RESEARCH ARTICLE

Biodegradation of *Camellia sinensis* (L.) O. Kuntze Wood by *Hypoxylon* sp. Isolates from Diverse Tea Growing Counties of Kenya

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Abstract

Many fungi play a vital role in the decomposition of wood in nature and nutrient cycling, yet some afflict serious damage to cultivated tree crops and forestry. Tea (*Camellia sinensis* (L) O. Kuntze) is affected by Hypoxylon wood rot (HWR) disease caused by the *Hypoxylon* sp. Pers ex Fr. In the recent years; there have been reports on increase of the disease in Kenya. This study aimed at determining disease incidence and biodegradation by 59 *Hypoxylon* isolates from diverse tea growing counties of Kenya on wood, from resistant (TRFK 6/25) and susceptible (TRFK 6/129) tea cultivars. The disease incidence varied ($p \leq 0.05$) in different counties (6.7 to 77.5%) and was more prevalent in tea cultivars (77.5%) than seedling type of teas (15.7%). Bio-degradation of wood was performed in Falcon tubes for 40 days. The 59 *Hypoxylon* isolates significantly ($p \leq 0.05$) bio-degraded tea wood. The weight loss ranged from 4.84 to 16.44% in the susceptible and 4.61 to 12.64% in the resistant cultivars. The results indicate the potential use of biodegradation to evaluate resistance of tea cultivars to damage by *Hypoxylon* sp. This study concludes that biodegradation is potentially usable technique to screen tea cultivars for resistance to HWR.

Keywords: Hypoxylon wood rot, Ascomycetes, disease incidence, tea cultivars, biodegradation, Kenya

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Introduction

Generally, wood inhabiting fungi play a vital role in decomposition of dead wood as a food base for growth, reproduction and nutrient cycling in nature.[1-4] Although, some wood-rotting species are necrotrophic parasites, a number of these fungi can invade and kill living sapwood (biotrophic parasites) and cause death of living trees.[1,5] Potential of wood decaying fungi in decomposition and recycling of natural polymers such as lignin and plant cell wall polysaccharides has been reported.[3,6] Finding by Fukasawa et al.[3] confirmed the decomposition abilities of 28 wood biodegrading fungi from the taxa Basidiomyceta, Ascomycota and Zygomycota on beech wood. Basidiomycete fungi such as *Heterobasidion parviporum* and *H. annosaum,* and some ascomycetes infect host stem wood of mature spruce trees causing soft-rot.[7] However, unwounded trees are less vulnerable from pathogen infection. Pruning is known to damage the xylem vessels rendering the tree vulnerable to wood decaying fungal infection.[6,8]

Gao et al.[4] successfully isolated and identified lignin degrading enzymes produced by ascomycetes of forestry disorders and further demonstrated 20.91% dry mass loss of wood and 22.99% w/w of lignin degradation. In addition, Liers et al.[9] demonstrated various patterns of wood structural components, lignin, hemicellulose, cellulose degrading and oxidative enzymes produced extracellularly by wood and litter colonizing basidiomycetes and Sordariomycetes. *Hypoxylon* sp. Pers ex Fr. belonging to the family Xylariaceae, in the class Sordariomycetes (*syn.* Pyrenomycetes), subdivision/subphylum Pezizomycotina and division/phylum Ascomycota is known to cause soft-rot of stem of mostly wounded host trees.[6,7,8] This is attributed to the production of enzymes such as laccase, peroxidase, hydrolases and esterase as potential ligninolytic enzymes.[2] The fungus exists as a saprophyte or facultative parasite or endophyte on dicotyledonous angiosperms including tea in tropical regions.[10-13]

Tea (*Camellia sinensis* (L.) O. Kuntze), is a perennial crop cultivated in many countries, Kenya being the third largest world producer after China and India[14], accounting for 8.36 % of the global production, which earned 1.39 billion US Dollar from 4.74 million tons in 2019. *C. sinensis* just like most other crops is affected by fungal pathogens, mainly *Hypoxylon* sp. *Armillaria* sp. *Phomopsis theae*, *Pestalotiopsis theae* and *Colletotrichum cameliae*. Hypoxylon Wood Rot (HWR) disease is caused by the fungus *Hypoxylon* spp.[15,16] In Kenya, the disease was first reported in farms located in Kericho, Nandi and Kiambu counties and is of economic importance to tea production.[15,17,18] In the recent past, there are increasing reports on the disease in the country leading estimated to account for about 20 to 30 % crop losses.[16,17,19] Yield losses become apparent when the numbers of tea bushes in the field have been reduced considerably. Pruning of tea bushes is a very important practice in tea cultivation but creates wounds (pruning cuts) on the primary branches thereby predisposing the bush to infection by *Hypoxylon* sp.[15,16] Tea pruning is a cultural practice that is done regularly after 3 to 4 years to maintain plucking table from 0.45 m to 1.2 – 1.5 m high (*syn.* harvesting height). Beyond 1.5 m, tea harvesting becomes

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unmanageable leading to decline in growth vigour which causes reduced productivity necessitating the tea to be down pruned to a height of 0.45 m.[10,16,20] The resultant large pruning cuts serve as points of infection by inoculum of *Hypoxylon* sp. Also the pathogen may also enter tea host plant through wounds caused by sun-scorch and hail damage.[8,15,16] The HWR disease is characterized by deterioration of the tea bush frame due to rotting and death of primary branches and in severe cases resulting to ultimate death of the whole tea bush.[10,15,17,21,22] The rotten branches bear superficial fructifications (stromata) of the fungus that appears as irregular dark-grey to black raised patches of different sizes. Ultimately, affected branches become porous and light in weight.[15-17] The disease is minimized by adherence to correct pruning methods. However, pruning that causes extensive wounding of branches which is often done at heights lower than recommended as well as any form of wounding below 45 cm, predisposes the tea bushes to infection by the pathogen.[10,22,24]

Despite the prevalence of HWR disease, limited information exists on pathogen diversity in different tea growing regions and factors influencing host–pathogen interactions as determined, for example, by cultivar genotypes in Kenya. This study aims to contribute into the development of an effective strategy for management of HWR disease by evaluating the potential use of wood biodegradation as a criterion for resistance screening in tea cultivars. Therefore, this study determined disease incidence in 6 counties on different tea types and evaluated 59 different isolates of *Hypoxylon* sp. from different counties of Kenya for biodegradation abilities on tea wood from different cultivars.

Methodology

Hypoxylon wood rot disease incidence

Pruned tea fields in 6 counties were used in the assessment of HWR disease. Kericho County was excluded as the tea fields had fully recovered from prune. In a pruned tea field, 25 pruned tea bushes were randomly selected in a row and the entire tea frame was thoroughly examined for presence of disease symptoms and signs which include wood rot and fungal fructifications, respectively[19,25] in three replicates. The data on the tea bushes with HWR symptoms and signs were used to calculate percent disease incidence (DI);

DI (%) = (Dt/Tt)*100. Where: Dt= the number of pruned tea bushes with disease symptoms and signs, Tt= the total number of pruned tea bushes. The type of tea cultivars grown on the fields used during the assessment was classified as pure or mixed cultivar or seedling teas and respective field altitude was recorded.

Sample collection

Dead tea wood was randomly sampled in August and September 2015 from seven pruned commercial tea fields located in seven tea growing counties in Kenya. The counties represented both east (Kirinyaga, Nyeri, Kiambu, and Meru North) and west (Nyamira, Nandi and Kericho) of the Great Rift Valley. The samples were stored in khaki paper bags, labelled appropriately, air dried in the laboratory and stored at room temperature.

Hypoxylon fungi isolation

Single spore isolation technique was used to isolate the *Hypoxylon* fungi as described by Henriques et al.[26] with slight modification. *Hypoxylon* stromata were excised moistened using sterile distilled water for 1 hour, and rinsed in sterile water by dipping twice for 1 minute each. They were then aseptically placed on plain agar (PA) medium (20 g Agar powder L^{-1}), inverted and incubated at room temperature for 12 hours to eject ascospores onto the petri dish lids. Ascospores were then plated on potato dextrose agar (PDA) (49 g of PDA powder L^{-1}) supplemented with 0.03 g/L streptomycin. The ascospore plates were examined visually and under a microscope three representative colonies arising from a single ascospore were selected and aseptically transferred to separate fresh PDA plates and incubated for 7 days. They were visually observed for consistency and one fungal colony growth was selected and used to make pure culture on PDA in duplicates.

Determination of biodegradation abilities of *Hypoxylon* isolates on wood from selected tea cultivars

Two tea cultivars TRFK 6/25 and TRFK 6/129 known to have low and high incidence of HWR disease respectively[19] were used in this study. The tea cultivars were planted in 1964, in Field 4 at Tea Research Institute. Wood branches of size 4 to 5 cm in diameter selected from the tea cultivars were cut and taken to the laboratory. The barks were then peeled off, wood washed and dried in an oven at 60°C for 48 hours.[9] The wood was sliced as described by Gao et al.[4] with slight modification. They were cut into slices of approximately 3 mm thick discs and subsequently into 1 cm by 1 cm to get wood chips approximately 1.0×1.0 cm×3 mm. Biodegradation was performed in liquid nutrient according to Gao et al.[4] The mixing ratios (weight by volume - w/v) of wood: wheat bran: nutrient solution was 16.4% tea wood chips, 3.6% wheat bran and 80% nutrient solution in to a clean 15 ml Falcon tubes, then autoclaved at 121°C for 20 minutes and allowed to cool. The sterilized biodegradation tubes were labelled with respect to *Hypoxylon* isolate and each was inoculated with 2 pieces of 2 mm inocula discs taken from hyphal tips of 7 days old *Hypoxylon* isolates in three replicates and a set of un-inoculated served as controls. All the inoculated and un-inoculated biodegradation tubes were incubated under solid-state fermentation at room temperature for 40 days.[4]

Recovery of biodegraded wood and data analysis

After 40 days of incubation, the biodegraded wood chips were removed and carefully washed in distilled water to remove the mycelium growth on wood surfaces.[4] Each of the wood chip samples were put in different paper envelopes and were then dried in an Oven at 60°C for 3 days. The samples were weighed after 48 hours drying, and thereafter at every 12 hours until the weight remained constant using a precision balance (KERN, EW 620-3NM, Germany). The wood weight loss for treatments and control (non inoculated) were determined by subtracting the final dry weights from initial weight and then expressed as percentage weight loss;

$$\text{Weight loss (\%)} = W_I\text{-}W_F/I*100.$$

Where: W_I= initial weight of wood chips; W_F= final weight of biodegraded wood chips.

The data on disease incidence and weight loss were subjected to ANOVA and a correlation between disease incidence and altitude was performed using GenStat 15th Edition, VSN International 2012 statistical software.

Results

Hypoxylon wood rot disease incidence

Hypoxylon wood rot disease incidence ranged from 6.7 to 77.5 % in different tea growing counties. The DI varied ($p \leq 0.05$), and

was higher in Kirinyaga county followed by Nandi and Nyamira Counties. On the other hand, Kiambu, Nyeri and Meru counties had significantly lower DI (Table 1). With regard to tea cultivar type, the DI was highest in pure or mixed cultivar than seedling teas by 77.5 % and 15.7 %, respectively (Table 2). There was no correlation ($p \leq 0.05$) ($r = 0.402$, $n = 6$) between disease incidence and altitude (agro-ecological attribute) of the tea growing counties indicating that the tea genotype mainly influence the occurrence of the disease.

Biodegradation by different *Hypoxylon* isolates on wood from selected tea cultivars

The wood chips in solid-state fermentation supported growth of all the *Hypoxylon* isolates at room temperature during the six weeks of incubation. Fourteen *Hypoxylon* isolates (e.g. NAND7, NAND26, NAND13) had significantly lower (4.84 – 7.54%) weight loss for the susceptible *C. sinensis* cultivar (TRFK 6/129) while eight (e.g. NAND20, NAND18, NAND27) had significantly higher (13.19 – 16.44%) weight loss and the rest of the isolates varied ($p \leq 0.05$) within the range (Table 3). Twenty two *Hypoxylon* isolates (e.g. NAND5, NAND14, KERC6) had significantly lowest effect (4.61 – 8.12%) on weight loss for the resistant cultivar (TRFK 6/25) while nineteen isolates (e.g. NAND2, NAND20, NYAM3) had highest (9.06 – 12.64%) effects on wood weight loss (Table 3). The rest of the *Hypoxylon* isolates varied significantly within the range.

Generally, nine *Hypoxylon* isolate (e.g. NAND11 NAND34, NAND24) had lowest (4.84–7.28%) effects on wood weight loss while six isolates (NAND6, NAND25, NAND3, NAND18, NAND20, and NAND2) had significantly ($p \leq 0.05$) high (11.82 – 14.16%) effect on wood weight loss (Table 3). The rest of the isolates varied within the range (Table 3). The pathogen isolates from Nandi County appear to have more diversity than for other counties since majority the isolate had mean high and low wood weight loss while few intermingled

Table 1: Hypoxylon wood rot disease incidence on *Camellia sinensis* in 6 counties of Kenya

County	*Mean[a] (%) incidence of HWR disease*
Kirinyaga	77.5±5.9a
Nyeri	8.3±2.9c
Meru	6.7±2.9c
Kiambu	16.7±5.8c
Nyamira	17.3±6.4bc
Nandi	27.9±8.6b
C.V. (%)	20.8
S.E.	3.5
p value	0.05

[a] Means followed by the same letter are not significantly different at ($p \leq 0.05$, LSD test)

Table 2: Hypoxylon wood rot disease incidence on *C. sinensis* genotypes in Kenya

Tea genotype	*Mean[a] (%) incidence of HWR disease*
Pure or mixed stand of a cultivar	77.5±5.9a
Seedling	15.7±0.8b
C.V. (%)	9.0
S.E.	4.2
p value	0.05

[a] Means followed by the same letter are not significantly different ($p \leq 0.05$, LSD test)

Table 3: Percent dry mass loss of tea wood through bio-degradation by different isolates of *Hypoxylon* sp.

	Tea cultivar/ Weight loss of wood (%)		
*Hypoxylon isolates**	*TRFK 6/129 (Susceptible)*	*TRFK 6/25 (Resistant)*	*Mean Hypoxylon isolate*
KIRN1	10.94	10.20	10.57
KIRN2	6.73	8.42	7.58
KIRN3	12.86	8.50	10.68
KIRN4	7.98	7.08	7.53
NYER1	11.83	8.34	10.08
MERU1	10.75	9.57	10.16
MERU2	10.02	8.65	9.33
KIAM1	10.72	10.15	10.43
KIAM2	9.68	8.98	9.33
NYAM1	8.35	7.91	8.13
NYAM2	8.54	6.61	7.57
NYAM3	12.12	9.39	10.75
NYAM4	8.82	6.27	7.54
NYAM5	10.23	8.83	9.53
NAND1	13.19	7.39	10.29
NAND2	13.96	10.64	12.30
NAND3	16.44	9.24	12.84
NAND4	10.38	9.68	10.03
NAND5	10.20	5.62	7.91
NAND6	14.41	9.24	11.82
NAND7	6.84	8.72	7.78
NAND8	10.42	8.68	9.55
NAND9	9.98	8.78	9.38
NAND10	7.98	4.80	6.39
NAND11	5.07	4.61	4.84
NAND12	12.74	7.17	9.96
NAND13	7.21	7.72	7.46
NAND14	8.83	6.06	7.45
NAND15	10.68	9.76	10.22
NAND16	4.84	7.46	6.15
NAND17	9.79	9.24	9.51
NAND18	15.82	8.18	12.00
NAND19	11.79	9.87	10.88
NAND20	15.67	12.64	14.16
NAND21	11.83	10.64	11.23
NAND22	8.35	7.13	7.74
NAND23	5.51	6.86	6.19
NAND24	7.54	6.00	6.77
NAND25	13.27	10.49	11.88
NAND26	5.12	8.65	6.88
NAND27	13.6	8.64	11.12
NAND28	6.36	7.98	7.17

NAND29	8.43	9.27	8.85
NAND30	9.68	7.95	8.82
NAND31	5.43	8.53	6.98
NAND32	12.23	9.42	10.83
NAND33	9.65	8.31	8.98
NAND34	6.43	8.12	7.28
KERC1	8.65	8.61	8.63
KERC2	12.87	7.80	10.33
KERC3	11.31	8.76	10.04
KERC4	7.13	7.79	7.46
KERC5	11.83	6.80	9.31
KERC6	9.53	7.73	8.63
KERC7	10.83	9.06	9.94
KERC8	11.57	10.19	10.88
KERC9	11.68	8.39	10.04
KERC10	10.21	9.35	9.78
KERC11	10.13	8.95	9.54
Control	1.92	1.75	1.84
Tea cultivar	9.95	8.29	
C.V. (%)			24.3
LSD ($p \leq 0.05$)	*Hypoxylon* isolates		2.52
	Tea cultivar		0.46
	*Hypoxylon**Tea cultivar		3.56

* County codes with respect to *Hypoxylon* isolate: KIRN – Kirinyaga, NYER – Nyeri, KIAM – Kiambu, KERC – Kericho, NYAM – Nyamira, NAND – Nandi

with others (Table 3). In addition, tea cultivars had significantly varied mean weight losses (9.95 and 8.29 %), respectively (Table 3).

As expected, lowest weight losses were observed in the non-inoculated (control) wood chips. The susceptible and resistant cultivar, and mean *Hypoxylon* isolate had 1.92, 1.75 and 1.84% respectively (Table 3). However, some *Hypoxylon* isolates (NAND31, NAND26, NAND11 and NAND16) did not have significant effects compared to control wood chips from susceptible cultivar and isolates NAND10 and NAND11 for the resistant cultivar. However, for mean *Hypoxylon* isolates was significant ($p \leq 0.05$) from the lowest weight loss as in isolate NAND11.

Discussion

In the present study, disease incidence was prevalent in all the tea growing counties covered in the study. However, the disease incidence was more prevalent in pure or mixed cultivar planting than in the seedling tea genotypes. This suggests that the type of tea genotype strongly influences the disease on a tea farm. In addition, seedling teas are genetically more diverse than clonal teas[28], hence the observed lower and varied incidence of HWR. Though altitude at which the tea was grown was positive, this was not significant, indicating that altitude may not be a single factor influence HWR prevalence but rather that the fungus is well adapted to the tea agro-ecological zones in the different tea growing counties. This finding is supported by earlier study by Onsando[27] which showed that *Hypoxylon* survives a wide range of the growth temperature 9°C – 30°C with an optimum at 25°C.

Wood biodegradation by certain wood colonizing fungi belonging to class Sordariomycetes and Bacidiomycetes produce ligninolytic peroxidases enzyme during wood decay.[2,4,9] It is also known that, lignin provides woody tissues with protection against pathogens owing to its resistance to biodegradation.[29] This study clearly demonstrated the difference in capacities among the 59 isolates of *Hypoxylon* isolates to biodegrade *C. sinensis* wood chips from resistant and susceptible tea cultivars under laboratory conditions. The cultivars showed distinction ($p \leq 0.05$) variation among those considered resistant or susceptible to the disease. This is also an indicator of existence in diversity across host spectrum among tea cultivars. Gao et al.[4] and Burcham et al.[30] reported similar findings, where host-pathogen interactions among wood decaying pathogenic fungi demonstrated variable ($p \leq 0.05$) effects on weight loss in Senegal mahogany (*Khaya senegalensis*) sap wood blocks. In addition, the varied diversity of the pathogen based on the effect on mean wood weight loss indicate possible severity of disease in any agro-ecological zone. This is supported by epidemiological studies[19] of the pathogen in that observations showed that a combination of a susceptible host tea and the pathogen led to serious effect of the disease. This led to replacement of the affected tea with other cultivar for profitable tea productivity. The varied weight loss obtained in the two tea cultivars (TRFK 6/25 and TRFK 6/129 6/25) can be attributed to their differences in preformed barriers conferring resistance to damage by *Hypoxylon* sp., a feature that should be investigated further in future. In addition, the findings by Schultz and Nicholas[31] that the sapwood of willows and birches was highly susceptible to decay-causing fungi owing to the chemical components in the wood which do not confer effective defence against decay fungi. Plants have evolved both preformed structural barriers to deter pathogen attack by interception of pathogen-derived effectors and elicitation of defence response.[32] Plant disease resistance involves interaction of plant molecular genetics and active biochemical processes that control host resistance in nature.[32,33,34]

In conclusion, this study found that the incidence of HWR disease in different tea growing counties of Kenya is mainly influenced by the host cultivar. In addition, the varied wood weight loss in the two tea cultivars studied indicate that wood structural components, which constitute the plant preformed barriers in a resistant cultivar, is one of the characteristics that could be responsible for deterrence to the pathogen attack. Also, biodegradation technique could be useful in screening for HWR resistant tea cultivars in future breeding programmes.

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