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FUNGAL DIVERSITY OF FOREST SOIL INFLUENCES DEAD WOOD DECOMPOSITION AND HEALTH OF FOREST ECOSYSTEM

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ABSTRACT

In forest ecosystem microorganisms play important role in dead wood decomposition and also effect forest health by providing manure to the vegetation. However, they are also involved in pathogenesis of tree. The fungal diversity in the soil of Achanakmar Biosphere of Chhattisgarh state of India was studied for three successive years from 2018 to 2021. Their involvement in wood composition was correlated. Overall 53 species belonging to 47 fungal genera were recorded. It was noted that seasonal variation impacted the diversity and decide the dominating fungal species in the habitat. It was also found that majority of the soil fungal species were positive for multiple enzyme assay involved in wood decomposition.

Key words: Biodiversity, fungal diversity, forest ecosystem, wood decay, enzyme assay

INTRODUCTION

Natural forests are home to a significant amount of deadwood, including branches, snags and stumps that provide a variety of habitats for organisms, such as bacteria, fungi and invertebrates **[1]**. It is important that the significant amount of dead wood and its continuous supply is maintained for the healthy fungal diversity even in the managed forest **[2]**. In many countries nature conservationists are working in creation, management and maintenance of dead wood habitats for the improvement of forest ecological conditions. Since, some of the tree pathogens especially specially require large root, snag and stumps for their survival. Thus the habitat of such organisms also increases as the volume of deadwood increases **[3]**. Moreover, not only the volume affects the fungal diversity, the size and type of dead wood do as well **[4, 5]**. It has been found that stable microclimatic conditions essential for certain species are provided by large logs **[6]**. However, small branches provide large surface area for suitable to support fungal species especially sporocarp are well supported by large surface instead of big logs **[7]**. Wood decay rates and fungal diversity composition is also influenced by the wood traits and its position in the forest **[8]**. In countries like India where forest is managed and the amount of deadwood is reducing day by day as compared to the natural forest is negatively affecting the deadwood dependent fungal diversity **[9, 10]**. However, there is always a dilemma between maintaining variation in dead wood sustainability of fungal diversity and the increase in tree pathogenic organism can be challenging in forest management.

MATERIALS AND METHODS

Study area: Achanakmar Wildlife Sanctuary forest, located in Mungeli District of Chhattisgarh India. Geographically it sits on the eastern from frontier of Satpuda Hill and to the north of river Mahanadi covering ≈551.55 square kilometers area. The main vegetation in the sanctuary is the decedous rain forest comprising Sal, Saja, Bija, and Bamboo. During the study period average minimum temperature remained between 12-15°C in December and maximum in May between 40-43°C. The maximum rain in falls noted was 225.35 mm and 236.55 mm in 2019 and 2020 respectively, whereas average wind speed was between 6.0-7.4 kmph in December and 9.8-10.5kmph in May. The humidity recorded between 44-48% in December and 19-20% during the month of May.

Sample collection: Samplings were done between 2018 and 2021 in all three seasons of year i.e. winter, summer and rainy. Sterilized Knife hand driven drill were used for wood sampling and were kept in properly labeled zip locked polybags. During the sampling bryophites coated stuff were separated before sampling so that only wood material is sampled. Five sub-sampless were collected perlog with the sub-samples aggregated prior to analysis. Larger pieces of wood were split into small pices and in situations where five sub-samples could not be obtained. Samples were kept in refrigerator until analysis.

Isolation of Fungi: Solidified Potato Dextrose Agar plate was used for isolation of fungi. The serially diluted dried and milled samples were placed over Potato Dextrose Agar plates and incubated at 28°C for one week. Morphologically distinct cultures were subcultures and maintained on PDA slants.

Identification of the Fungal Isolates

The Fungal isolates were characterized on the basis of their growth pattern and morphological features such as conidial morphology and colony pigment using by slide culture method **[10]**.

This was confirmed and authenticated using the Color atlas of mycology **[11]**.

Molecular characterization of fungal isolates: Molecular identification of isolates was done to its nearest species on the basis of partial ITS sequence. To start with, genomic DNA was isolated and its ~0.6-0.8kb, ITS fragment was amplified through PCR. The PCR product was then sequenced and the data were analyzed to know their nearest species.

Enzyme assays:

Crude enzyme was extracted for assaying wood degrading activity. One gram of crushed sample was added to 4 ml water. Tubes were shaken for 1hr at room temperature. In a stainless steel filter of 2mm dia enzyme was extracted by pressing the samples mix. The filtrate was further centrifuged at 10,000 rpm for 10 min at cooled condition. The supernatant was collected and stored at -20°C until the analysis.

Laccase assay: For estimation Laccase activity 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)) oxidation by Bourbonnais and Paice **[12]** was followed.

Manganese peroxidase (MnP) assay: Manganese peroxidase activity in enzyme extract was measured following the method described by Daniel *et al*. (1994). One milliliter of final reaction mixture consisted of 50mM sodium malonate (pH 4.5), 2,6, dimethoxyphenol, 1mM $MnSO_4$ and enzyme extract.

Cellulose and Hemicellulase assay: Cellulose and Hemicellulose assay was done using the same reaction process except the substrate mixture. Remazol brilliant blue R with carboxymethyl cellulose and Remazol brilliant blue R withbirch wood xylan as substrate for Cellulose and Hemicellulase assay respectively. The common reaction mixture included 200µl of Azo-CM cellulose (2%) and 200µl of enzyme extract. After 30 min incubation at 40°C 1ml of solution containing 20% sodium acetate trihydrate and 3% zinc acetate was added to stop the reaction. Optical density read at 590nm.

RESULTS AND DISCUSSION

Over 160 isolates consisting of 44 genera and 65 species were isolated from the forest soil during the study period from 2018 to2021. During the first study year (2018-19) in winter season. It was found that *Penicillium* species dominated the population with 18.75% which was followed by *Aspergillus sp.,* 13.75% Rest all species of 35 genera shared 1.25% -3.13% of the population **(Figure 1)**. Next year in same season 94 isolates belonging to 32 genus and 57 species were isolated. Among those species of *Aspergillus* contributing 22% of the population was the most dominating genus and *Penicillium* being the second most dominating population in the diversity shared 11.70% of total diversity recorded as second. Rest 24 genus consisting of 42 species shared 1.6-5.3% population in the diversity. In the year 2020-21, 62 species belonging to 35 genera were recorded. Both *Aspergillus* and *Penicillium* dominated population with their individual share of 17% population in the diversity. *Cladosporium sp.,* remained as second most dominating population with 8.0% contribution. Other 32 genus with 43 species existed in the range between 1-4% populations.

During the summer of the first study year, 59 species belonging to 38 genera isolated, among this *Aspergillus sp.,* with 21.11% occurrence was the most dominant in population. This was followed by *Penicillium sp,* with 10.0% and *Chaetomium sp. with 6.67*% occurrences whereas remaining 35 species shared 1.11-5.56% population in the diversity. Second year of this season 25 genus and 44 fungal species were isolated from all studies sites. *Aspergillus* (23%) was found to be the most dominating genus followed by *Penicillium sp.,* (17.78%) in the sampling season. *Gliocladium roseum (6.67%)* was a third most dominating species of the population. The percentage contribution of remaining 22 genus ranged from 1.11%-4.44% population in the diversity. Third year, in this season 52 species belonging to 31 genera were isolated and *Penicillium* sharing 17.78% in the population was the most dominating genus. *Aspergillus* (12.22%) stands as second most dominating population in the diversity, whereas *Fusarium,* 7.78% was a third largest population. Rest all 34 species belonging to 28 genera existed in ranged between 1.11-4.44% individual shares in the diversity.

In rainy season 50 species belonging to 31 genera were isolated in first year. During this sampling season *Aspergillus sp*., dominated the diversity with 20.59% population. This was followed by *Penicillium sp*., with 15.69

%. Remaining 29 genera found in the range from 1.96-3.92% during the season. Next year in this season 55 species belonging to 32 genera were isolated among which *Penicillium sp.* shared 18.89% in the diversity, whereas *Aspergillus* spans 15.56% in the population was second most dominating genus. Rest 55 species belonging to 31 genera populated the diversity in the range of 1.11-5.56% individual share. Third year 64 species belonging to 39 fungal genera were recorded and *Penicillium* was found to be most dominating genus in the diversity with 17.50 % population share followed by *Aspergillus* with 13.33% of the population. Other 62.5% population in the diversity was contributed by 44 species belonging to 36 genera.

Based on the observation and critical data analysis it was noted that a significant numbers of fungal species belonging to various genera were consistently present throughout the year during the study period and the seasonal changes mealy influenced their existence. Among those the highest populated fungi was subjected to molecular characterization and identified as *Aspergillus brunneoviolaceus* **(Figure 2)**. The following ITS sequence (494bp) was recorded

ACTAGAGACCGGGGCCTGCCCCCGGG
ACCGCGCCCGCCGGAGACCCCAATGG
AACACTGTCTGAAAGCGTGCAGTCTG
AGTCGATTGATACCAATCAGTCAAAA
CTTTCAACAATGGATCTCTTGGTTCCG
GCATCGATGAAGAACGCAGCGAAATG
CGATAACTAATGTGAATTGCAGAATT
CAGTGAATCATCGAGTCTTTGAACGC
ACATTGCGCCCCCTGGTATTCCGGGG
GGCATGCCTGTCCGAGCGTCATTTCTC
CCCTCCAGCCCCGCTGGTTGTTGGGCC
GCGCCCCCCCGGGGGCGGGCCTCGAG
AGA.

Enzyme activity in fungal isolates from forest soil: Laccase (p-diphenol:dioxygen oxydoreductase; EC 1.10.3.2) is particularly found in white rot fungi and degrade aromatic phenolic compounds. They are capable of degrading the whole wood components. This is often associated with lignin peroxydase or MnP or both **[13]**. More than 50% of the species of different genera were found to be active for laccase **(Figure 3)**. All species of *Aspergillus* (8 species) and 8 of 9 *Penicillium* showed laccase activity. One of *Chaetomium* along with 15 different species belonging to *Chaetomella*, *Acromonium, Alterneria*, *Arthrobotrys, Cladosporium, Cladosporium, Memnoniella, Monodictys, Nigrospora, Rhizoctonia, Verticillium, Absidia, Mucor, Rhizopus* were found to be laccase positive. This indicates the presence of lignocellulasic material degrading fungi in soil to decompose the debrise in soil. However almost 50% of organism studied did not show laccase activity, their significance might be different in terms of other enzymatic activities.

Manganese peroxydase (E.C. 1.11.1.13) is an oxidorecuctase enzyme catalyzing the oxidation of Mn^{2+} to Mn^3 which then oxidizes lignin like phenolic substrates. Microbial lignolytic enzymes like laccase, Mn peroxides are key contributors in the microbial lignolitic sytem. Lignin peroxydase require H_2O_2 as an oxidant **[14]**. A 58% of the test iolates were found to be Mn peroxydase positive. Among the most populated *Aspergillus* genus, 50% species showed MnP peroxydase activity, whereas, 40% of *Penicillium sp*. were MnP active. Two out of three of *Fusarium* and one out of two *Trichoderma* species showed MgP activity. Among others 23 species belonging to genera *Chaetomium, Alternaria, Colletotrichum, Mucor. Cheatomella, Beltrania, Beltraniella, Bipolaris, Cuwularia, Cylindrocladium, Graphium, Macrophomina, Monodictys, Pestalotiopsis, Pyrenochaeta, Rhizoctonia, Thozetellopsis, Verticillium ter, Absidia, Cunninghamella, Mycogone, Syncephalastrum* and *Zygorhynchus* were also found MnP positive.

Cellulases (EC 3.2.1.4) are group of enzymes synthesized by wide variety of microorganisms. Their synergetic effects are important for lignocellulosic polymer hydrolysis. This is a mixed action of exoglucanases, endoglucanases and β-glucosidase that determines process of cellulose degradation. β-1,4 linkage is targeted in polymeric structure **[15-17]**.

Out of the total isolates tested 60% of species were found cellulase positive. Among those all members of genera *Aspergillus, Colletotrichum, Chaetomium, Fusarium, Trichoderma.* Eight out of nine *Penicillium* along with *6* other species belonging to genera *Verticillium, Absidia, Cunninghamella, Mycogone, Rhizopus, Syncephalastrum* were found cellulose active. Hemicellulases are group of enzymes hydrolysing glactance, xylans, mannans and arabans. Among the hemicellutytic anzymes Endoxylanase (EC 3.2.1.8) targets 1,4-β-D xylanopyranosyl linkages of Xylan, β-D xylanosidase (EC 3.2.1.3) act on non-reducing end for hydrolysis of xyl-oligosaccharides and produce D-xylose sugar. Out of total isolates studied 50.2% species were found hemicellulase positive. It was observed that all species of *Aspergillus,* four out of nine *Penicillium* species and one of three *Trichoderma* was hemicellulase positive. One each out of two *Cheatomium* and *Cladosporium* was tested hemicelluloses positive. Among others, 18 different fungal species belonging to genera *Cheatamella*, *Acremomium, Arthrobotrys, Cephalosporium, Cuwularia, Cylindrocladium, Graphium, Macrophomina, Monodictys, Nigrospora, Pestalotiopsis, Pyrenochaeta, Thozetellopsis, Trichurus, Mycogone, Rhizopus, Syncephalastrum* and *Zygorhynchus* were also hemicelluloses active.

Thirteen percent of the isolates produced all of four enzymes tested. These included all of *Aspergillus,* three of *Penicillium* and one of *Mucor* species. Twenty one percent of test species were found active for three of the enzymes **(Figure 3)**. They are *Chaetomium, Cheatomella*, four species of *Aspergillus* along with one each of *Graphium, Monodictys, Penicillium, Verticillium, Absidia, Cunninghamella, Mycogone*, *Syncephalastrum racemosum* and *Zygorhynchus.* Activity of multiple enzymes shows the involvement of multienzyme process in wood decay. Forty percent of fungal population in diversity was active for two of the enzyme tested whereas 21 % of the population was active for only one enzyme.

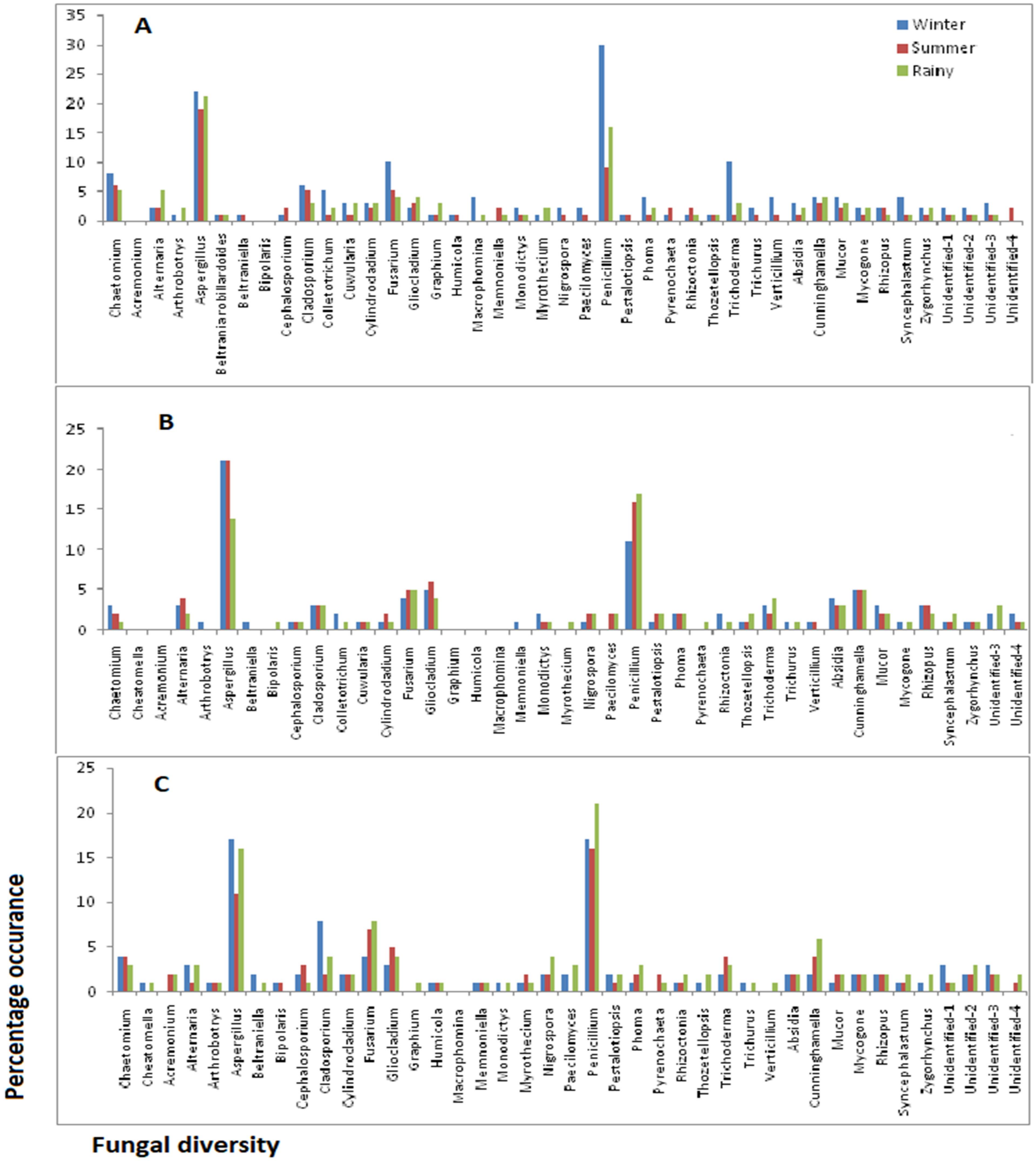

Figure 1: Fungal diversity percentage in the population (A) year 2018-2019 (B) year 2019-2020 (C) year 2020-2021

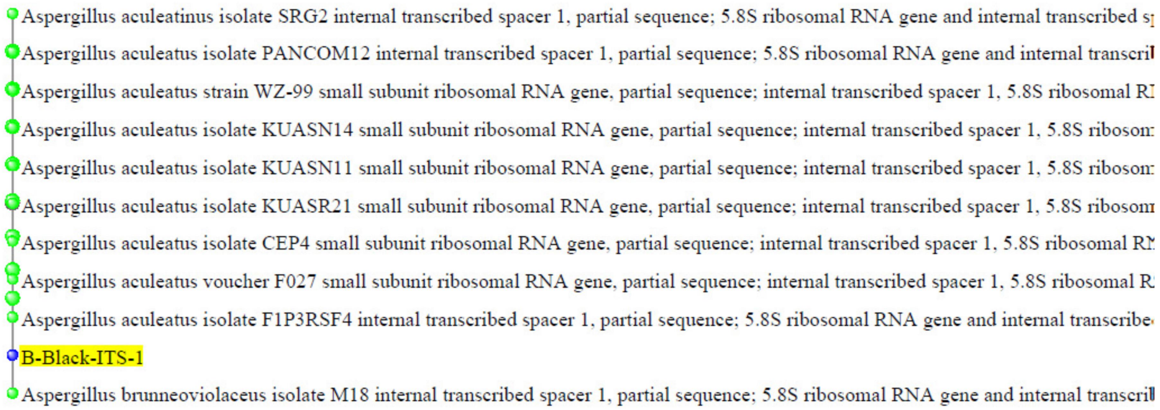

Figure 2: Dendogram constriction of the basis of ITS sequence

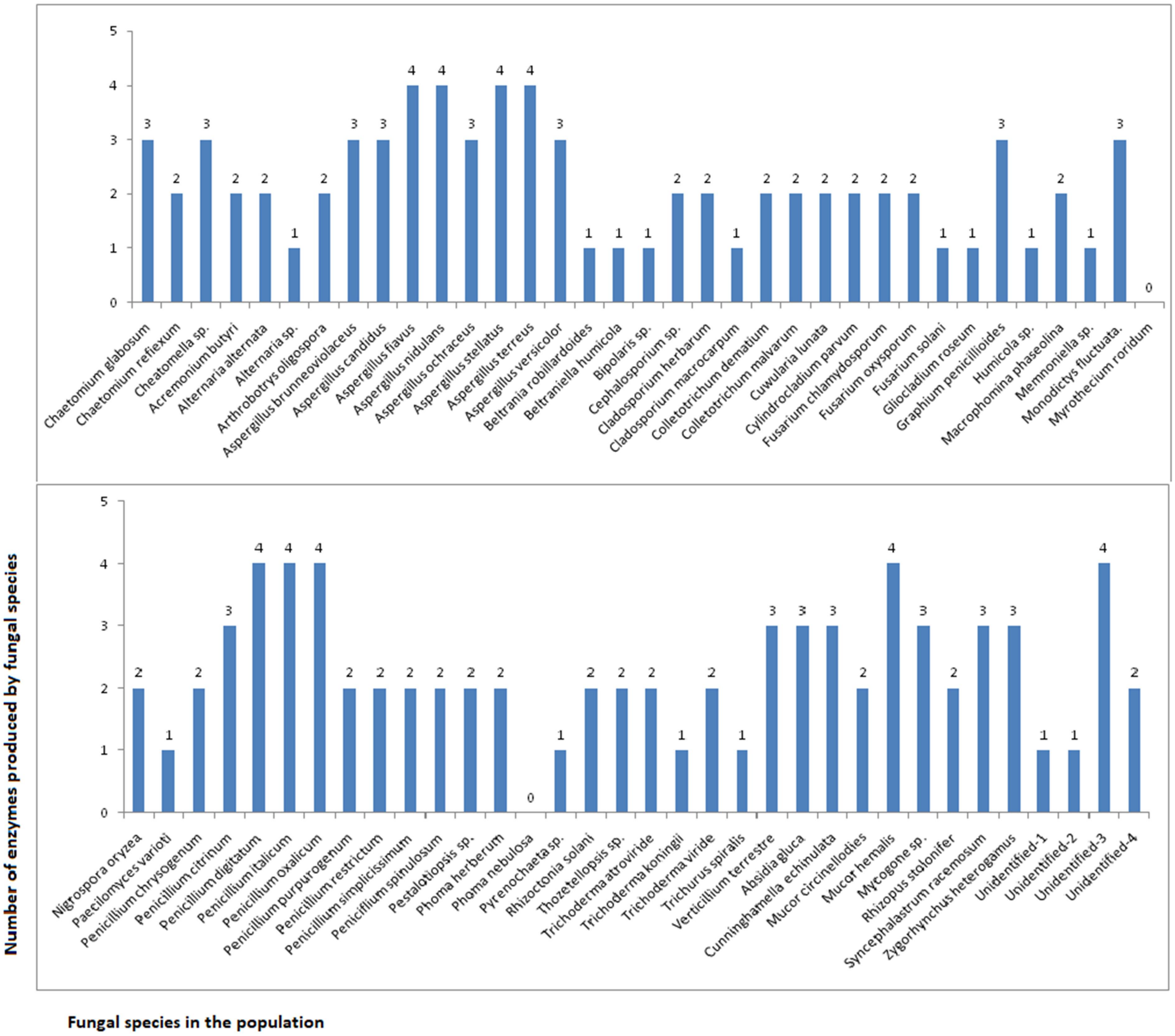

Figure 3: Pattern of wood decaying enzyme production by fungal species

CONCLUSION

Fungal diversity in the study site revealed presence of 65 fungal species belonging to 44 genera suggests that their forest ecosystem is rich in fungal diversity. The effect of seasonal variation on the diversity is partially influenced. Dominance of species/genera in the population is not so clear with the data. However, there are species of few genera such as *Aspergillus,* and *Penicillium* remained persistent throughout the study period. Correlation of diversity richness and forest health is still to be studied for study site.

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REFERENCES

[1] Cornelissen., J.H.C. Cornelissen, *et al.* (2012) Controls on coarse wood decay in temperate tree species: birth of the LOGLIFE experiment Ambio, 41, 231-245.

[2] Heilmann-Clausen, J. and Christensen, M. (2003) Fungal diversity on decaying beech logs– implications for sustainable forestry. Biodivers. Conserv. 953-973.

[3] Garbelotto, M. and Pautasso, M. (2011) Impacts of exotic forest pathogens on Mediterranean ecosystems: four case studies. *Eur J Plant Pathol*. DOI 10.1007/s10658-011-9928-6.

[4] Lassauce A., Y. Paillet, H. Jactel, C. Bouget (2011) Deadwood as a surrogate for forest biodiversity: meta-analysis of correlations between deadwood volume and species richness of saproxylic organisms Ecol. Ind., 11, 1027-1039.

[5] Junninen and Komonen, A. Komonen (2011) Conservation ecology of boreal polypores: a review Biol. Cons., 144, 11-20.

[6] Stokland J., H. Kauserud (2004) *Phellinus nigrolimitatus*—a wood-decomposing fungus highly influenced by forestry For. Ecol. Manage., 187, 333-343.

[7] Nordén, M. Ryberg, F. Götmark, B. Olausson (2004) Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests Biol. Conserv., 117, 1-10.

[8] Van der Wal A, E. Ottosson, W. de Boer (2015) Neglected role of fungal community composition in explaining variation in wood decay rates Ecology, 124-133.

[9] Penttilä, R. Siitonen, J. Kuusinen. M. (2004) Polypore diversity in managed and old-growth boreal Picea abies forests in southern Finland Biological conservation, 117, 271-283.

[10] Ódor P., J. Heilmann-Clausen, M. Christensen, E. Aude, K. Van Dort, A. Piltaver, I. Siller, M. Veerkamp, R. Walleyn, T. Standovár (2006), Diversity of dead wood inhabiting fungi and bryophytes in semi-natural beech forests in Europe Biol. Cons., 131, 58-71.

[11] Oyeleke, S.B. and Manga, S.B. (2008). Essentials of laboratory practicals in microbiology. Tobest Publishers Minna, Nigeria, Pp.36-75.

[12] Love, Gordon L. and Ribes Julie A. (2018) Color Atlas of Mycology, Pub. College of American Pathologists.

[13] Bourbonnais, R. and Paice, M.G. (1990) Oxidation of Non-Phenolic Substrates—An Expanded Role for Laccase in Lignin Biodegradation. FEBS Letters, 267, 99-102.

[14] Daniel G, Volc J, and Kubatova E (1994) Pyranose oxidase, a major source of H2O2 during wood degradation by Phanerochaeī techrysosporium, Trametes versicolor and Oudemansiella mucidaAppl. Environ. Microbiol. 60: 2524–2532

[15] Vantamuri1A B, Adhoni1 S. A., Nadaf1, P. D., Payamalle, S., Guruvin, S. K. and Manawadi S. I. (2015) Isolation and characterization of Laccase producing fungi from different environmental samples. *International Journal of Recent Scientific Research*. Vol. 6, Issue, 10, pp. 6853-6857.

[16] Hatakka A., Hammel K.E. (2011) Fungal Biodegradation of Lignocelluloses. In: Hofrichter M. (eds) Industrial Applications. The Mycota (A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research), vol 10. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-11458-8_15.

[17] Jayasekara S and Ratnayake R (2019) Microbial Cellulases: An Overview and Applications Cellulose, DOI: 10.5772/intechopen.84531.