
Executive Summary of the Thesis entitled

**Biochemical and Histological Study on Deconstruction of Lignin
for Its Application in Bio-Pulping**

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INTRODUCTION

Research Background:

Woody plants account for the largest portion of Earth's terrestrial biomass, playing a crucial role in global ecosystems and carbon sequestration (Favero et al., 2020; Nunes et al., 2020; Chaturvedi et al., 2021; Zeng and Hausmann 2022). Tropical forests alone are estimated to account for nearly half of this substantial biomass (Xu et al., 2021). The carbon stored in woody biomass is approximately equivalent to 58 % of the total atmospheric carbon, underscoring its pivotal role in regulating the global carbon cycle (Chave et al., 2005; Xu et al., 2021). The release of stored carbon from these woody plants has a profound influence on the global climate system. The enduring utility of wood as both a versatile material and a long-term carbon reservoir reinforces its significance across historical and contemporary contexts, ranging from basic construction applications to advanced climate mitigation strategies (Chave et al., 2005; Xu et al., 2021).

Wood is a structural tissue and the main tissue responsible for mechanical support, and it plays a crucial role in the conduction of nutrients. It is composed of various elements such as fibres, tracheids, vessels, axial and ray parenchyma, particularly in Eudicots. In gymnosperms, it is composed of only tracheids (axial and ray), while vessels are absent in most of them, except in the species of the order Gnetales. The secondary xylem of dicot trees is known as hardwood, while the wood of the gymnosperm is called softwood (Fahn 1982; Pallardy 2008). However, these terms are misleading regarding the actual physical hardness of the wood, because some of the softwoods can be harder than certain hardwoods and vice versa.

Wood is the product of cambial activity and the most important natural product of arboreal plants. It is not only an inexhaustible and renewable source of natural energy but also acts as a sink for the increasing concentration of carbon dioxide. Therefore, it is a major source of cheap, environmentally friendly and alternative fuel in the near future against fossil fuels (Plomion et al., 2001). Wood not only provides energy but also serves as an important, energy-sufficient material for buildings, furniture, and many other products (FAO 2017). Besides these, the newly developing wall of the wood cell represents the most important sinks for excess atmospheric carbon dioxide, thereby decreasing the concentration of CO₂, which is one of the major contributors to global warming (Phillips et al., 2002; Schnitzer and

Bongers 2011). Wood is one of the important products in the world trade and plays significant role in national economies (FAO 2017). Every year, massive quantities of trees are logged by foresters to provide fuel, fibres (for pulp, paper products and boards) and sawn timber (for house building, and furniture) as commodities (Plomion 2001). According to Wang et al. (2021) and Plomion et al. (2001), the complex chemical composition of wood (cellulose, hemicellulose, lignin and pectin) also brands it as a perfect raw material for future ligno-chemical industries, which could replace the petrochemical industry by not only providing plastics and other kinds of chemical products, but also food and textile products. Wood cells are composed of different cells with a unique composition of their cell wall constituents. It is primarily composed of three polymeric components (called lignocellulosics), i.e. cellulose, hemicellulose (xylans) and lignin. These constituents comprise nearly 95% of the total dry weight of the wood, from which cellulose and hemicellulose occupy nearly 70% matter, whereas lignin is about 20-30%, depending upon the species (Parand 2001; Koyani et al., 2010; Koyani et al., 2011; Huang et al., 2022).

Lignin (Latin *lignum* = wood) is the most abundant and widely distributed renewable aromatic polymer in the terrestrial ecosystem and is considered to be the second most abundant renewable biopolymer in nature, after cellulose (Koyani et al., 2011; Vasile and Baican 2023). It is the substrate, which is resistant to chemical and biological processes and provides resistance to wood. Among all the natural chemical compounds, lignin is extremely recalcitrant due to its chemical structure, which is significantly more resistant to degradation in the absence of oxygen (Kirk 1983; Koyani 2011; Koyani et al., 2011; Xu et al., 2021). However, anaerobic lignin degradation is possible through various microorganisms and microbial consortia, often occurring at slower rates (Khan and Ahring 2019). It is mineralised in an obligate aerobic oxidative process, and its degradation yields no net energy gain (Pointing 2001). Lignin degradation lies in a central position in the Earth's carbon cycle, because the most renewable carbon is stored either in lignin or in compounds like cellulose and hemicellulose, which are protected by lignin from enzymatic degradation (Kirk 1983; Eichlerová and Baldrian 2020).

Though various cell types compose the wood, each cell has its own unique chemical composition (Parand 2001; Schwarze 2007; Deflorio et al., 2008; Koyani et al., 2010; Lehringer et al., 2010; Crang et al., 2019) with respect concentration of cellulose, hemicellulose and lignin. Available literature indicates that even type of lignin differs between fibres and vessels of the same wood (Koyani et al., 2015; Pramod et al., 2015; Bhatt

et al., 2016). However, wood is prone to degradation by the enzymatic activity of microbes, particularly bacteria and fungi. After bacteria, fungi are the only microbe that has an exceptional capability of breaking down lignin, cellulose, and hemicellulose (Kirk and Cullen 1998). Their slow-growing nature, stress tolerance ability, and production of secondary metabolites that act as an antagonistic and self-defensive weapon against other microbes, provide them unique ability to utilise these cell wall constituents as a source of carbon (Rayner and Baddy 1988; Holmer and Stenlid 1997). Most wood-dwelling fungi are strictly saprotrophic in nature and exploit dead wood as a source of food.

Among different groups of fungi, only white rot fungi have the proficient quality of mineralising lignin competently (Kirk and Cullen 1998; Goodell et al., 2020; Holmer and Stenlid 1997; Rayner and Baddy 1988; Wang et al., 2021). These fungi degrade wood by releasing the extracellular enzymes that deconstruct the molecules of cellulose, hemicellulose and lignin (Eriksson et al., 1990; Blanchette 1991, 1992, 2000; D'Souza-Ticlo et al., 2009; Paço et al., 2017). Hence, such wood rot fungi are under the main focus for their biotechnological application. This is due to the fact that enzymes from white rot fungi can depollute contaminants, xenobiotic compounds and delignify the agricultural wastes, and leave behind only cellulose as a cost-effective commercial raw material extensively used in industrial fermentations. Additional, white rot fungi that has the skill of selective delignification, which may be used in the process of bio-pulping of wood for the paper industry, biodegradation of chemical wastes, and to improve the digestibility of lignocellulosic materials that are used for animal feeding (Eriksson and Kirk 1985; Eriksson et al., 1990; Adaskavege et al., 1995; Akhtar et al., 1992, 1998; Anagnost 1998; Luna et al., 2004; Lagaert et al., 2009; Koyani et al., 2010; Sanghvi et al., 2010; Abdel-Aziz et al., 2015).

REVIEW OF LITERATURE

Though various cell types compose the wood, each cell has its own unique chemical composition (Parand 2001; Schwarze 2007; Deflorio et al., 2008; Koyani et al., 2010; Lehringer et al., 2010; Crang et al., 2019) with respect concentration of cellulose, hemicellulose and lignin. Available literature indicates that even type of lignin differs between fibres and vessels of the same wood (Koyani et al., 2015; Pramod et al., 2015; Bhatt et al., 2016). However, wood is prone to degradation by the enzymatic activity of microbes, particularly bacteria and fungi. After bacteria, fungi are the only microbe that has an exceptional capability of breaking down lignin, cellulose, and hemicellulose (Kirk and Cullen 1998). Their slow-growing nature, stress tolerance ability, and production of secondary metabolites that act as an antagonistic and self-defensive weapon against other microbes, provide them unique ability to utilise these cell wall constituents as a source of carbon (Rayner and Baddy 1988; Holmer and Stenlid 1997). Most wood-dwelling fungi are strictly saprotrophic in nature and exploit dead wood as a source of food.

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al., 1995; Anagnost 1998; Luna et al., 2004; Lagaert et al., 2009; Koyani et al., 2015; Pramod et al., 2015; Bhatt et al., 2016). Based on the type of decay, wood shows various changes in physical, chemical and morphological features of wood which are characteristic of each taxonomic group of fungi (Schwarz 2007; Bari et al., 2021). Depending on micro-morphological and chemical appearances, wood-degrading fungi are classified into three main categories: i) Soft rot fungi, ii) Brown rot fungi, and iii) White rot fungi.

In white rot, all cell constituents are degraded in two ways: a) selective delignification and b) simultaneous rot. In selective delignification, hemicellulose and lignin are preferentially degraded in the early stages. This type is anatomically characterised by the separation of fibres due to the dissolution of the middle lamella. In contrast, in simultaneous rot, lignin and structural polysaccharides are degraded at more or less the same time (Worrall et al., 1997; Schwarze and Fink 1998; Koyani et al., 2011, 2015; Pramod et al., 2015; Bhatt et al., 2016). They often cause a bleaching of normal wood colouration and appear whiter than normal wood and therefore are referred to as white rot. From a commercial point of view, the other two types, i.e. soft rot and brown rot, are not of much importance and lead to economic loss in the timber industry.

The process of lignin biodegradation involves extracellular oxidations of lignin by the production of ligninolytic enzymes. The major fungal enzymes involved are laccases, lignin peroxidases (LiPs) and manganese peroxidases (MnPs) (Kirk and Cullen 1998). Discovery of these nonspecific extracellular peroxidases (LiPs in 1983 and MnPs in 1984) fascinated researchers throughout the world for their considerable industrial applications such as bio-pulping, bio-bleaching, treating of pulp mill effluents and bioremediation of non-degradable compounds such as polycyclic aromatic hydrocarbons, textile dyes and other thousands of environmentally detrimental chemicals (Kirk and Farrell 1987; Worrall, et al., 1997; Schwarze and Fink 1998; Schwarz 2007).

Review of literature indicates that white rot basidiomycetes play a pivotal role in timber damage by degrading highly complex natural molecules like lignin. Though every year, 20-80% of the world's timber is destroyed by white rot fungi, the enzymes produced by these fungal species not only have potential biotechnological applications in various industrial products but also play a crucial role in the bioremediation of non-degradable and xenobiotic compounds.

Therefore, **the main objectives of the present study are as follows:**

- Collection, isolation, and purification of wood rot fungi and standardisation of growth media
- Screening of fungi for various ligninolytic enzyme production for the selection of a potential fungus
- Molecular identification of the selected fungi
- Solid-state fermentation using wood material for enzyme production
- To study the enzyme kinetics (temperature, pH, substrate concentration, etc.)
- Partial purification and quantification of different ligninolytic enzymes such as laccases, manganese peroxidase, and lignin peroxidase.
- Histological study of wood material used as SSF media to understand the pattern and location of cell wall components removal by the fungus.

MATERIALS AND METHODS

Source of material for the present study: In the present investigation, nearly 30 fungal strains were collected from the dead wood logs and infected living trees growing in different forest areas throughout the state. Wood blocks adjacent to fungus fruiting bodies, measuring 60x20x60 mm in height, width and depth, respectively were excised with the help of a chisel and hammer from the main trunk. They were packed in sterile polyethylene bags and brought to the laboratory. As described by Berlyn and Miksche (1976), half of the samples were fixed in FAA (Formaldehyde-Acetic acid-Alcohol), aspirated with a vacuum aspirator to remove the air from the wood, while the rest of the wood blocks were used for the isolation of fungus. The remaining samples were used for the establishment of a pure culture of the infecting/causal organisms. About fixed samples, After 24 hrs of FAA fixation, samples were transferred in 70 % alcohol after 24 hrs of fixation for further processing and storage.

Isolation, identification and establishment of pure culture: Collected fruiting bodies and wood samples were surface sterilised by the routine method of 0.1% HgCl₂ with intermediate washing by sterile distilled water, followed by a treatment of 70% ethanol. Finally, these samples were treated with absolute alcohol and again washed with sterile distilled water before inoculating on different growth media. Pure cultures of all these fungal strains were maintained in refrigerated conditions. All the isolated strains were subjected to Bavandamm's test to distinguish between the white rot and brown rot fungi. From the test, positive strains were only 11 potent strains, viz. *Bjerkandera adusta*, *Coprinellus micaceus*, *Daedaleopsis flavidia*, *Flavodon flavus*, *Hexagonia tenuis*, *Inonotus hispidus*, *Lenzites betulinus*, *Microporus ochrotinctus*, *Pleurotus ostreatus*, *Polyporus tricholoma*, and *Schizophyllum commune*; and *Lenzites betulinus* and *Daedaleopsis flavidia* were selected for further studies on the biodegradation of lignin and for the production of lignin peroxidase and manganese peroxidase enzymes. Both the strains have been identified by morpho-taxonomic features, and their identity was confirmed by Agharkar Research Institute, Plant Pathology Division, Pune (Maharashtra State).

Enzyme studies, Isolation, Production and Purification of Ligninolytic Enzymes:

Isolation, production and purification of manganese peroxidase, manganese independent peroxidase and laccase by *Lenzites betulinus* and *Daedaleopsis flavida* were carried out by solid-state fermentation using different agricultural wastes. Influence of various parameters such as temperature, pH, carbon sources, nitrogen sources, effect of metal ions, etc., was evaluated with respect to enzyme production. To study the effect of carbon source on enzyme production, different sugars such as sucrose, lactose, fructose, dextrose and maltose, etc., were utilised while ammonium sulphate, sodium nitrate, sodium nitrite, asparagine and urea were used as a source of nitrogen. Similarly, the effect of different particle sizes of substrates on enzyme production was also carried out, and it showed a significant effect on the quantity of enzyme production. Partial purification of the enzyme was also carried out using the ammonium sulphate precipitation method. Production of the enzyme was recorded as maximum in the SSF media with one mm particle size, while it was less in the SSF media with four mm particle size. Further purification of the enzyme was carried out using a DEAE Sephadex G-75 column, and the molecular weight of the enzyme was determined by using the SDS PAGE electrophoretic method as described by Laemmli (1970). Metal ions are considered to play an important role in enzyme production and its activity. As an inducer, different metal ions like Mn^{2+} , Zn^{2+} , Ca^{2+} , Mg^{2+} , Cu^{2+} , Co^{2+} , Fe^{3+} were added to Solid State Fermentation (SSF) media to study the effect of these supplements on the enzyme activity. Extraction and purification of these enzymes were carried out using the same process as described above.

In-vitro Study, Sample preparations, sectioning and photography: Besides enzyme production potential, an *in-vitro* decay test was carried out by using healthy wood samples of *Leucaena leucocephala*. Autoclaved and sterilised wood samples were inoculated with both the fungal strains for 30, 60, 90 and 120 days. After an incubation period, these samples were fixed in FAA (Berlyn and Miksche 1976), and after 24 hrs of fixation, they were processed for paraffin embedding to obtain 10 to 12 μ m thick sections. Suitably trimmed samples were dehydrated with tertiary butyl alcohol series and processed by the routine method of paraffin embedding. Transverse, radial and longitudinal sections of 10-12 μ m thickness were taken with a rotary microtome (Leica RM 2035, Germany). Sections were de-waxed in xylene-ethanol series and stained with safranin-Astra blue (Sigma, Germany) combinations (Messner and Srebotnik 1994). After dehydration in an ethanol-xylene series, the sections were mounted in Dibutyl Phthalate Xylene (DPX). Important results were micro-photographed

using a Leica DM 2000 trinocular research microscope with a digital camera (Canon S70D, Germany).

In-vitro infected wood samples were also studied using a confocal laser scanning microscope. These samples were washed in water followed by 0.01M phosphate buffer (pH 9.0). Hand sections (approximately 40-80 μ m thickness) were taken from the wood block and mounted in buffered glycerol (pH 8-9). Slides were examined with a Zeiss confocal laser scanning microscope using a Krypton/argon laser emitting at wavelengths of 488 and 568nm (Donaldson and Lausber 1998).

Confocal Laser Scanning Microscopy (CLSM): Samples were washed in water followed by 0.01M phosphate buffer (pH 9.0). Hand sections (approximately 40-80 μ m thickness) were taken from the wood block and mounted in Fluoroshield mounting medium (Sigma, Germany). Slides were examined with a Zeiss confocal laser scanning microscope using a Krypton/argon laser emitting at wavelengths of 488 and 568nm (Donaldson and Lausber 1998). For localisation of fungal mycelia, sections were mounted on a clean glass slide with a drop each of calcofluor white and 10% potassium hydroxide (Kitin et al., 2010) followed by observation at blue excitation (λ_{exc} = 300–440 nm).

Scanning Electron Microscopy (SEM): For SEM studies, wood samples were fixed in 2.5% glutaraldehyde in 0.2M phosphate buffer (pH 7.2) overnight. Samples were cut into small blocks of 2-3 mm thickness using a sharp razor blade, dehydrated in acetone-isoamyl alcohol series and coated with gold using Quaram Sputter coating unit, Model SC 7610 and observed with LEO 440i SEM at 10 KV.

Transmission electron microscopy (TEM): Infected test samples after trimming to 2 x 5 mm size pieces were fixed in a mixture of 0.1 % glutaraldehyde and 4% paraformaldehyde in 50mM sodium cacodylate buffer for 4 hrs., at room temperature. After washing in buffer, samples were dehydrated in a graded series of ethanol and embedded in London Resin (LR) white as described elsewhere (Pramod et al., 2019). Semi-thin (1-2 μ m) and ultrathin sections (60-80 nm) were obtained with glass and diamond knives, respectively using the RMC, Powertome X (USA) ultra-microtome. Ultrathin sections were taken on nickel grids and stained with 0.1% KMnO₄ in citrate buffer for 45 minutes at room temperature for lignin (Donaldson and Lausber 1998) and observed under TEM (Philips Tecnai 20) at an acceleration voltage of 80 kV. Micrographs of important observations were taken using a CCD camera (Mega View III, Olympus Soft Imaging Solutions, USA).

RESULTS AND DISCUSSION

The present investigation aims to understand the mechanism of lignin degradation so that the delignified wood can be exploited in the paper industry for biopulping. Removal of lignin using biological agents like bacteria or fungi is an environmental friendly mechanism for the mitigation of environmental pollution. If compared with the traditional chemical treatment method, the application of such a biological agent is beneficial. Therefore, it is essential to understand the histological and biochemical mechanisms. As per available information, the majority of the studies are focused on the biodegradation of polyaromatic hydrocarbons (PHAs) or xenobiotic compounds. However, there is a lack of information on the mechanism by which these fungi affect timbers at the cellular level (histological) in tropical trees remains less explored. In contrast, available information on this aspect is based on the temperate species. Thus, it is necessary to explore similar possibilities in the Indian scenario. On the basis of the above information, we tried to understand the mechanism of wood decay and the removal of lignin by an *in-vitro* decay test. Based on the time taken by the fungal strain, the potential of the strains for ligninolytic enzyme production helped us for further studies. Therefore, the present work is divided into:

- I) Biochemical and Enzymatic Study
- II) Histological Study (both, naturally infected and in-vitro infected wood)

I) Biochemical and Enzymatic Study

Based on this primary screening, further research work has been performed using agro-industrial waste (Wheat straw and Rice Straw) and wood chips (*Eucalyptus* and *Leucaena*). To provide an in-depth understanding and to analyse their potential industrial application for biopulping for the production of fungal ligninolytic enzymes, different factors are also considered for their optimisation. In this context, considering the exceptional advantages of SSF over other techniques and plentiful availability in nature, in the present work SSF technique was used for the production of ligninolytic enzymes using two fungal strains, *Daedaleopsis flavidia* and *Lenzites betulinus*. Various agro-industrial wastes as substrates, like wheat straw, rice straw, sugarcane bagasse, sawdust, etc., were employed to scale up the production of ligninolytic enzymes like manganese peroxidase (MnP), laccase

(Lac), and lignin peroxidase (LiP) for primary investigation for the production of ligninolytic enzymes.

Enzyme production by white rot fungi depends upon the substrate selection, fermentation conditions, and medium composition. Therefore, the primary objective of the present study has been carried out for the production of different ligninolytic enzymes using fungal strains *Daedaleopsis flavidia* and *Lenzites betulinus* with agro-industrial wastes (Wheat straw and Rice Straw) and wood chips (*Eucalyptus* and *Leucaena*) as an exclusive source of solid substrate without any external mineral supplements. All the substrates showed excellent growth for both fungal strains. Maximum enzyme productivity exhibited by *Daedaleopsis flavidia* for laccase (106.67 IU/ ml) was observed with wheat straw; for MnP (97.03 IU/ ml) with *Eucalyptus* wood chips; for LiP (17.21 IU/ ml) with *Leucaena* wood chips. Though the maximum enzyme activity was observed for laccase with wheat straw, all other substrates exhibited efficient productivity for Laccase, LiP, and MnP, viz. For LiP - *Eucalyptus* wood chips (15.47 IU/ ml), wheat straw (14.24 IU/ ml), rice straw (13.11 IU/ ml); for MnP-*Leucaena* wood chips (82.86 IU/ ml), wheat straw (87.17 IU/ ml), rice straw (78.86 IU/ ml); For Laccase- *Eucalyptus* wood chips (87.22 IU/ ml), *Leucaena* wood chips (91.94 IU/ ml), rice straw (91.11 IU/ ml). In the case of *Lenzites flavidia*, maximum enzyme productivity for laccase (102.22 IU/ ml) was observed with wheat straw; for MnP (74.72 IU/ ml) with wheat straw; for LiP (16.08 IU/ml) with *Eucalyptus* wood chips. In general, all substrates demonstrated significant productivity with *Lenzites flavidia* viz. For LiP - *Leucaena* wood chips (15.98 IU/ ml), wheat straw (14.45 IU/ ml), rice straw (12.90 IU/ ml); for MnP-*Leucaena* wood chips (73.20 IU/ ml), *Eucalyptus* wood chips (70.19 IU/ ml), rice straw (78.86 IU/ ml); For Laccase- *Eucalyptus* wood chips (87.22 IU/ ml), *Leucaena* wood chips (91.94 IU/ ml), rice straw (91.11 IU/ ml).

Besides individual potential of the fungus/micro-organisms, various factors like particle size of solid lignocellulosic substrate, incubation time of the fungus, reaction time for enzyme to interact with the substrate, temperature and pH represent one of the significant aspects to be considered for SSF that certainly influence the nutrient availability for microbial growth and enzyme productivity of microorganisms involved in solid substrate fermentation. Among various sizes of solid lignocellulosic substrate, the particle size of 1mm was found to be more suitable, as both the fungal strains showed the highest enzyme production, while the particle size of 4 mm showed the minimal enzyme production. In SSF, the particle size determines the extent of surface area available for microbial interaction, and the porosity,

which collectively influence the efficiency of microorganisms for inhibition and utilisation of the substrate during fermentation. Incubation time plays a vital role in the process of delignification because the organism needs to adapt to the type of agricultural waste. Both strains started enzyme production on the 3rd day of inoculation. *Lenzites betulinus* displayed the highest enzyme activity of laccase at the 9th day of incubation with maximum activity on wheat straw as a substrate (102.22 IU/ml), whereas maximum LiP activity was noticed on the 12th day of inoculation on *Eucalyptus* wood chips, while it was relatively less on other substrates, viz., Wheat straw, Rice straw, and *Leucaena* wood chips. Enzymes are highly sensitive to pH and are an important factor in controlling the activity of different ligninolytic enzymes. Therefore, the effect of pH on ligninolytic enzymes was investigated at room temperature in the present study. Considering this important factor, the effect of pH on ligninolytic enzymes was evaluated for maximum enzyme production at room temperature. In the case of *Dadaleopsis flavida*, the highest activities of Laccase (106.67 IU/ml), MnP (97.36 IU/ml), and LiP (17.22 IU/ml) were recorded at pH 5, 5.5, and 4.5, respectively. In the case of *L. betulinus*, maximum enzyme activities of Laccase (102.22 IU/ml), MnP (74.71 IU/ml), and LiP (14.47 IU/ml) were observed at pH 5 for all three ligninolytic enzymes. Detailed information is also provided for the effect of temperature on the enzyme activity and potential of both strains of fungi.

II) Histological Study (both, naturally infected and in-vitro infected wood):

i) *Xylaria polymorpha* is known to cause root rot disease in hardwood trees. In the present study, trees of *Lannea coromandelica* naturally infected with *Xylaria polymorpha* caused root rot and showed a soft rot decay pattern. Bright field microscopy, Confocal Microscopy and Scanning Electron Microscopy revealed that fungal mycelium bores through the S₂ layer of the fibre wall, while axial parenchyma was found relatively resistant without much visible damage. Occasionally separation of parenchyma adjacent to fibres was noticed due to the dissolution of the compound middle lamella. Ray parenchyma cells showed several boreholes having irregular shape and size. Enlargement of pits on axial and ray parenchyma was common in all the samples investigated. Xylem fibres were the most susceptible cell types and developed several tunnels through the S₂ layer. Tunnels formed in the S₂ layer of the fibre wall by the mycelium showed 'L' or 'T' bending. Initially, the tunnels were narrow in diameter, but an increase in their diameter extends into the S₃ layer. Occasionally, complete removal of the S₃ layer and fusion of the tunnel with the fibre lumen appears as a

“U” shaped erosion trough. At the advanced stage of decay, extensive damage was observed in the vessel walls, leaving the middle lamella and wall layer facing the vessel lumen intact.

ii) *Ailanthus excelsa* Roxb., requires less care and thrives in arid and semi-arid regions. Therefore, becomes an important choice for the social forestry program. Young branches of these trees are frequently invaded by *Bjerkandera adusta* (Willd.) P. Karst., fungus. The main aim of the present study was to characterise the wood decay pattern and to evaluate the extent of damage caused by the fungus. Infected samples were studied by using Light Microscopy, Confocal Laser Scanning Microscopy, Transmission Electron Microscopy and Scanning Electron Microscopy. Wood of the dead branches became pale white and very soft. *In vitro* decayed wood showed extensive weight loss of wood blocks (68.60 %) at the end of three months. In the xylem, fibres were the most severely affected cell types, whereas the wall structure of the axial parenchyma remained virtually unaltered. Unlike axial parenchyma, the walls of the ray cells formed several bore holes and finally collapsed in the advanced stage of decay. In contrast, the middle lamella of all the xylem elements remained intact even at the end of three months. In the early stage, vessels appeared to be resistant to the fungal action, but they also became eroded at the advanced stage. The degradation pattern showed anatomical characteristics of both white rot and soft rot types of decay. Thinning of the wall from the lumen side and appearance of U-notches resemble white rot, while tunnels through the S₂ layer of fibre wall added the characteristics of soft rot decay pattern. Extensive weight loss of wood blocks suggests *B. adusta* as an aggressive wood-degrading fungus.

iii) Structural alterations induced in response to degradation by *Lenzites betulinus*, and *Daedaleopsis flavidia* (white rot Basidiomycetes) in the walls of secondary xylem of *Leucaena leucocephala* (Lam) de Wit, and *Eucalyptus globulus* were compared. An *in vitro* decay test was employed to investigate the pattern of wood delignification in two timber species using *Lenzites betulinus* and *Daedaleopsis flavidia*. Wood samples inoculated with both strains were analysed for different periods, viz., 30, 60, 90 and 120 days after fungal inoculation. Initially, there was no appreciable percentage weight loss of the wood blocks, but later on (after 60 days) it increased rapidly and was found to be similar for both the strains (43–46% of wood mass). Samples inoculated with both the strains showed a dual pattern of degradation, i.e., selective delignification in the initial stage followed by simultaneous rot during the advanced stage of decay. Separation of the cells due to dissolution of the middle lamella was the characteristic feature of both strains, but in the advanced stage of decay, formation of erosion troughs was conspicuous in all the cell types. Other features such as cell

wall thinning, rounded pit erosion, formation of erosion channels and bore holes were also observed frequently. Initially, fungal invasion started through the vessel lumen, followed by all the cell types of the xylem. From the vessels, mycelia entered into the adjacent rays and parenchyma cells through the pits. In the advanced stage, degradation was so pronounced that rays were partially or even completely destroyed while many cells, including vessels, were either deformed or destroyed due to loss of rigidity of their walls.

Under a bright field light microscope, transverse sections of the fibres showed two distinct types of cell wall thinning; one was characterised by the localised degradation of cell wall and middle lamellae, while the second pattern showed general erosion of the cell walls adjacent to hyphae growing on the lumen surface. Tangential sections also revealed the thinning of secondary walls through general erosion from the lumen surface during the advancement of decay, indicating typical simultaneous white rot. Large erosion channels formed through simultaneous degradation along the cell axis and merging of boreholes were detected after 60 days of infection. Colonisation of fungal mycelia occurred through the formation of penetration hyphae passing through the simple pits between adjacent cells. The tangential wall of fibre showed progression of erosion channels, which appeared as 'U' shaped notches. The fungal hypha within the erosion channel was contrasted with astra blue staining. Multi-seriate rays often showed a hollow region due to the complete removal of some of the cell wall constituents. Separation of fibres at cell corners due to dissolution of compound middle lamellae became evident at advanced stages of decay. Bore holes composed due to the progression of bordered pit erosion were detected in inter-vessel walls. Radial longitudinal sections revealed the extensive degradation of the ray cell wall, resulting in unstained patches of cell wall regions often inhabited by fungal mycelia.

The major cell wall structural changes during the advancement of the degradation process include the formation of boreholes, erosion troughs, pit erosion, enlargement of erosion channels through merging of boreholes and progressive degradation along the length of the cell with U-shaped notches and thinning of cell walls. It has been reported that the presence of erosion troughs and channels, thinning of cell walls, boreholes, and round or oval-shaped pit erosion are characteristic features associated with the simultaneous degradation pattern of white rot fungi. Therefore, the majority of anatomical features observed during the *in vitro* wood decay test by both species can cause simultaneous degradation in *Leucaena* wood.

In general, vessels and ray parenchyma are considered to be resistant to fungal degradation due to the presence of more guaiacyl lignin units and polyphenolic substances, respectively. Our result showed that the cell walls of fibres, axial parenchyma, ray parenchyma and vessels were subjected to considerable degradation after 120 days of inoculation. Multiseriate rays appeared hollow in the middle region. Degradation of pectin-rich middle lamellae commenced after the delignification of secondary walls, resulting in conspicuous hollowing of multiseriate xylem rays in beech wood. Confocal microscopy also revealed the strong degradation of lignin from the cell walls of all cell types undergoing fungal degradation.

SUMMARY AND CONCLUSION

In the present study, naturally infected wood samples were compared with *in vitro* decayed test samples of the wood. All the investigated wood samples (naturally infected and *in vitro* decayed) go hand in hand and share similar features. After thorough screening of several samples, we selected four strains of wood rot fungi, viz., *Xylaria polymorpha*, *Bjerkandera adusta*, *Daedaleopsis flavidula* and *Lenzites betulinus*. Fungi showing different types of wood decay fungi that show softrot and whiterot properties, while brownrot fungi are investigated because of their usefulness in the paper industry for paper and biopulp making. From the above-mentioned fungi, *X. polymorpha* (Ascomycota) showed soft rot characteristics, while the remaining three species showed whiterot features such as selective and simultaneous removal of lignin. Among the investigated fungal species, specifically *Bjerkandera adusta*, always behaved unusually and showed various patterns of wood degradation. Therefore, it is necessary to understand their actual nature; the above species were selected in the current investigation.

Xylaria polymorpha affected *Lannea* wood significantly by forming the boreholes in the S₂ layer of cell walls. These boreholes were irregular in shape and size is characteristic of soft rot degradation. Xylem fibres were the most susceptible cell types and developed several tunnels through the S₂ layer. Tunnels formed in the S₂ layer of the fibre wall by the mycelium showed 'L' or 'T' bending. Initially, the tunnels were narrow in diameter, but an increase in their diameter extends into the S₃ layer. Occasionally, complete removal of the S₃ layer and fusion of the tunnel with the fibre lumen appears as a "U" shaped erosion trough. At the advanced stage of decay, extensive damage was observed in the vessel walls, leaving the

middle lamella and wall layer facing the intact vessel lumen. The degradation of the cell wall and middle lamella of xylem cells suggests that *Xylaria polymorpha* is an aggressive saprobe with strong lignolytic activity.

The second example was *Bjerkandera adusta*, which demonstrated a simultaneous type of white-rot decay in the wood of *Ailanthus excelsa*. However, the degradation pattern at an advanced stage of decay suggests that it shares the characteristics of both soft and white rot types of decay. Among the different cell types, the secondary wall of fibres is more prone to decay from the initial stage, while non-contact rays and axial parenchyma resist degradation till the advanced stage of decay. Confocal and TEM studies show the wall regions of high lignin distribution, such as vessel wall and compound middle lamellae, are relatively resistant to degradation. A higher percentage of weight loss by *B. adusta* in *Ailanthus* wood suggests that this could be a potentially aggressive decay fungus with high ligninolytic activity. This study also highlights the need for necessary action to monitor the spreading pattern and host nature of this fungus in urban parts of Baroda, where a large number of *Ailanthus* trees are grown under a social forestry program.

Daedaleopsis flavidia and *Lenzites betulinus* belong to basidiomycota, and both fungi showed a white-rot pattern of wood decay. During the *in vitro* decay test, both species invade sound wood of *Leucaena leucocephala* through the vessel elements, and it was subsequently invade other cell types of the secondary xylem. Invasion was facilitated by axial and ray parenchyma cells. In the beginning, the weight loss of woodblocks was slow might be due to the presence of low molecular weight compounds in the wood cells, but it became fast after a month. Both the fungi (*Daedaleopsis flavidia* and *Lenzites betulinus*) initially show selective delignification, which is characterised by the separation of fibres and even vessels at the advanced stage of decay, cell wall thinning, formation of erosion holes on fibre walls, and axial and ray parenchyma cells. If compared with axial and ray parenchyma cells, vessels were more resistant. In the later stage of cell wall degradation, the formation of boreholes in all investigated samples was similar to simultaneous rot. However, it differs in the absence of erosion troughs in the beginning, but with the passage of time (i.e., at the end of 120 days), “U” shaped erosion troughs were observed on the fibre wall facing cell lumen, indicating that both the strains cause a simultaneous pattern of white-rot.

Enzyme studies on *Daedaleopsis flavidia* and *Lenzites betulinus* showed potential strains for their exploitation in the paper industry. Both fungi were influenced by SSF media

(i.e. agroindustrial waste), their particle size, incubation time, pH of the buffer solution, and incubation temperature. *Daedaleopsis flavidia* had more potential than *Lenzites betulinus*. Among different SSF media, laccase production was highest on wheat straw, while a 1 mm particle size was found ideal for the production of different ligninolytic enzymes. Production of these enzymes initiates after the third day of inoculation, and maximum enzyme production was recorded on the 9th day by *D. flavidia* and 12th day on *Eucalyptus* wood chips.

REFERENCES

- Abdel-Aziz N.A; Salem A.Z.M., El-Edawy M.M.; Camacho L.M., Kholif A.E., Elghandour M.Y. and Borhami B.E. 2015. Biological treatments as a mean to improve feed utilization in agriculture animals-An overview. *Journal of Integrative Agriculture* 14(3): 534–543
- Adaskaveg, J. E., Gilbertson, R. L. and Dunlap, M. R. 1995. Effects of incubation time and temperature on in vitro selective delignification of silver leaf Oak by *Ganoderma colossum*. *Applied and Environmental Microbiology* 61: 138-144.
- Akhtar, M., Attridge, M. C., Blanchette, R. A., Myers, G. C., Wall, M. B., Sykes, M. S., Koning, J. W., Jr, Burgess, R. R., Wegner, T. H. and Kirk, T. K. 1992. The white-rot fungus *Ceriporiopsis subvermispora* saves electrical energy and improves strength properties during biomechanical pulping of wood. In: Kuwahara M, Shimada M (eds) *Biotechnology in pulp and paper industry*. University of Tokyo, Japan, pp 3-8.
- Akhtar, M., Scott, G. M., Swaney, R. E. and Kirk, T. K. 1998. Overview of biomechanical and biochemical pulping research. In: Eriksson KL, Cavaco-Paulo A (eds) *Enzyme applications in fibre processing*. ACS Symposium Series 687, American Chemical Society, pp 15-26.
- Anagnost, S. E. 1998. Light microscopic diagnosis of wood decay. *IAWA J.* 19: 141-167.
- Bari, E., Ohno, K., Yilgor, N., Singh, A. P., Morrell, J. J., Pizzi, A., Tajick Ghanbary, M. A., and Ribera, J. 2021. Characterizing Fungal Decay of Beech Wood: Potential for Biotechnological Applications. *Microorganisms* 9(2): 247.
- Berlyn, G.P. and Miksche, J.P. 1976. *Botanical microtechnique and cytochemistry*. Iowa State University Press. Ames, p 326.
- Bhatt, I. M., Pramod, S., Koyani, R. D., and Rajput, K. S. 2016. Histological changes in the cell wall structure during wood decay by *Trametes hirsuta* and *Trametes versicolor* in

- neem (*Azadirachta indica* A. Juss), *J. Sustain. Forest* 35:578–590, <https://doi.org/10.1080/10549811.2016.1236277>, 2016.
- Blanchette, R. A. 1991. Delignification by wood-decay fungi. *Annual Review of Phytopathology* 29: 381-398.
- Blanchette, R. A. 1992. Anatomical responses of xylem to injury and invasion by fungi. In: Blanchette RA, Biggs AR, eds. *Defence mechanisms Woody Plants Against fungi*. Berlin, Germany: Springer Verlag, pp 76-92.
- Blanchette, R. A. 2000. A review of microbial deterioration found in archaeological wood from different environments. *Intl. Biodeterioration and Biodegradation* 46: 189-204.
- Chaturvedi, O. P., A. K. Handa, R. Kaushal, A. R. Uthappa, S. Sarvade, and P. Panwar. 2021. Biomass Production and Carbon Sequestration through Agroforestry. *Range Management and Agroforestry* 37 (2): 116–27.
- Chave, J., Andalo, C., Brown, S., Cairns, M. A., Chambers, J. Q., Eamus, D., Fölster, H., Fromard, F., Higuchi, N., Kira, T., Lescure, J. P., Nelson, B. W., Ogawa, H., Puig, H., Riéra, B., and Yamakura, T. 2005. Tree allometry and improved estimation of carbon stocks and balance in tropical forests. *Oecologia* 145(1), 87–99.
- Crang R, Lyons-Sobaski S, Wise R. 2019. Correction to: Plant Anatomy. In *Plant Anatomy: A Concept-Based Approach to the Structure of Seed Plants*. Cham: Springer International Publishing. pp.C1-C7
- Deflorio, G., Johanson, C., Fink, S. and Schwarze, F. M. W. R. 2008. Decay development in living sap wood of coniferous and deciduous trees inoculated with six wood decay fungi. *Forest Ecology and Management* 255: 2373-2383.
- Donaldson, L. A. and Lausberg, M. J. F. 1998. Comparison of conventional transmitted light and confocal microscopy for measuring wood cell dimensions by image analysis. *International Association of Wood Anatomist Journal*, 19, 321–336.
- D'Souza-Ticlo, D., Sharma, D. and Raghukumar, C. 2009. A Thermostable Metal-Tolerant Laccase with Bioremediation Potential from a Marine-Derived Fungus. *Mar Biotechnology* 11: 725-737.
- Eichlerová, I., and Baldrian, P. 2020. Ligninolytic enzyme production and decolorization capacity of synthetic dyes by saprotrophic white rot, brown rot, and litter decomposing basidiomycetes. *Journal of Fungi* 6(4) :301. DOI: 10.3390/jof6040301
- Eriksson, K. E. and Kirk, T. K. 1985. Biopulping, biobleaching, and treatment of kraft bleaching effluents with white rot fungi, pp 271–294. In C. L. Cooney and A. E. Humphrey (ed.), *Comprehensive biotechnology*. Pergamon Press, Toronto.

- Eriksson, K. E. L., Blanchette, R. A. and Ander, P. 1990. *Microbial and Enzymatic Degradation of Wood and Wood Components*. Springer, Berlin, New York.
- Fahn, A. 1982. *Plant anatomy*. Pergamon Press, Oxford, New York, pp. 534.
- FAO, 2017. *State of the World's Forests*. Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla. 00100 Rome, Italy. ISBN 92-5-103977-1
- Favero, A., Daigneault, A., and Sohngen, B. 2020. Forests: Carbon sequestration, biomass energy, or both? *Science Advances* 6(13): eaay6792.
- Goodell, B., Nicholas, D. D., and Schultz, T. P. 2020. Fungi involved in the biodeterioration and bioconversion of lignocellulose substrates. In K. Esser & J. Bennett (Eds.), *The Mycota II: Genetics and Biotechnology*. pp. 369–389.
- Holmer, L. and Stenlid, J. 1997. Competitive hierarchies of wood decomposing basidiomycetes in artificial systems based on variable sizes. *Oikos* 79: 77-84.
- Huang, C., Xu C., Meng X., Wang L. and Zhou X. 2022. Editorial: Isolation, Modification, and Characterization of the Constituents (Cellulose, Hemicellulose, Lignin, et al.) in Biomass and Their Bio-Based Applications. *Frontiers in Bioengineering and Biotechnology* 10:866531.
- Khan, M. U. and Ahring B. K. 2019. Lignin degradation under anaerobic digestion: Influence of lignin modifications -A review. *Biomass and Bioenergy* 128, 105325.
<https://doi.org/10.1016/j.biombioe.2019.105325>
- Kirk, T. K. 1983. Degradation and conversion of lignocelluloses. In: Smith JE, Berry DR, Kristiansen B (eds) *The filamentous fungi*, vol. 4. *Fungal technology*. Edward Arnold, London, pp 266-295.
- Kirk, T. K. and Farrell, R. L. 1987. Enzymatic "combustion": the microbial degradation of lignin. *Annual Review of Microbiology* 41: 465-505.
- Kirk, T. K. and Cullen D. 1998. *Enzymology and Molecular Genetics of Wood Degradation by White Rot Fungi*. *Environmentally Friendly Technologies for the Pulp and Paper industry*, ISBN 0-471-15770-8: 273-307.
- Kitin, P., Voelker, S. L., Meinzer, F. C., Beeckman, H., Strauss, S. H. and Lachenbruch, B. 2010. Tyloses and phenolic deposits in xylem vessels impede water transport in low-lignin transgenic poplars: a study by cryo-fluorescence microscopy. *Plant Physiology*, 154(2), 887-898.
- Koyani, R.D. 2011. *Screening of wood rot fungi for bioremediation of xenobiotic compounds*. PhD thesis, the Maharaja Sayajirao University of Baroda, Vadodara.

- Koyani, R. D., Sanghvi, G. V., Bhatt I. M. and Rajput, K. S. 2010. Pattern of delignification in *Ailanthus excelsa* Roxb, wood by *Inonotus hispidus*. Mycology: Journal of Fungal Biology 1(3): 204-211.
- Koyani, R. D., Sanghvi, G. V. and Rajput, K. S. 2011. Comparative study on the delignification of *Azadirachta indica* (L) Del., wood by *Chrysosporium asperatum* and *Tricoderma harzianum*. International Biodeterioration and Biodegradation 65: 179-184.
- Koyani, R. D., Pramod, S., Bhatt, I. M., Rao, K. S., & Rajput, K. S. 2015. The delignification pattern of *Ailanthus excelsa* wood by *Inonotus hispidus* (Bull.: Fr.) P. Karst. Journal of Sustainable Forestry, 34(5), 502-515.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
- Lagaert, S., Beliën, T. and Volckaert, G. 2009. Plant cell walls: Protecting the barrier from degradation by microbial enzymes. Seminars in Cell and Developmental Biology 20:1064-1073.
- Lehringer, C., Hillebrand, K., Richter, K., Arnold, M., Schwarze, F. W. M. R. and Miltz, H. 2010. Anatomy of bioincised Norway spruce wood. International Biodeterioration and Biodegradation 64: 346-355.
- Luna, M. L., Murace, M. A., Keil, G. D. and Otano, M. E. 2004. Patterns of decay caused by *Pycnoporus sanguineus* and *Ganoderma lucidum* (Aphyllophorales) in poplar wood. IAWA J. 25: 425-433.
- Messner, K. and Srebotnik, E. 1994. Biopulping: An overview of developments in an environmentally safe paper-making technology. FEMS Microbiological Review 13: 351-364.
- Nunes, L. J. R., Matias, J. C. O., and Catalão, J. P. S. 2020. Woody biomass in the Mediterranean region: Potential and utilization for energy production. Renewable and Sustainable Energy Reviews 119: 109562. <https://doi.org/10.1016/j.rser.2019.109562>.
- Paço, A., Duarte, K., Silva, A. M. S., and Freitas, A. C. 2017. Biodegradation of lignin by fungal cultures. Journal of Pharmacognosy and Phytochemistry 6(4): 1840–1842.
- Pallardy, S.G. 2008. Physiology of woody plants (3rd ed.). Academic Press, Amsterdam, New York, pp 454.
- Parand, M.2001. Feasibility Study of Fungal Bioremediation of a Flare Pit Soil Using White Rot Fungi. Ph.D. thesis submitted to the University of Calgary, Alberta.

- Phillips O.L., Vásquez Martínez R., Arroyo L., Baker T.R., Killeen T., Lewis S.L., Malhi Y., Monteagudo Mendoza A., Neill D., Núñez Vargas P., Alexiades M., Cerón C., Di Fiore A., Erwin T., Jardim A., Palacios W., Saldias M., Vinceti B. 2002. Increasing dominance of large lianas in Amazonian forests. *Nature* 418: 770–774.
- Plomion, C., Leprovost G. and Stokes A. 2001. Wood formation in trees. *Plant Physiology* 127: 15-23.
- Pointing, S. B. 2001. Feasibility of bioremediation by white-rot fungi. *Applied Microbiology and Biotechnology* 57: 20-33.
- Pramod, S., Koyani, R. D., Bhatt, I., Vasava, A. M., Rao, K. S. and Rajput, K. S. 2015. Histological and ultrastructural alterations in the *Ailanthus excelsa* wood cell walls by *Bjerkandera adusta* (Willd.) P. Karst. *International Biodeterioration & Biodegradation*, 100, 124-132.
- Pramod, S., Rajput, K. S. and Rao, K. S. 2019. Immunolocalization of β -(1–4)-D-galactan, xyloglucans and xylans in the reaction xylem fibres of *Leucaena leucocephala* (Lam.) de Wit. *Plant Physiology and Biochemistry*, 142, 217-223.
- Rayner, A. D. M. and Boddy, L. 1988. *Fungal Decomposition of Wood: Its Biology and Ecology*. John Wiley, Chichester, UK.
- Sanghvi, G. V., Koyani, R. D. and Rajput, K. S. 2010. Thermostable xylanase production and partial purification by solid state fermentation using agricultural waste wheat straw. *Mycology: Journal of Fungal Biology* 1(2): 106-112.
- Schnitzer S.A. and Bongers F. 2011. Increasing liana abundance and biomass in tropical forests: emerging patterns and putative mechanisms. *Ecology Letters* 14: 397–406.
- Schwarze, F. W. M. R. 2007. Wood decay under the microscope. *Fungal Biology Reviews* 21: 133-170.
- Schwarze, F. W. M. R. and Fink, S. 1998. Host and cell type affect the mode of degradation by *Meripilus giganteus*. *New Phytologist* 139: 721-731.
- Vasile, C. and Baican, M. 2023. Lignins as promising renewable biopolymers and bioactive compounds for high-performance materials. *Polymers*. 15:3177.
- Wang, L, Li, G., Chen, X., Yang, Y., Liew, R. K., Abo-Dief, H. M., Lam, S. S., Sellami, R., Peng, W., and Li, H. 2024. Extraction strategies for lignin, cellulose, and hemicellulose to obtain valuable products from biomass. *Advanced Composites and Hybrid Materials* 7: 219.

- Wang, X., Li, Y., Zhang, H., Chen, J., and Liu, Q. 2021. Mechanism of lignin degradation via white rot fungi explored using spectral analysis and gas chromatography–mass spectrometry. *BioResources*, 20(1), 145–160
- Worrall, J. J., Anagnost, S. E. and Zabel, R. A. 1997. Comparison of wood decay among diverse lignicolous fungi. *Mycologia* 89: 199-219.
- Xu, L., Saatchi, S. S., Yang, Y., Yu, Y., Pongratz, J., Bloom, A. A., Bowman, K., Worden, J., Liu, J., Yin, Y., Domke, G., McRoberts, R. E., Woodall, C., Nabuurs, G.-J., de-Miguel, S., Keller, M., Harris, N., Maxwell, S., Schimel, D., and Pan, Y. 2021. Changes in global terrestrial live biomass over the 21st century. *Science Advances* 7(40): 9829.
- Zeng, N., and Hausmann, H. 2022. Wood Vault: remove atmospheric CO₂ with trees, store wood for carbon sequestration for now and as biomass, bioenergy and carbon reserve for the future. *Carbon Balance Manage* 17(2).<https://doi.org/10.1186/s13021-022-00202-0>.

LIST OF PUBLICATIONS

- 1) Thacker K.D., Joshi E.B., Joshi P.N., **Vasava A.M.** & Rajput K.S. 2025. *Commiphora shanakarsinhiana* (Burseraceae): A new species from Gujarat, India. *Rheedea* 35(1): 07-12. <https://dx.doi.org/10.22244/rheedea.2025.35.01.03>
- 2) Patil S.M., **Vasava A.M.**, Raole V.M. & Rajput K.S. 2022. *Ipomoea laxiflora* H.J. Chowdhery & Debta: A new record for Western Ghats and Semiarid regions of India. *Journal of Threatened Taxa* 14(1): 20526-20529. <https://doi.org/10.11609/jott.59.14.1.20526-20529>
- 3) Patel R.S., Vanjara A.G., Patel N.R., **Vasava A.M.**, Patil S.M. & Rajput K.S. 2021. *In silico* discovery of fungal metabolites bergenin, quercitrin and dihydroartemisinin as potential inhibitors against main protease of SARS-CoV-2. *Coronaviruses* (Bentham Science) 2(8): e260721189437. <https://doi.org/10.2174/2666796701999201223163604>
- 4) Rajput K.S., Kachhiyapatel R.N., Patil S.M., **Vasava A.M.**, Patel R.S., Patel S.K. & Raole V.M. 2021. Checklist of pteridophytes of Gujarat State. *Phytotaxa* 514(2) 89-104. <https://doi.org/10.11646/phytotaxa.514.2.1>

- 5) Pramod S., **Vasava A.M.**, Koyani R.D. & Rajput K.S. 2021. Post infectious alterations caused by *Xylaria polymorpha* in the secondary xylem of *Lannea coromandelica* (Houtt.) Merr. *Forestists* 71 (2): 93-101.
<https://doi.org/10.5152/forestist.2020.20036>
- 6) Patel R.S., **Vasava A.M.** & Rajput K.S. 2020. Morphological and molecular identification of *Geastrum* from western India. *Kawaka* 54: 50-54.
<https://doi.org/10.36460/Kavaka/54/2020/50-54>
- 7) Patel R.S., **Vasava A.M.** & Rajput K.S. 2019. Distribution of *Trichaleurina javanica* (Rehm) M. Carbone, Agnello & P. Alvarado (Chorioactidaceae) in India. *Plant Biosystems* 153(2) 231-234. <https://doi.org/10.1080/11263504.2018.1461704>
- 8) Patel R.S., **Vasava A.M.** & Rajput K.S. 2018. Morphological and molecular evidence for the occurrence of *Itajahya galericulata* (Fungi, Phallaceae) in India and Asia. *Plant and Fungal Systematics* 63(2): 39-44. <https://doi.org/10.2478/pfs-2018-0006>
- 9) Patel R.S., **Vasava A.M.** & Rajput K.S. 2018. New distribution record of *Cyathus stercoreus* De Toni (Nidulariaceae) for India from Gujarat. *Studies in Fungi* 3(1): 227-233.
- 10) Patel R.S., **Vasava A.M.** & Rajput K.S. 2018. New distribution record of *Clathrus delicatus* Berk. & Broome (Phallaceae) from Gujarat. *Journal of Indian Botanical Society* 97(1): 54-57.
- 11) **Vasava A.M.**, Patel R.S., Koyani R.D. & Rajput K.S. 2018. Distribution and diversity of Polyporaceae in Western India: An overview and addition to mycoflora of the Gujarat state. *Plant Science Today* 5(1): 32-38.
<https://doi.org/10.14719/pst.2018.5.1.353>
- 12) **Vasava A.M.**, Koyani R.D., Patel R.S. & Rajput K.S. 2017. Diversity and distribution of Agaricaceae in western part of India with special reference to Gujarat State. *Journal of Indian Botanical Society* 96(1&2): 119-135.
- 13) Koyani R.D., Bhatt I.M., Patel H., **Vasava A.M.** & Rajput K.S. 2016. Evaluation of *Schizophyllum commune* Fr. for its biobleaching potential in lignin degradation: Light microscopic analysis. *Wood Material Science and Engineering* 11(1): 46-56.
- 14) **Vasava A.M.**, Koyani R.D., Singh A.P. & Rajput K.S. 2015. Diversity and distribution of myxomycetes in western part of India, with special reference to the state of Gujarat. *Current Research in Environmental and Applied Mycology* 5(4): 381-388

- 15) Rajput K.S., Koyani R.D., Patel H.P., **Vasava A.M.**, Patel R., Patel A. & Singh A.P.
2015. Preliminary checklist of fungi of Gujarat state, Western India. *Current Research in Environmental and Applied Mycology* 5(4): 285-306