. aculeatus, Trichoderma harzianum, B. ochroleuca, P. lilacinum* and *F. oxysporum* (Table 1).

In living termites, 25 – 60 % colonisation frequencies were observed for *F. solani*, *Fusarium* sp. 1 and *Fusarium* sp. 2, while *A. tamari, A. aculeatus*, *N. fischeri* and *R. variabilis* showed the frequencies in the range of 10 – 15 %. It was noted that *N. fischerri* was only present in living termites while both *Lasiodiplodia* sp. and *B. ochroleuca* were not observed in living termites. However, *Lasiodiplodia* sp. (40 – 42 %) and *B. ochroleuca* (5 – 7 %) assemblages were detected in termite galleries and decayed tea stumps, and *A. tamari, A. aculeatus, N. fischerri, T. harzianum, P. lilacinum* were absent in termite galleries.
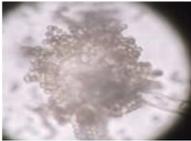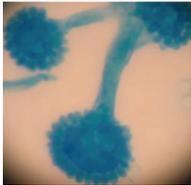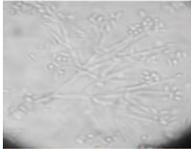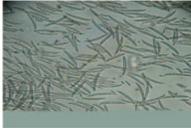
Table 2 presents the frequencies of occurrence of wood decaying fungi species common in decayed stumps, living termites and termite galleries. The results revealed that the frequencies of four fungal species, *F. solani, Fusarium* sp.1, *Fusarium* sp. 2 and *R. variabilis* are significantly different (F = 48.91, df = 3, p < 0.0015) among decayed stumps, living termites and termite galleries. Mean separation indicated that the frequency of occurrence (50 ± 1.12) of *F. solani* in decayed stumps was significantly (p < 0.05) higher than those of living termites (25 ± 1.12) and termite galleries (30 ± 2.24),

while the frequency of occurrence was not significantly different (p > 0.05) between living termites and termite galleries. The frequency of occurrence (36.67 ± 2.9) of *Fusarium* sp.1 in decayed stumps was significantly lower (p < 0.05) than that of living termites (60 ± 1.94), and termite galleries (55 ± 1.94), while it was not significantly different (p > 0.05) between living termites and termite galleries. Frequencies of occurrence of *Fusarium* sp. 2 were not significantly different (p < 0.05) among decayed stumps, termite galleries and living termites. The frequencies of occurrence of *R. variabilis* among decayed stumps, termite galleries and living termites were also not significantly different (p > 0.05).

It has been reported that *Fusarium* sp. and *Aspergillus* sp. belonging to the family deuteromycetes cause soft rot of wood in trees, while *T. corrugata* belonging to the family basediomycetes cause wood decay (Eaton & Hale, 1993). Further it has also been reported that *Lasiodiplodia* sp. are associated in tea stems and cause stem canker (Pradeepa *et al*., 2014). In the present study, these fungi were also isolated from decayed stumps, termite galleries and living termites indicating that they facilitate wood decaying in the tea stem. Schubert *et al*. (2008) have reported that *T. harzianum* is a parasitic fungus on other fungi and it is used as an effective bio-control agent to control other fungi. Therefore *T. harzianum* could be a parasitic fungus on wood decaying fungi associated in decayed tea stumps. *Rizomucor variabilis* is common in wood at ground contact and it is less important for wood deterioration (Eaton & Hale, 1993). Therefore *Fusarium* spp., *Aspergillus* spp., *T. corrugata* and *Lasiodiplodia* spp. are the potential fungi that cause soft rot in wood of plants.

Utilisation of carbon sources by selected wood decaying fungi associated with decayed tea stems, termite galleries and living termites

Colonisation of the host tissues by wood decaying fungi involves the digestion of constituents of plant cell wall, and that can be achieved by enzymatic digestion of epidermal cell layers. Lignin, cellulose and pectin are the main components of the cell wall and it is reported that phytopathogens can produce different types of enzymes to digest the components of the cell walls. The present study was conducted with fungi isolated from two and a half years old decayed stems of the cultivar TRI 4042, living termites (*G. dilatatus*) and termite galleries. The ability of the selected fungi to produce extracellular enzymes that degrade plant cell wall components and storage materials was investigated. The results revealed that all the test fungi; *F. solani, Fusarium* sp. 1, *Fusarium* sp. 2, *A. tamari*, *A. aculeatus*, *R. variabilis* and *N. fischerii*

Sequence based identification	Sequence with best match	Identity (%)	Sporulating structure (10×40×1)
Aspergillus aculeatus	KJ653817.1	100	
Aspergillus tamari	JX110981.1	100	
Bionectria ochroleuca	EU552110.1	99	
Fusarium solani	KF751073.1	99	
Fusarium sp. 1	KC478529.1	99	
Fusarium sp. 2	DQ780425.1	99	
Fusarium oxysporum	KJ562372.1	99	
Lasiodiplodia sp.	AB734791.1	99	
Purpureocillium lilacinum	KM2466754.1	100	
Rhizomucor variabilis	HM623314.1	99	

Sequence based identification	Sequence with best match	Identity (%)	Sporulating structure (10×40×1)
Trametes corrugate	EU661875.1	99	
Trichoderma harzianum	KM207772.1	100	
Unidentified sp.			
Unidentified sp.			
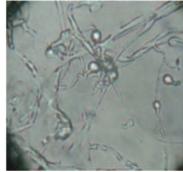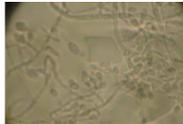
Figure 1: Fungal species isolated from decayed stumps, termite occupied galleries and living termites occupied in stems of cultivar TRI 4042

Table 1: Frequency of occurrence of different fungal species isolated from surface sterilised living termites, termite galleries and decayed stumps of TRI 4042

Fungal species	Frequency of occurrence (%)		
	Living termites	Termite galleries	Decayed tea stumps
A. aculeatus	10 ± 2.24	ND	3.33 ± 0.65
A. tamari	10 ± 1.94	ND	1.67 ± 0.65
B. ochroleuca	ND	5 ± 1.12	6.67 ± 2.58
F. oxysporum	ND	ND	3.33 ± 0.65
F. solani	25 ± 1.12	30 ± 2.24	50 ± 1.12
Fusarium sp. 1	60 ± 1.94	55 ± 1.94	36.7 ± 2.9
Fusarium sp. 2	30 ± 1.12	25 ± 1.12	30 ± 2.24
Lasiodiplodia sp.	ND	40 ± 2.96	41.67 ± 2.33
N. fischeri	15 ± 1.12	ND	ND
P. lilacinum	ND	ND	5 ± 0
R. variabilis	10 ± 1.94	10 ± 1.12	16.67 ± 0.65
T. corrugata	ND	ND	33.3 ± 1.29
T. harzianum	ND	ND	11.67 ± 2.81

ND = not detected

Table 2: Mean percentage frequencies of occurrence of fungal species common to decayed tea stumps, termite galleries and living termites

	% Frequency of occurrence ± SE			
	F. solani	*Fusarium* sp. 1	*Fusarium* sp. 2	*R. variabilis*
Living termites	25 ± 1.12[b]	60 ± 1.94[a]	30 ± 1.12[a]	10 ± 1.94[a]
Termite galleries	30 ± 2.24[b]	55 ± 1.94[a]	25 ± 1.12[a]	10 ± 1.12[a]
Decayed tea stumps	50 ± 1.12[a]	36.67 ± 2.9[b]	30 ± 2.24[a]	10.67 ± 1.29[a]

Each data point represents the mean of three replicates ± SE; means followed by the same letter(s) in each column are not significantly different according to the Tukey's studentised range test and ANOVA ($p \leq 0.05$).

were able to utilise starch (Table 3). Cellulose utilisation ability was exhibited by *Fusarium* sp. 1, *F. solani* and *A. tamari*. It has been reported that *F. solani* and *Fusarium* sp. isolated from leaf litter in a forest were able to produce cellulase (Agustini *et al*., 2012), and these findings are in agreement with the present study.

The production of both pectin and polygalacturonic acid degrading enzymes, pectate lyase and polygalctouranase, which are assumed to be responsible for the degradation of cell wall and middle lamella during penetration and colonisation of the host tissue by fungi, was evaluated. The results revealed that five test fungi species, *Fusarium* sp. 1, *F. solani* , *A. tamari, Fusarium* sp. 2 and *A. aculeatus* utilised pectin by producing pectate lyase, whereas *R. variabilis* and *N. fischeri* did not produce pectate lyase.

Production of polygalctouranase was observed in *Fusarium* sp. 1, *F. solani, A. tamari* and *Fusarium* sp. 2, while *R. variabilis, A. aculeatus* and *N. fischeri* were not able to produce polygalctouranase. The third major component of wood cell wall is lignin. The ability to utilise lignin (lignin degradation) was evaluated by using the production of three enzymes; laccase, peroxidase and tyrosinase. Lignin degradation by white rot fungi (basidiomycetes) has been studied extensively (Eaton & Hale, 1993). The results revealed that all test fungi can utilise lignin by producing peroxidase but only three fungi, *Fusarium* sp. 2, *Fusarium solani* and *A. aculeatus* could produce tyrosinase to support the digestion of lignin, while none of the test fungi could produce laccase (Table 3). *Rhizomucor variabilis* and *N. fischeri* have less contribution for the deterioration of wood because it did not produce cellulose, pectate lyase and polygalctouranase.

Table 3: Substrate utilisation patterns of fungi isolated from surface sterilised *G. dilatatus*

Fungal species	Cellulose	Starch	Pectin		Lignin		
			Polygalactouronase	Pectate lyase	P	L	T
R. variabilis	–	+	–	–	+	–	–
Fusarium sp. 1	+	+	+	+	+	–	–
Fusarium sp. 2	+	+	+	+	+	–	+
A. tamari	+	+	+	+	+	–	–
F. solani	+	+	+	+	+	–	+
A. aculeatus	+	+	–	+	+	–	+
N. fischeri	–	+	–	–	+	–	–

Number of replicates = 3   L = Laccase   P = Peroxidase   T = Tyrosinase

CONCLUSION

Decayed tea stumps of TRI 4042 cultivar occupied by *G. dilatatus* contained fourteen species of fungi and twelve species were identified as *F. solani, Fusarium* sp. 1, *Fusarium* sp. 2, *T. corrugata, Lasiodiplodia* sp., *R. variabilis, A. tamari, A. aculeatus, T. harzianum,*

B. ochroleuca, P. lilacinum and *F. oxysporum*. Six pure cultures were isolated from the termite galleries and identified as *F. solani, Fusarium* sp. 1, *Fusarium* sp. 2, *Lasiodiplodia* sp., *R. variabilis* and *B. ochroleuca*. Seven pure cultures of fungi isolated from surface sterilised living termites were identified as *F. solani, Fusarium* sp. 1, *Fusarium* sp. 2, *A. tamari, A. aculeatus, N. fischeri* and *R. variabilis*.

The results showed that *F. solani*, *Fusarium* sp. 1, *Fusarium* sp. 2, *A. tamari* and *A. aculeatus* could utilise cellulose when they grow in cellulose containing medium. Starch utilisation of wood decaying fungi indicated that all the test fungal species have the ability to digest starch when they grow in starchy medium. *F. solani*, *Fusarium* sp. 1, *Fusarium* sp. 2 and *A. tamari* could produce both polygalactouronase and pectate lyase to utilise pectin. The results also revealed that *A. aculeatus* produced only pectate lyase. *Rhizomucor variabilis* and *N. fischeri* did not produce these two enzymes. Tests carried out for lignin degradation revealed that none of the test fungi produce laccase, while all the fungi were able to produce peroxidase. *Fusarium solani*, *A. aculeatus* and *Fusarium* sp. 2 were able to produce tyrosinase enzyme and the other four species of test fungi were negative for tyrosinase production.

Observations of the substrate utilisation tests on pure substrates indicated that the test fungi could degrade structural components, cellulose, lignin and pectin, and the storage component starch in stems of the tea plant. Therefore, it will be useful if cultivar screening programme is evaluated for termite resistance as well as resistance to wood rot.

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