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Comparative pyrolysis of agricultural biomass for bio-oil production and *in vitro* antifungal analysis of developed bio-oil based formulations

VAIBHAV BADONI[1], ASHUTOSH DUBEY[1], R. N. PATERIYA[2] and A.K. VERMA[1*]

[1]*Department of Biochemistry,*[2]*Department of Farm Machinery and Power Engineering, G.B. Pant University of Agriculture and Technology, Pantnagar, 263145(U.S. Nagar, Uttarakhand)*
[*]*Corresponding author's email id: akvermapht@gmail.com*

ABSTRACT: Biomass feedstocks including corn cob, pine needles, rice straw, and bamboo leaves were pyrolyzed to obtain bio-oils. Among these, corn cob yielded the highest bio-oil output (19.6% w/w), followed by pine needles (18.9%w/w), rice straw (13.8% w/w), and bamboo leaves (13.5% w/w). Bio-oil based formulations (BOBFs) were prepared from each bio-oil by mixing 40 mL of bio-oil, 30 mL of ethanol, 2 g of saponin, and 30 mL of the aqueous phase of bio-oil. These BOBF were evaluated for antifungal activity under *in vitro* conditions and showed complete inhibition of *Alternariabrassicae* and *Botrytis cinerea* at higher concentrations of 600 ppm and 400 ppm, respectively. Among the formulations, corn cob BOBF exhibited the highest percent inhibition at 1500 ppm, outperforming those derived from other biomass sources. The remaining formulations also demonstrated strong dose-dependent antifungal activity, with inhibition increasing by more than 1.2-fold as concentrations rose. Overall, corn cob residue proved to be the most efficient feedstock for producing pyrolysis bio-oil and holds strong potential as a sustainable agent for crop protection against fungal pathogens.

Key words: *Alternaria brassicae*, bio-oil, BOBF, *Botrytis cinerea*, pyrolysis

Globally the biomass production from agriculture and forest residues is about 2.9 billion tonnes of collectable biomass, largely comprised of crop residues, sugarcane bagasse, oilseed residues, and forest byproducts (Sani *et al.*, 2024; Antar *et al.*, 2021). In India large agricultural and forest residue is generated with total potential of 750 million tonnes of agricultural biomass produced annually, with about 225–230 million tonnes as surplus. Within India, the major biomass generating crops are rice and wheat, followed by sugarcane (bagasse and trash), with additional contributions from maize, millets, oilseeds, cotton etc. (Negi *et al.*, 2023; Reddy *et al.*, 2025). Production estimates place India's 2023–24 rice output at about 137.8 million tonnes and maize at about 37.7 million tonnes (Singh and Jana., 2024;MAFW, 2024). Beyond field crops, India also generates notable lignocellulosic waste from forest landscapes and non-timber sources, with pine needles in Himalayan states recognized as a seasonal, fire prone biomass and bamboo leaf litter forming a diffuse but locally significant biomass resource. Around 6–8 million tonnes of pine needle leaves are generated per year across the Himalayan belt. Uttarakhand estimates near 2.06 million tonnes per year of dry pine needles (Halba and Arora.,2024). Bamboo is an important non-wood forest resource found in the forest as well as non-forested areas. As per the official website of the National Bamboo Mission, it covers a 13.96 million hectare area with 136 species in India (National Bamboo Mission.,2025).

Pyrolysis of biomass is the thermal decomposition of organic material heated without oxygen to form three main products, such as liquid bio oil, solid biochar, and non condensable gases. Pyrolysis temperature parameters typically span about 350–800°C, with the chosen window determining whether liquids, solids, or gases dominate the products (Mandal *et al.*, 2018). Pyrolysis bio oil is a dark, acidic liquid formed by rapidly heating biomass without oxygen. It typically contains broad chemical families including organic acids (acetic and formic acids), carbonyls such as aldehydes and ketones, furans and furan derivatives (furfural, hydroxymethylfurfural), phenolics, from lignin depolymerization (guaiacol, syringol, eugenol, vanillin and related methoxyphenols) (Staš *et al.*, 2020). Pyrolysis bio oils and their aqueous fractions, often termed as wood vinegar or pyroligneous acids exhibit antimicrobial activity because of the oxygenated phenolics, furans, organic acids, and carbonyls produced from lignin and carbohydrate depolymerization during thermolysis (Kim *et al*., 2012). Fractionated fast pyrolysis oils have shown antibacterial effects against gram negative and gram positive bacteria, including *Escherichia coli* and *Salmonella typhimurium* (Patra *et al*., 2016), and antifungal inhibition of wood decay fungi such as *Trametes versicolor* and *Gloeophyllum trabeum* (Theapparat *et al*., 2015). Volatile constituents can exert antifungal activity against plant pathogens including *Ralstonia solanacearum*, *Phytophthora capsici, Colletotrichum orbiculare, Valsamali, Cochliobolus sativus, Helminthosporium sativum* and *Phytophthora infestans in vitro* (Hossain *et al*., 2015). Pine needle derived pyrolysis oils have also been reported to inhibit foodborne pathogens such as *Bacillus cereus* and *Listeria monocytogenes* with potency varying by feedstock, pyrolysis temperature, and downstream purification or fractionation (Patra *et al*., 2015). The aqueous pyroligneous fraction typically contains about 2–4% acetic acid with minor formic and propionic acids along with phenolics and furans, contributing to bactericidal and fungicidal effects (Mattos *et al*.,2019).

Alternaria leaf blight is among the most destructive diseases of mustard, caused primarily by necrotrophic fungi, *Alternaria brassicae* and *A. brassicicola*, which infect leaves, stems, and siliquae and thrive under high humidity with moderate temperatures. Alternaria blight typically causes average yield losses of about 35–38% in Indian mustard and 46–47% in yellow sarson, with reported extremes up to 70% under highly conducive conditions (Singh *et al*., 2017). Similarly, Botrytis grey mould is a major disease of chickpea caused by *Botrytis cinerea*, capable of rapid epidemic spread in cool, humid conditions and dense canopies, often leading to severe yield and quality losses. Botrytis grey mould is a major disease of chickpea that commonly causes large losses in humid, cool-wet seasons, with average annual yield losses around 50% and documented epidemics reaching near total crop failure under highly conducive conditions (Pande *et al*., 2006).

This study focuses on determination of *in vitro* antifungal efficacy of bio-oil based formulation (BOBF) prepared from pyrolysis bio-oil of waste biomass residues of pine needles, corn cobs, rice straw and bamboo leaves against plant pathogenic fungi *A.brassicae* and *B. cinerea*. Pyrolysis bio-oils exhibit broad antimicrobial and antifungal activity largely attributed to rich phenolic pools and organic acids that disrupt membranes, denature proteins, and impair sporulation and biofilm formation promoting bio-oil based antimicrobials by valorisation of residue, cutting synthesis related footprints. Their potential to reduce reliance on synthetic chemicals aligns with sustainable agriculture practices, offering a renewable, biodegradable, eco-friendly, safer and non-toxic, approach for crop protection.

MATERIALS AND METHODS

Collection of fungal material and plant biomass residues

Pine (*Pinus roxburghii*) needle litters were collected from the Kumaon region of the Himalayas, and corn (*Zea mays*) cobs and rice straw were collected from the Norman E. Borlaug Crop Research Centre, Pantnagar. Bamboo leaves (*Bambusa vulgaris*) were collected from Agroforestry Research Center, Pantnagar. Fungal pathogens, *Alternaria brassicae* was procured from the Department of Plant Pathology at the College of Agriculture, G.B.P.U.A. and T, Pantnagar. *Botrytis cinerea* (ITCC No. 8651) was procured from ITCC, Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi.

Preparation of bio-oil and Bio-oil based formulation (BOBF)

Pyrolysis of biomass was done as published by our laboratory (Mandal *et al*., 2018). A batch pyrolyzer reactor was used to prepare bio-oil from pine needles, corn cob, rice straw and bamboo leavesat the Department of Farm Machinery and Power Engineering, College of Technology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Four hundred gram of biomass was sun dried for 5-7 days, cut into 4-5 mm pieces by a hand operated chopping machine and fed into the bio-oil reactor to produce pyrolysis oil.

Formulation of each bio-oil frompine needle,corn cobs, rice straw and bamboo leaves were prepare BOBF by mixing 40 mL bio-oil, 30 mL ethanol, 2 g saponin and 30 mL aqueous phase (Bhatnagar *et al*., 2025).

In vitro* antifungal efficacy of BOBF against *Alternaria brassicae* and *Botrytis cinera

The formulation was prepared for different working concentrations of 100-700 ppm and antifungal activity was analysed against *Alternaria brassicae* and *Botrytis cinerea* (Nene and Thapilyal, 2002). Double strength Potato Dextrose Agar (PDA) was prepared by dissolving 78g/L PDA powder and equal volumes of BOBF was mixed before plating to obtain desired concentrations. Control plate was prepared using single strength PDA without BOBF. The media was supplemented a pinch of streptomycin to avoid bacterial contamination. Mycelia discs 5mm in diameter, was carefully placed on the surface of the PDA plates. Inoculated plates were incubated in triplicate at 27 ºC and growth was monitored. Growth inhibition was quantified using following formula:

Growth inhibition % = $\{(R-r)/R\}^{*}100$

Where, R=radial growth of fungal mycelia on control plate

r= radial growth of fungal mycelia on the plate treated with formulation.

Statistics analysis

The experiment were conducted in triplicate and standard error was calculated manually. All data were analysed by two-way analysis of variance (ANOVA) and post-hoc tests analysed by Duncan Multiple Range test for Least Significant Difference. All the data were testedat a significance level of (alpha= 0.05).

RESULTS AND DISCUSSION

Bio-oil yield

The comparative analysis of pyrolysis product yields from different plant residues suggested that among the biomass used, corn-cob exhibited the highest bio-oil yield (19.6%w/w), surpassing pine needles (18.9 %w/w), rice straw (13.8 %w/w), and bamboo leaves (13.5 %w/w). The aqueous phase yield was maximal in corn cob (33.6 %w/w) followed by bamboo leaves (33.5 %w/w) and rice straw (31.6 %w/w) residues, indicating a substantial fractional generation. While the pine needles had the lowest aqueous phase yield at 27.8 %w/w (Table 1).

In vitro* antifungal efficacy against *Alternaria brassicae

In vitro antifungal efficacy analysis showed a complete inhibition of the fungal growth across all the concentrations of BOBF at 4th day of inoculation. Thereafter a significant dose dependent percent inhibition of fungal growth was observed with increasing concentrations of all BOBF. In pine needleBOBF, the percent inhibition increased from 80.54% at 400 ppm to 85.05% at 500 ppm with a relative fold increase of 1.05. Further this inhibition reached to 100% at concentrations of 600 and 700 ppm, corresponding to an overall fold increase of 1.17 relative to 400 ppm. Similarly, corn cob BOBF demonstrated a similar trend with increasing percent inhibition from 83.31% at 400 ppm to 87.71% at 500 ppm with a relative fold increase of 1.05. That showed a 100% inhibition at 600 and 700ppm, with an overall fold increase of 1.20 compared to 400 ppm.Rice straw BOBF exhibited an increase in percent inhibition from 77.20% at 400 ppm to 81.12% at 500 ppm with a relative fold increase of 1.05, followed by maximum inhibition of 100% at

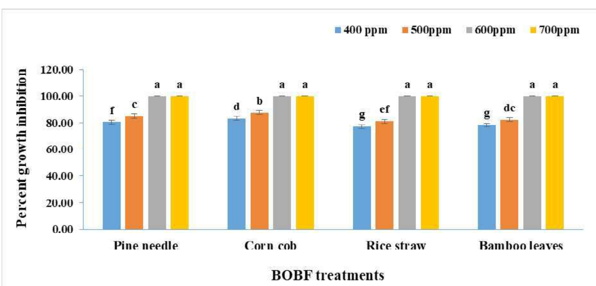
Table 1: Percent weight (W/W) yield of bio-oils from different biomass residues

Plant Residue	%Weight yield of bio-oil	%Weight yield of aqueous phase of bio-oil	% Weight yield of biochar (%)	Gas escaped (%)
Pine needles	18.9%	27.8%	23.8%	29.5%
Corn-cob	19.6%	33.6%	21.3%	25.5%
Rice straw	13.8%	31.6%	26.8%	27.8%
Bamboo leaves	13.5%	33.5%	23.4%	29.6%

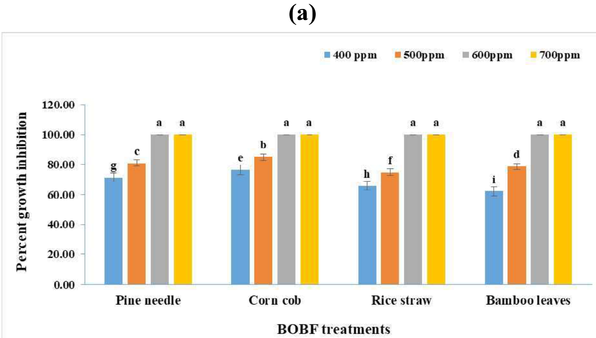
600 and 700 ppm, corresponding to an overall relative fold increase of 1.296 compared to 400 ppm. Bamboo leaves oil showed a similar increase from 78.37% at 400 ppm to 82.31% at 500 ppm with 1.05 fold increase, further reaching to 100% inhibition at 600 and 700 ppm, equivalent to a relative fold increase of 1.27(Fig.1).

Comparing the maximum inhibition among BOBF all exhibited 100% inhibition at 600 and 700 ppm. At 500 ppm, corn cob BOBF showed the highest percent inhibition of 87.71%, which was 1.03 fold higher than pine needle (85.05%), 1.08 fold higher than rice straw (81.12%), and 1.06 fold higher than bamboo leaves BOBF (82.31%). It was also observed that at 400 ppm concentration of among different BOBF revealed that the corn cob BOBF exhibited the highest percent inhibition of 83.31%, which was 1.03 fold higher than pine needle (80.54%), 1.07 fold higher than rice straw (77.20%) and 1.06 fold higher than bamboo leaves BOBF (78.37%).

At day 10 the percent inhibition exhibited by all different BOBF increased markedly with increasing concentrations, indicating a clear concentration dependent antifungal response as observed previously. For pine needle BOBF, percent inhibition increased from 71.55% at 400 ppm to 80.88% at 500 ppm, corresponding to a relative fold increase of 1.13, further reaching to 100% inhibition at higher concentrations of 600 and 700 ppm, with an overall fold increase of 1.40 compared to 400 ppm. Similarly, corn cob bio-oil showed an increase from 76.41% at 400 ppm to 85.02% at 500 ppm with a fold increase of 1.11, and further achieving 100% inhibition at 600 and 700 ppm concentrations, indicating a 1.31 overall fold increase relative to 400

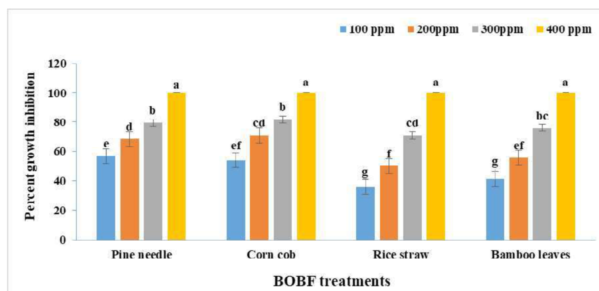


(a)



(b)

Fig. 1:*In vitro* anti-fungal efficacy of BOBF from pin needle, corn cob, rice straw and bamboo leaves against *Alternariabrassicae* (a) at day 7 and (b) 10 post inoculation. Values represents mean± S.E. (n=3). The ANOVA indicated that Factor A, Factor B, and their interaction (A × B) were highly significant ($p < 0.001$). Post-hoc comparison of means using DMRT indicate treatments sharing the same superscript letter did not differ significantly



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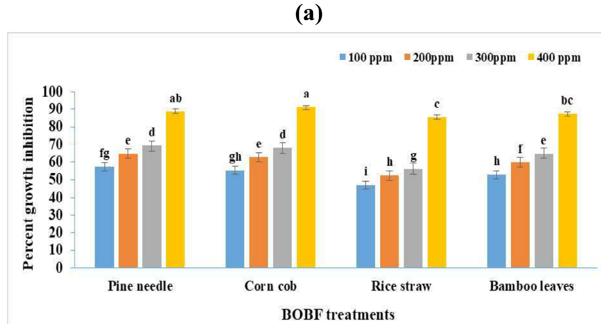

(b)

Fig. 2:*In vitro* anti-fungal efficacy of BOBF from pin needle, corn cob, rice straw and bamboo leaves against *Botrytis cinerea*(a) at day 4 and (b) 7 post inoculation. Values represents mean± S.E. (n=3). The ANOVA indicated that Factor A, Factor B, and their interaction (A × B) were highly significant ($p < 0.001$). Post-hoc comparison of means using DMRT indicate treatments sharing the same superscript letter did not differ significantly

ppm.Rice straw BOBF exhibited a lower inhibition of 65.94% at 400 ppm, which increased to 74.92% at 500 ppm with a relative fold increase of 1.14 and further reaching to complete inhibition of 100% at 600 and 700 ppm, corresponding to an overall relative fold increase of 1.52 comparedto 400 ppm. Bamboo leaves bio-oil formulation demonstrated a similar pattern, with percent inhibition increasing from 62.18% at 400 ppm to 78.66% at 500 ppm with fold increase of 1.26 and further attaining complete inhibition at higher concentrations, accounting to an overall fold increase of 1.6 relative to 400 ppm.

When comparing the maximum inhibition across different BOBF, all treatments achieved complete inhibition at 600 and 700 ppm. However, at 500 ppm, corn cob BOBF recorded the highest inhibition of 85.02%, which was higher than pine needle (80.88%), bamboo leaves (78.66%), and rice straw BOBF (74.92%). At the lowest concentration of 400 ppm, corn cob BOBF again exhibited the highest inhibition (76.41%) compared to pine needle (71.55%), rice straw (65.94%), and bamboo leaves (62.18%) (Fig.1).

In vitro antifungal efficacy against Botrytis cinerea

At day 4 post inoculation a progressive and concentration dependent enhancement in antifungal inhibition across all four BOBF was observed. For pine needle BOBF, percent inhibition increased from 56.82% at 100 ppm to 68.46% at 200 ppm with a fold increase of 1.21, and further to 79.42% at 300 ppm with a fold increase of 1.40 fold increase. Complete inhibition of 100% was achieved at 400 ppm, corresponding to an overallfold increase of 1.76 relative to the lowest concentration of 100 ppm.Corn cob bio-oil formulation showed a similar trend, with increasing percent inhibition from 53.97% at 100 ppm to 70.95% at 200 ppm with a fold increase of 1.31 and further to 81.66% at 300 ppm with a fold increaseof 1.51,and complete inhibition at 400 ppm with an overall fold increase of 1.85 compared to 100 ppm.Rice straw bio-oil formulation exhibited a lower initial inhibition of 35.81% at 100 ppm, which increased to 49.98% at 200 ppm with an increase of 1.40 foldsand furtherto 70.83% at 300 ppm with a fold increase of 1.98, ultimately reaching 100% inhibition at 400 ppm with an overall 2.79 fold increase relative to100 ppm concentration. Similarly, bamboo leaves bio-oil formulations increased percent inhibition from 41.10% at 100 ppm to 55.71% at 200 ppm with 1.36 fold increase and 76.09% at 300 ppm with 1.85 fold increase, further achieving complete inhibition at 400 ppm with an overall fold increase of 2.43 fold compared to 100 ppm.

When comparing the maximum inhibition across bio-oil types, all four achieved 100% inhibition at 400 ppm. However, at 300 ppm, corn cob BOBF showed the highest inhibition of 81.66%, which was about 1.03 fold higher than pine needle (79.42%), 1.15 fold higher than bamboo leaves (76.09%), and 1.15 fold higher than rice straw (70.83%) BOBF. At 200 ppm, again corn cob oil again showed the highest percent inhibition of 70.95% that was about 1.04 fold higher compared to the pine needle (68.46%), 1.29 fold higher than bamboo leaves (55.71%), and 1.42 fold higher than rice straw (49.98%) bio-oil formulation. At the lowest concentration of 100 ppm, pine needle BOBF exhibited the highest percent inhibition (56.82%), which was 1.05 fold higher than corn cob (53.97%), 1.38 fold higher than bamboo leaves (41.10%) and 1.59 fold higher than rice straw (35.81%) (Fig. 2).

At day 7 post inoculation a similar concentration dependent increase in percent inhibition across all BOBF was observed, highlighting that antifungal efficacy improved consistently with higher doses. For pine needle BOBF, percent inhibition increased from 57.47% at 100 ppm to 64.91% at 200 ppm, representing a 1.13 fold increase, and further to 69.21% at 300 ppm with a 1.20 fold increase. The percent inhibition reached 88.80% at 400 ppm, which corresponds to an overall 1.54 fold increaserelative to the lowest concentration of 100 ppm. Corn cob BOBF exhibited a similar trend, with increasing percent inhibition from 55.23% at 100 ppm to 62.86% at 200 ppm with a fold increase of 1.14, thanto 68.10% at 300 ppm with a fold increaseof 1.23 and further to 91.05% at 400 ppm with an overall relative fold increase of 1.65 compared to 100 ppm. Rice straw bio-oil displayed a lower percent inhibition compared to other BOBF with 47.01% at 100 ppm, which rose to 52.42% at 200 ppm with a relative fold increase of 1.12, 56.17% at 300 ppm with 1.19 fold increase, and further reached to 85.45% at 400 ppm with an overall fold change of 1.82 relative to 100 ppm). Similarly, bamboo leaves bio-oil showed progressive percent inhibition from 52.98% at 100 ppm to 59.70% at 200 ppm with 1.13 fold increase, 64.92% at 300 ppm with 1.23 fold increase, and further to 87.30% at 400 ppm, amounting to an overall relative increase of 1.65 fold compared to100 ppm.

When comparing the maximum percent inhibition among different BOBF, all exhibited highest percent inhibition at 400 ppm. At this concentration, corn cob BOBF recorded the highest inhibition of 91.05%, followed by bamboo leaves (87.30%), pine needle (88.80%), and rice straw (85.45%) respectively. At 300 ppm, pine needle bio-oil base formulation showed the highest percent inhibition of 69.21%, followed by corn cob (68.10%), bamboo leaves (64.92%), and rice straw (56.17%) BOBF. At 200 ppm, again pine needle BOBF had the highest percent inhibition of 64.91%, followed by corn cob (62.86%), bamboo leaves (59.70%), and rice straw (52.42%). At the lowest concentration of 100 ppmpine needle BOBF showed 57.47% inhibition which was higher compared to corn cob (55.23%), bamboo leaves (52.98%) and rice straw (47.01%) at the same concentration (Fig. 2).

These results indicate that while all BOBFs achieved complete inhibition at higher concentrations, a pronounced fold increase in antifungal efficacy was observed with increasing concentration, suggesting a strong dose responsive behaviour and potent inhibition potential at higher concentrations.

The yield of bio-oil is greatly influenced by several factors, including the type of biomass residue, reaction temperature, retention time, and gas flow rate. These parameters can vary under different pyrolysis conditions, which may explain variation in the bio-oil yield obtained in the present study compared to the previous reports. For instance, Mandal *et al*. (2018) reported a bio-oil yield of 43.76% w/w from pine needle biomass at 550 °C, while Shezi and Kiambi (2025) observed yields of 45% w/w and 37% w/w from pine and bamboo wood biomass, respectively. Similarly, Mandal *et al*. (2025) recorded bio-oil yields of 31.3% w/w and 33.3% w/w from paddy straw at 450 °C and 500 °C, respectively. In the case of corn cob biomass, Demiral and Şensöz (2012) reported a yield of 26.44% w/w at 500 °C, whereas Zubiolo *et al*. (2023) achieved 39.72% w/w under similar conditions.

Pyrolysis bio-oils derived from waste biomass residues possess a complex chemical composition that confers broad spectrum efficacy against diverse microbial groups, including bacteria and fungi. In the present study, pyrolysis bio-oils obtained from four different biomass feedstocks demonstrated significant antifungal activity against two major plant pathogens, *A. brassicae* and *B. cinerea*. Complete growth inhibition of *A. brassicae* was achieved at a concentration of 600 ppm, whereas *B. cinerea* was fully inhibited at 500 ppm.Previous studies have similarly reported the antifungal potential of pyrolysis bio-oils and their products, thereby underscoring their effectiveness against a wide range of pathogenic fungi. Shiny *et al* (2014) reported strong antifungal activity of coconut shell pyrolysis bio-oil, achieving 81.5% and 90% inhibition against *Polyporus sanguineus* (white rot fungi) and *Tyromyces palustris* (brown rot fungi) respectively, at a concentration of 2500ppm, that was considerably higher compared to those employed in the present study. The pronounced antifungal activity was attributed to the high phenolic content of the coconut shell pyrolytic oil. Similarly, pyroligneous acids from walnut branches was prepared by Wei *et al.* (2010) at three temperature ranges of 90–230 °C, 230–370 °C. 370–450 °C. Extracts obtained at higher temperature ranges (230–370 °C and 370–450 °C) demonstrated superior antifungal activity against *Phytophthora capsici, Colletotrichum orbiculare, Valsamali, Cochliobolus sativus, Helminthosporium sativum* and *Phytophthora infestans* within the concentration range of 625–40,000 ppm. These findings highlight the critical role of pyrolysis temperature in determining the chemical composition and, consequently, the bioactivity of the resulting bio-oil. Pyrolysis bio-oils derived from lignin showed efficacy at 300 ppm against key plant pathogenic fungi such as *Pythium ultimum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. This effective concentration closely align with the range observed in present study. Chemical characterization of these bio-oils identified polycyclic aromatic hydrocarbons (e.g., anthracene, pyrene, phenanthrene, fluoranthene, benzoanthracene, 1-phenylnaphthalene), likely contributing to their antifungal activity (Hossain *et al*., 2015).

Tar oil showed antifungal activity against brown-rot fungi (*Tyromyces palustris, Lentinus lepideus*), white-rot fungi (*Pleurotus ostreatus, Coriolus versicolor*), and the sap-staining fungus *Ophiostoma piliferum*. Growth inhibition was observed across dilutions ranging from 2000 ppm to 100 ppm. Similar to the ineffectiveness of the lower BOBF concentrations in completely inhibiting *A. brassciae* and *B.cinerea*, tar oil also inhibited *P. ostreatus* and *C. versicolor* at all higher dilutions compared to lower concentration of 100 ppm. Brown-rot fungi were also completely inhibited at higher tar oil concentrations of 2000 ppm along with *O. piliferum* that was inhibited at 2000 and 1000 ppm dilutions (Kartal *et al*., 2011). Most studies reported the antifungal activity of the bio-oils to be comparatively at higher concentrations, such as fungicidal activity of pistachio shell pyrolysis bio-oil,which was largely due to its high phenolic content, against *Trichoderma viridae*, *Coriolus versicolor*, *Trichophyton rubrum*, and *Aspergillus niger* was observed at much higher concentrations of 10,000 to 50,000 ppm (Okutucu *et al*., 2011). The antimicrobial activity of 5% NaHCO*f* extracts of walnut shell pyroligneous acid with inhibition rates of 75–78.17% at 5,000/ ppm were reported against similar pathogens such as *Alternaria solani*, *Botrytis cinerea,* along with *Verticillium dahlia* and *Glomerella cingulate* (Ma *et al*., 2011), which was much higher compared to the inhibitory concentrations of 600 and 400 ppm reported in the present study. However, Cintra *et al* (2025) reported lower inhibitory concentrations for bio-oil aqueous phases (BOAP) obtained from the pyrolysis of *Cocos nucifera* (coconut), *Syagrus coronata* (licuri) and *Terminalia catappa* (almond) against *C. auris*, demonstrating notable antifungal efficacy, with licuri derived BOAP showing the inhibitory activity, with MIC ranging from 35-560 ppm, aligning well with the similar inhibitory concentration range of 100ppm- 600 ppm that was used in our study. *S. jamaicensis* bio-oil was screened for their antimicrobial potency showed remarkable antimicrobial efficacy with a MIC of 6.25 µg/mL (6.25 ppm) against *C. albicans* (Chongloi *et al*., 2024), though it was much lower compared to the inhibitory concentration observed against *A. brassicae* and *B. cinerea* in the present study. Chandra (2022) further, specifically reported that pine needle bio-oil exhibiting 100% inhibition of *A. brassicae* at 10^{-1} dilution, with further inhibition of 52%, 48% and 42% at 10^{-2}, 10^{-3} and 10^{-4}dilutions respectively. Another study focusing exclusively on corn cob and pine needle BOBF showed complete growth inhibition of maize pathogens,including prominent fungi such as *Alternaria spp., Curvularia lunata, Bipolaris maydis, Bipolaris zeicola*, and *Fusarium* spp. at 400 ppm, as well as highlighting the phenolic as the major constituents of the pine needle and corn cob bio-oils (Bhatnagar *et al*., 2025).

The present findings are in line with earlier reports highlighting the antifungal activity of pyrolysis bio-oils, primarily attributed to their complex chemical composition, largely including the phenolic compounds (e.g., phenol, 2, 6-dimethoxyphenol, guaiacol etc.), along with ketones, aldehydes, carboxylic acids, and furans, which disrupt membranes, denature proteins, and impair metabolic pathways. Phenolics and related aromatics are known to induce oxidative stress, exerting fungistatic and fungicidal effects (Kim *et al*., 2012; Lourençon *et al*., 2016). Pine have been extensively been used for pyrolysis in several studies such as use of pine and oak, wood and bark by Mohan *et al*. (2008) to obtain phenol rich, lignin fractions exhibiting strong antifungal activity against *Gloeophyllum trabeum* (brown rot) and *Trametes versicolor* (white rot). Methanol diluted bio-oil treatments (5–25%) and whole bio-oil (25%) provided excellent decay resistance, largely due to the phenolic constituents in the lignin-rich fractions. Similarly, *Pythium ultimum* was reported to be strongly inhibited by tobacco bio-oil fractions containing high levels of nicotine and phenols (Booker *et al*., 2010). Theapparat *et al*., (2015) derived pyroligneous acids from a diverse biomass sources such as *Eucalyptus camaldulensis*, *Leucaena leucocephala*, *Azadirachta indica*, *Hevea brasiliensis* (rubberwood), and *Dendrocalamus asper* (bamboo) that were tested against *Trametes versicolor*, *Rigidoporus amylospora*, *Gloeophyllum trabeum* and *Botryodiplodia theobromae*. Similar to our study that utilised the bamboo leaves bio-oil, the pyroligneous acids from bamboo and rubber wood exhibited strong antifungal activity, with the highest inhibitions of 89.7 and 85.03% respectively.Again the study highlighted the elevated phenolic content, notably 2-methoxy-4-propylphenol and 2-methylphenol as the primary fungal inhibitory constituents. Meier *et al*. (2001) reported the fungicidal role of crude pyrolysis oil derived from pine wood against brown rot basidiomycetes *Coniophora puteana*, *Poria placenta*, and *Lentinus lepideus*. Their study highlighted the strong protective effect of crude bio-oil,inhibiting colonization and degradation by these wood destroying fungi. Similarly, crude pyroligneous acid derived from oil palm kernel shells, along with its dichloromethane extract, exhibited pronounced antifungal activity against *Aspergillus niger* and *Botryodiplodia theobromae*, primarily due to the presence of phenolic compounds and their major derivatives (Mahmud *et al*., 2016).Consistent with our findings, presence of high phenolic content along with aldehydes, carboxylic acids and furans are the major constituents responsible for the strong antifungal property exhibited by the pyrolysis bio-oils from various biomass residues.

CONCLUSION

A pronounced difference in pyrolysis product such as bio-oil, syn gas and char were found among the biomass residues. Corn-cob biomass yielded the highest proportion of bio-oil with 19.6% by weight followed by pine needles (18.9%), rice straw (13.8%) and bamboo leaves (13.5%). The *in vitro* antifungal assay suggested all BOBF displayed strong dose responsive inhibition against *A.brassicae* and *B. cinerea*. Complete inhibition of fungal growth was observed across all BOBF at concentrations of 600 and 700 ppm for *Alternaria* and at 400 ppm for *Botrytis*. Corn-cob BOBF consistently showed most effective antifungal response, recording the highest percent inhibition of 87.71 at day 7 and 85.02at day 10 at 500 ppm and complete inhibition at 600ppm post inoculation against *A.brassicae*. Similarly, against *B. cinerea*, corn cob BOBF was found superior to others with complete inhibition of fungal growth at day 4 and 91.05% at day 7 at 400 ppm concentration post inoculation. These findings highlight the critical role of feedstock selection for optimising both, pyrolysis yield and biocidal potential.Collectively the outcomes position bio-oils as a promising alternative to conventional chemical fungicides, offering an eco-friendly, non-toxic, and carbon neutral solution. Owing to its sustainable origin and broad-spectrum antifungal efficacy, bio-oil holds significant potential for further value addition and the development of bio-oil based fungicidal formulations.

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