CONTENT ANALYSIS AND ANTIMICROBIAL ACTIVITY OF PYROLYSIS LIQUID OBTAINED FROM OAK RESIDUE (*QUERCUS SP.*)

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Abstract. The search for organic pesticides continues due to the adverse effects of chemical pesticides used against plant pathogens such as bacteria, and fungi. The evaluation of agricultural and forestry residues within this framework is of vital importance from many perspectives. This study was conducted to determine the activity of pyrolysis liquid (PL) prepared at different concentrations ranging from 1% to 25%, v/v against certain plant pathogenic bacteria and fungi. The PL was obtained from a company that produces charcoal through carbonization at temperatures ranging from 100 to 550 °C. The study was carried out *in vitro* under a Randomized Plot Design with four replications. The content analysis of PL was determined using solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS). The detection of antifungal activity was performed using the Poisoned Food Method on Malt Extract Agar, while antibacterial activity was determined using the Agar Well Diffusion Method on Mueller-Hinton Agar. Statistical analyses of the obtained datasets (factorial ANOVA) indicated that both the statistical significance of the concentration (P=0.001) and its practical importance (effect size=0.9953) were significant. It was determined that concentrations of PL at 2% and above (v/v) completely inhibited fungi populations. It is suggested that the PL used in this study has potential antifungal activity and can be utilized for various purposes, particularly in plant protection activities.
Keywords: *biopesticide, circular economy, plant biomass*

Introduction

Cultivated plants are subjected to qualitative and quantitative losses due to various diseases and pests (Tudi et al., 2021). Among these pests, pathogenic fungi can cause yield losses of up to 14% annually, posing a significant problem for global food security and agricultural sustainability (Anand and Rajeshkumar, 2022). Pathogenic fungi of the genus Fusarium are responsible for significant yield losses in small-grain cereals and corn worldwide (Ferrigo et al., 2016). Verticillium species also cause vascular wilting in plants (Klosterman et al., 2009). Similarly, phytopathogenic bacteria pose a risk to global food production (Martins et al., 2018; Sánchez-Hernández et al., 2023) and result in economic losses (Martins et al., 2018). Currently, synthetic pesticides are widely used as part of pest control strategies (Khan et al., 2023). However, these chemicals can have significant adverse effects, particularly on non-target organisms and the environment (Arora and Sahni, 2016; Kowalska, 2021; Tudi et al., 2021). Therefore, in addition to controlling pesticide pollution and its negative impacts on environmental and non-target organisms, the development of biopesticides is necessary (Tudi et al., 2021). Pyrolysis liquid (PL), produced from the pyrolysis of biomass, has shown biopesticide activity in numerous studies (Koç et al., 2017, 2018, 2024; Öğün and Koç, 2019; Koç, 2019; Guo et al., 2019; Oramahi et al., 2021; Yin et al., 2023; Sivaram et al., 2024). PL is a liquid with a characteristic smoky odor, dark brown color, and an elemental composition similar to biomass (Kan et al., 2016; Uddin et al., 2018; Jerzak et al., 2024). This liquid is also

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referred to as bio-oil, crude bio-oil, pyrolysis oil, wood oil, bio-slurry, pyrolytic tar, pyroligneous liquor, wood liquid, wood distillate, pyroligneous tar, and smoke condensate (Zhang et al., 2007; Uddin et al., 2018). Composed largely of water, PL contains many chemical components, including acetic acid, phenol, furan, esters, and ketones (Mahmud et al., 2016; Omulo et al., 2017; Theapparat et al., 2018; Ray et al., 2022; Feng et al., 2023; El-Fawy et al., 2023). In a sample study using GC-MS for the chemical composition of PL (Liu et al., 2018), 48 organic compounds were identified. In the analysis of PL produced from *Litchi chinensis* using GC-MS, a total of 17 chemical compounds were identified, representing 83.96% of the compositions (Yang et al., 2016). PL produced from *Coptis chinensis* at various temperatures through slow pyrolysis up to 400 °C showed the presence of over 70 different oxygenated organic compounds, including acids, alcohols, aldehydes, catechols, esters, furfural, carboxylic acids, and ketones (Yin et al., 2023). One of the studies conducted on PL's effects on plant pathogenic bacteria and fungi was by Koç et al. (2017), which found that PL produced from broiler chicken bedding exhibited antifungal activity against *Aspergillus niger* and *Penicillium digitatum*. Another study on antifungal activity showed that PL produced from hazelnut shells was effective against *A. niger* and *P. digitatum* (Koç et al., 2018). Yin et al. (2023) reported that PL produced from *Coptis chinensis* at various temperatures exhibited antifungal effects against *Trichoderma viride, Aspergillus niger*, and *Penicillium*. Guo et al. (2019) indicated that PL had fungistatic effects on *Setosphaeria turcica*. Oramahi et al. (2021) observed the highest inhibition effect of PL produced from empty fruit bunches against *Phytophthora citrophthora* at 450 °C. Öğün and Koç (2019) examined the activities of PLs produced from broiler chicken bedding and hazelnut shells against certain plant pathogenic bacteria (*Erwinia amylovora, Pseudomonas syringae* pv. lachrymans, and *Xanthomonas axonopodis* pv. phaseoli). As a result, it was reported that the studied pathogenic bacteria were sensitive to the vinegar produced from hazelnut shells, but resistant to other vinegars. In summary, it is observed that plant residues contain many biologically active substances, which can be extracted through pyrolysis. However, parameters such as raw material type, pyrolysis temperature, pressure, and reactor type affect the structure of PL (Okolie et al., 2022; Mong et al., 2022; Roy et al., 2023). There is a significant amount of biomass on Earth, such as agricultural and forestry residues, which can lead to environmental pollution. The conversion of this biomass into value-added products like biopesticides constitutes the main theme of the circular economy. Literature searches indicate that there is a substantial amount of oak residues, and the effects of PL produced from this raw material on plant pathogenic bacteria and fungi have not been investigated. In Sakarya Province (Türkiye), a commercial company producing charcoal from oak residues has been observed to generate tons of PL as a by-product. Investigating the chemical composition and antimicrobial activity of this product is of great importance in many aspects. In this context, the present study investigates the content analysis and antimicrobial activity of PL produced from oak residues (*Quercus sp.*) through slow pyrolysis.

Materials and methods

PL

PL was obtained from a commercial company producing charcoal through slow pyrolysis of oak residues (*Quercus sp.*) in Sakarya Province, Türkiye. PL is produced as a by-product from the condensation of vapors and smoke released during the

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carbonization of biomass at temperatures ranging from 100 to 550 °C. The raw PL obtained from this company was collected from the top of plastic barrels after being stored for one year, allowing the tar to settle at the bottom.

Procurement of test microorganisms

The plant pathogenic bacterial isolates used to determine antibacterial activity (*Clavibacter michiganensis* subsp. michiganensis, *Pseudomonas syringae* pv. syringae, *Erwinia amylovora*, and *Xanthomonas euvesicatoria*) and fungi (*Fusarium oxysporum* and *Verticillium dahliae*) were obtained from the Department of Plant Protection, Faculty of Agriculture, Van Yüzüncü Yıl University, Türkiye.

Content analysis of PL by GC-MS

In the identification of aromatic compounds, a gas chromatograph model "Shimadzu GC-2010 Plus" and the associated mass spectrometer "Shimadzu GCMS-QP2020" were utilized. The separation of aromatic compounds was performed using a DB-HeavyWax column (60 m x 0.25 mm x 0.25 μm). The injection temperature was set to 250 °C, and the oven temperature was initially set to 40 °C, then increased by 3 °C per minute until reaching 80 °C. At this temperature, it was held for 1 minute, and then increased by 5 °C per minute until reaching 240 °C, where it was maintained for 6 minutes. Helium was used as the carrier gas, with a flow rate of 1.05 mL/min. A sample of 2 mL was taken into a 20 mL vial. After placing the fiber in the vial at 45 °C, adsorption was achieved from the headspace for 50 minutes. The fiber used was 50/30 μm, 2 cm in length, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, Supelco Inc., USA) (Genovese et al., 2015; Korkmaz, 2023).

Determination of antimicrobial activity

Antifungal activity

The antifungal activities of PL were determined using the Poisoned Food Method reported by Balouiri et al. (2016). For this purpose, Malt Extract Agar (Merck, Germany) plates containing PL at concentrations of 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, and 25% (v/v) were prepared. Sections with a diameter of 10 mm were transferred from cultures of *Fusarium oxysporum* and *Verticillium dahliae* onto the prepared Petri plates. Additionally, sections with a diameter of 10 mm from positive control cultures of *F. oxysporum* and *V. dahliae* were transferred onto Malt Extract Agar plates containing 50 μL of cycloheximide (prepared by dissolving in ethanol to a stock solution of 35 mg/mL). Malt Extract Agar was used as a negative control. After inoculation, the Petri dishes were incubated at 27 °C for 7 days. The study was conducted in a Randomized Plot Design with four replications. Following incubation, the fungi in the Petri dishes were examined, and the colony diameters were measured and recorded. The percentage of mycelial growth inhibition was calculated using the formula below (Yahyazadeh et al., 2008).

$$MGI\ (\%) = [(C - T)/C] \times 100 \qquad \text{(Eq.1)}$$

where, MGI (%) is the percent mycelial growth inhibition, C the colony radius of the pathogen when growing without PL (control); T the colony radius of the pathogen for each PL concentration.

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Antibacterial activity

The antimicrobial activity of the PL against plant pathogenic bacteria (*Clavibacter michiganensis* subsp. michiganensis, *Pseudomonas syringae* pv. syringae, *Erwinia amylovora*, and *Xanthomonas euvesicatoria*) was determined using the Agar Well Diffusion Method reported by Balouiri et al. (2016). Accordingly, the turbidity of 24-hour cultures of the pathogenic bacterial strains was adjusted to match the 0.5 McFarland standard (Anonymous, 2019). From the cultures with standard turbidity, 100 µL was added to the surface of Mueller-Hinton Agar (Merck, Germany) medium and spread across the surface using a sterile glass rod. Subsequently, 10 mm diameter wells were created on the surface of the medium using a cork borer. To the wells, 50 µL of stock solutions of PL and diluted solutions of 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, and 25% (v/v) with distilled water were applied. Ciprofloxacin (5 µg, Oxoid, UK) was used as the positive control, and 100 µL sterile Tryptic Soy Broth was used as the negative control. Plates were incubated at 35 °C for 24 h, and inhibition zone diameters were measured in millimeters after incubation.

Statistical analysis

A two-way analysis of variance (ANOVA) was performed to statistically evaluate the data. This analysis assessed the main effects of species (*Fusarium oxysporum* and *Verticillium dahliae*) and application concentration (1% and positive control) on the fungal inhibition rate, as well as the interaction between these factors. Because concentrations of 2% and higher resulted in complete (100%) inhibition of fungal growth in all samples, no variance was observed at these levels; therefore, they were excluded from the statistical analysis. Pairwise comparisons were conducted for factors that showed significant effects according to the ANOVA results. Differences between mean values are indicated by letter groupings based on Tukey's HSD post hoc test ($p < 0.05$). All statistical analyses were performed using the Minitab software package (Version 17) (Winer et al., 1971).

Results

GC-MS analysis

In the content analysis of PL using GC-MS, 146 components were identified. The most important of these components are Propanoic acid, methyl ester (CAS) (10.07%), Benzene, 1-methoxy-3-methyl- (6.75%) and Phenol, 2-methoxy- (3.56%), shown in bold in *Table 1* (*Table 1, Figure 1*).

Antifungal activity

After one week of incubation, the fungal growth in the Petri dishes was visually inspected, and the diameters of the growing colonies were measured in millimeters. No growth was observed in the Malt Extract Agar plates containing PL at concentrations of 2%, 3%, 4%, 5%, 10%, 15%, 20%, and 25% (v/v). In other words, all PL concentrations between 2% and 25% exhibited complete antifungal activity. Growths of *F. oxysporum* and *V. dahliae* were observed only in the plates containing 1% (v/v) PL (*Figure 2*).

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Table 1. *Volatile components of PL*

No	Name	m/z	*R.T.	*R.I.	Area (%)
1	Formic acid, methyl ester (CAS)	31.00	5.455	841	0.05
2	Propanal	29.00	5.580	852	0.07
3	Acetone	43.00	5.940	882	0.50
4	Methanol (CAS)	31.00	7.365	946	3.90
5	Propanoic acid, methyl ester (CAS)	57.00	7.660	958	**10.07**
7	2-Pentanone (CAS)	43.00	9.695	1022	0.79
8	2-Butanone, 3-methyl- (CAS)	43.00	9.895	1026	0.31
9	Butanoic acid, methyl ester (CAS)	74.00	10.000	1029	0.87
10	Acetonitrile (CAS)	41.00	10.655	1044	0.15
11	Butanoic acid, 2-methyl-, methyl ester	88.00	10.765	1047	0.12
12	Methyl isovalerate	74.00	11.115	1055	0.33
13	Acetic acid, 2-propenyl ester (CAS)	43.00	11.585	1066	0.11
14	2-Butenal	70.00	11.885	1073	0.04
15	3-Hexanone	43.00	12.260	1082	0.11
16	Methyl 3-butenoate	41.00	12.470	1086	0.05
17	2,3-Pentanedione (CAS)	43.00	12.765	1093	0.20
18	Acetic acid, butyl ester (CAS)	43.00	13.105	1101	0.05
19	2-Hexanone	43.00	13.400	1107	0.07
20	Methyl valerate	74.00	13.620	1111	0.16
21	2-Butenal, 2-methyl-	84.00	13.985	1118	0.05
22	Tetradeuteromethane	20.00	14.180	1122	0.08
23	2-Butenoic acid, methyl ester, (E)-	69.00	14.540	1128	0.05
24	1-Butanol, 3-methyl-, acetate (CAS)	43.00	15.005	1137	0.03
25	Pentanoic acid, 3-methyl-, methyl ester	74.00	15.320	1143	0.23
26	3-Pentenoıc acid, methyl ester	55.00	17.950	1193	0.20
29	Cyclopentanone	55.00	18.310	1200	0.50
30	Methyl tiglate	55.00	18.660	1208	0.23
31	4-Heptanone, 3-methyl-	43.00	18.805	1211	0.10
32	Cyclohexanone, 3-methyl-, (R)- (CAS)	69.00	19.205	1219	0.09
33	Cyclopentanone, 3-methyl-	69.00	19.490	1225	0.21
34	Cyclopentane, (1-methylethyl)-	68.00	19.665	1229	0.10
35	5-Hexenoic acid, methyl ester	74.00	19.910	1234	0.13
36	1-Pentene, 4,4-dimethyl- (CAS)	57.00	20.235	1241	0.09
37	Furan, 2-(methoxymethyl)-	81.00	20.385	1244	0.06
38	4-Hexenoic acid, methyl ester	55.00	20.465	1246	0.05
39	Pentanenitrile, 4-methyl- (CAS)	55.00	20.715	1251	0.08
40	Pyridine	79.00	20.995	1257	0.10
41	3-Hexen-2-one, 5-methyl-	43.00	21.155	1260	0.02
42	3-Pentenoic acid, 4-methyl-, methyl ester	69.00	21.845	1275	0.03
43	2-Pentenoic acid, 4-hydroxy-	43.00	21.935	1277	0.02
44	Heptanoic acid, methyl ester (CAS)	74.00	22.055	1279	0.14
45	Cyclopentanone, 2-ethyl-	84.00	22.390	1286	0.08
46	Pyridine, 2-methyl- (CAS)	93.00	22.615	1291	0.04
47	2-Butanone, 3-hydroxy- (CAS)	45.00	23.605	1314	0.48
48	2-Propanone, 1-hydroxy-	43.00	24.120	1327	2.83
49	2-Cyclopenten-1-one, 2,3-dimethyl-	67.00	24.675	1341	0.70

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50	Propanoic acid, 2-hydroxy-, methyl ester (CAS)	45.00	24.920	1347	0.07
51	2-Cyclopenten-1-one, 3-methyl-	81.00	25.205	1354	0.08
52	2-Cyclopenten-1-one	82.00	25.780	1369	1.91
53	2-Cyclopenten-1-one, 2-methyl-	67.00	26.630	1390	0.10
54	1-Hydroxy-2-butanone	57.00	26.890	1397	1.71
55	Acetic acid, hydroxy-, methyl ester	31.00	27.040	1400	0.15
56	Acetic acid	43.00	27.285	1408	3.46
57	2H-Pyran-3(4H)-one, dihydro-	42.00	27.845	1424	1.02
58	Benzene, 1-methoxy-3-methyl-	122.00	28.115	1432	**6.75**
59	Vinyl butyrate	43.00	28.875	1454	0.29
60	2-Furancarboxaldehyde (CAS)	96.00	29.250	1465	2.89
61	2-Butanone	43.00	29.795	1481	0.41
62	2-Acetyl-5-methylfuran	109.00	29.975	1487	0.46
63	Formic acid	46.00	30.130	1491	1.11
64	Ethanone, 1-(2-furanyl)- (CAS)	95.00	30.520	1503	1.09
65	2,5-Hexanedione (CAS)	43.00	30.820	1513	0.22
66	2-Furanmethanol, tetrahydro- (CAS)	71.00	30.925	1516	0.27
67	Propanoic acid	74.00	31.080	1522	3.46
68	2-Butanone, 1-(acetyloxy)-	43.00	31.425	1533	0.23
69	2-Cyclohenen-1-one, 3,4-dimethyl-	96.00	31.565	1538	0.78
70	Propanoic acid, 2-methyl-	43.00	32.045	1554	0.39
71	2-Cyclopenten-1-one, 3,4,4-trimethyl-	109.00	32.270	1561	0.09
72	2-Furancarboxaldehyde, 5-methyl-	110.00	32.590	1572	1.34
73	2-Propenal, 3-phenyl- (CAS)	131.00	32.960	1584	0.38
74	2-Cyclopenten-1-one, 3,4,5-trimethyl- (CAS)	109.00	33.135	1590	0.04
75	2(3H)-Furanone, dihydro-3-methyl- (CAS)	41.00	33.360	1597	0.03
76	Benzonitrile	103.00	33.455	1600	0.03
77	2-Cyclohexen-1-one, 6-(1-hydroxy-1-methylethyl)-3-methyl-	82.00	33.545	1604	0.03
78	Butanoic acid (CAS)	60.00	33.775	1612	1.57
79	1,2-Ethanediol, monoacetate (CAS)	43.00	34.135	1625	0.21
80	Cyclohexane, (1-methylethylidene)-	124.00	34.215	1628	0.11
81	Butanoic acid, 4-chloro-	42.00	34.530	1639	0.97
82	Ethanone, 1-phenyl- (CAS)	105.00	34.755	1648	0.17
83	Butanoic acid, 3-methyl- (CAS)	41.00	34.925	1654	0.72
84	3-Fluorobenzoic acid, 6-ethyl-3-octyl ester	131.00	35.295	1667	0.04
85	Benzaldehyde, 2-hydroxy- (CAS)	122.00	35.535	1676	0.24
86	1-Nonene (CAS)	43.00	35.760	1684	0.49
87	2-Butenoic acid, (Z)- (CAS)	86.00	35.930	1690	0.18
88	Benzene, 1,2-dimethoxy-	138.00	36.510	1712	0.33
89	Pentanoic acid	60.00	36.680	1719	0.30
90	2(5H)-Furanone, 3-methyl- (CAS)	41.00	36.755	1722	0.30
91	4-Isopropylcyclohexanone	55.00	37.220	1740	0.12
92	Bis(2,4-dimethylamino)pyrimidine	166.00	37.335	1745	0.05
93	2-Butenoic acid, (E)-	86.00	37.580	1754	0.47
94	2-Butenoic acid, 2-methyl- (CAS)	55.00	37.845	1765	0.09
95	Pentanoic acid, 3-methyl- (CAS)	60.00	38.065	1773	0.06
96	4-Pentenoic acid	55.00	38.475	1790	0.22
97	3,4-Dimethoxytoluene	152.00	38.660	1797	1.78
98	Ethanone, 1-(4-hydroxyphenyl)- (CAS)	121.00	38.770	1801	0.14

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99	1-Allylcyclohexanol	81.00	39.025	1812	0.05
100	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	112.00	39.320	1824	1.43
101	2-Hexenal, 2-ethyl- (CAS)	55.00	39.605	1836	0.19
102	Phenol, 2-methoxy-	109.00	40.090	1856	**3.56**
103	2-Methoxy-5-methylphenol	123.00	40.290	1865	2.21
104	2-Methyl-3(2-furyl)acrolein	79.00	40.490	1873	0.13
105	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	126.00	40.805	1886	0.42
106	Phenol, 2,6-dimethyl-	107.00	40.940	1892	0.80
107	4-Methyl-5H-furan-2-one	69.00	41.110	1899	0.17
108	1-Cyclopentyl-2-propen-1-ol	57.00	41.340	1909	0.07
109	Cyclohexanone, 2-(2-butynyl)-	135.00	41.580	1920	0.09
110	Phenol, 4-ethyl-2-methoxy- (CAS)	137.00	41.720	1926	2.68
111	Creosol	123.00	42.335	1953	2.81
112	1H-Inden-1-one, 2,3-dihydro-2-methyl-	131.00	42.745	1971	0.08
113	Phenol	94.00	42.990	1982	2.49
114	3,4-Dihydroxyacetophenone	137.00	43.195	1991	0.16
115	6-Heptenoic acid	68.00	43.305	1996	0.18
116	Phenol, 2-methoxy-4-propyl-	137.00	43.425	2001	1.49
117	2-Methoxy-4-ethyl-6-methylphenol	151.00	43.665	2012	0.39
118	Octanoic acid (CAS)	60.00	44.180	2035	0.22
119	Phenol, 2-ethyl- (CAS)	107.00	44.380	2043	1.80
120	p-Cresol	108.00	44.885	2066	1.40
121	Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-	149.00	45.180	2079	0.21
122	4-Hydroxy-2,4,5-trimethyl-2,5-cyclohexadien-1-one	137.00	45.295	2084	0.20
123	Phenol, 2-ethyl-4-methyl- (CAS)	121.00	45.800	2106	0.50
124	Phenol, 3-(1-methylethyl)-	121.00	45.935	2112	0.95
125	Phenol, 2,3-dimethyl-	107.00	46.070	2118	0.50
126	Benzene, 1-ethyl-4-methoxy-	121.00	46.400	2133	1.00
127	Phenol, 2-ethyl-4-methyl-	121.00	46.515	2138	0.25
128	2-Hydroxy-gamma-butyrolactone	57.00	47.000	2159	0.44
129	2,4,5-or 2,3,5-Trimethylphenol	121.00	47.345	2174	0.93
130	Phenol, 3,4-dimethyl-	107.00	47.615	2186	0.74
131	Butanoyl chloride	71.00	47.785	2194	0.58
132	Phenol, 4-(1-methylpropyl)- (CAS)	121.00	48.020	2204	0.53
133	Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	164.00	48.495	2225	0.69
134	Phenol, 2,6-dimethoxy-	154.00	48.670	2233	1.23
135	Galactose, 4,6-O-octylidene-	191.00	49.190	2256	0.86
136	Propanoic acid, 2-methyl-, 2-methylpropyl ester	71.00	49.340	2263	0.30
137	1,1-Bis(trİdeuteromethyl)-3-methyl-1-silacyclobutane	78.00	49.655	2276	0.21
138	Pentanoic acid, 4-oxo-	43.00	49.815	2284	0.61
139	Ehylthiophosphonamide, o-methyl ester	78.00	50.045	2294	0.35
140	Butanedioic acid, monomethyl ester	101.00	50.175	2299	0.27
141	Phenol, 4-methoxy-3-(methoxymethyl)-	168.00	50.415	2310	0.63
142	Trichloroacetic acid, dodecyl ester	57.00	50.675	2322	0.36
143	Ethanol, 2,2'-oxybis-, dipropanoate	57.00	51.370	2352	0.16
144	CIS-cyclohexen-3,5-diol	78.00	51.470	2357	0.10
145	Tetrahydropyrrole-3-amino-2,5-dione	78.00	51.950	2378	0.19
146	Benzoic acid	105.00	52.225	2390	0.18

*R.T.: Retention time, *R.I.: Retention index

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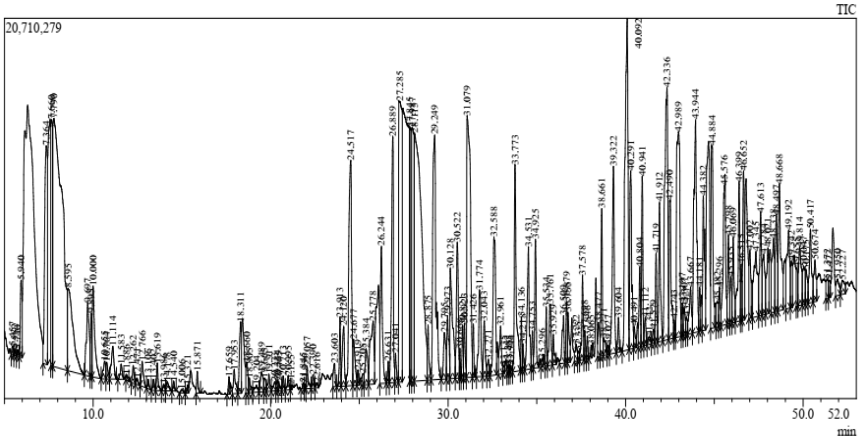

Figure 1. *Volatile components chromatogram of PL*

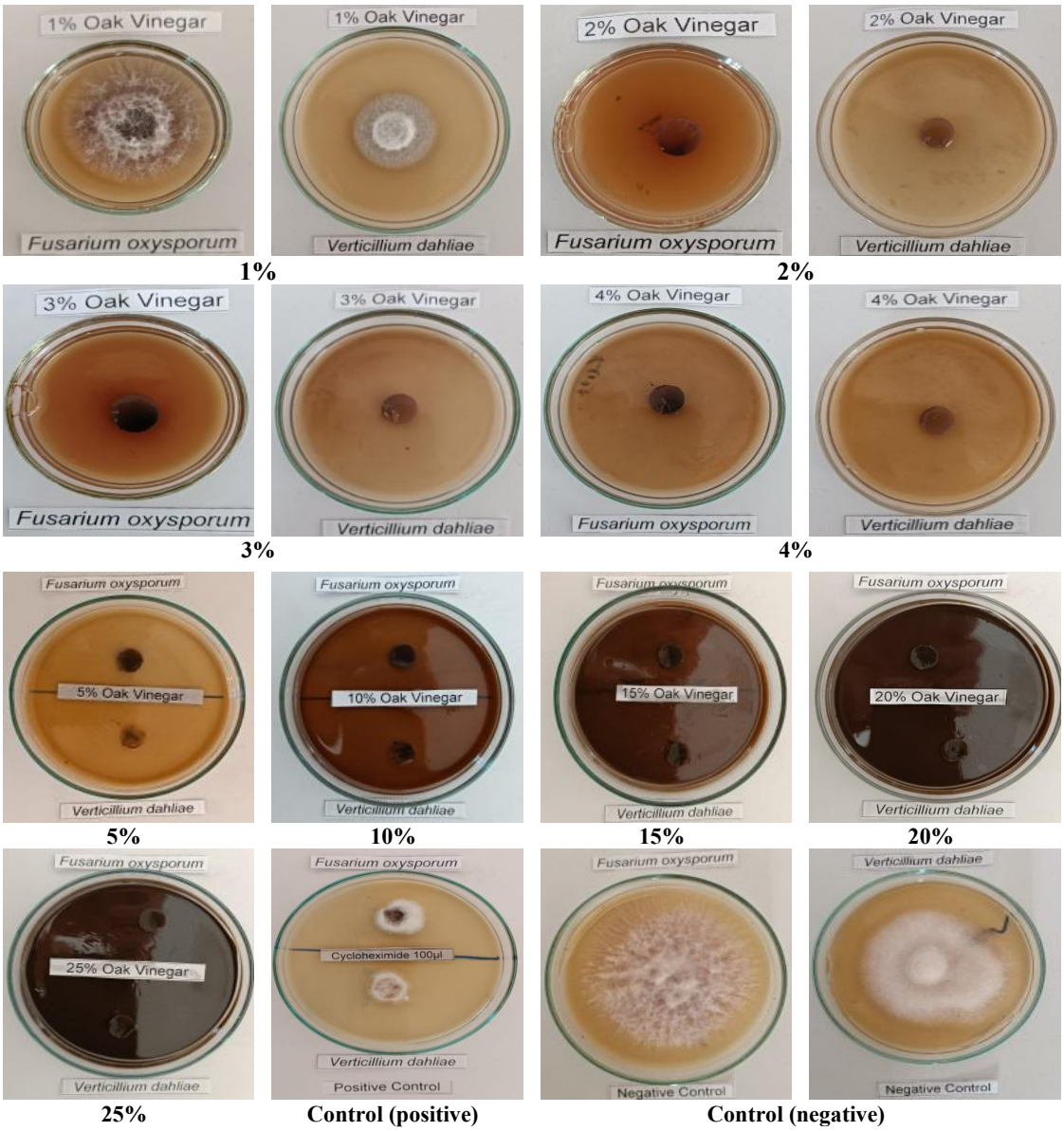

Figure 2. *Sample images of antifungal activity of PL at different concentrations*

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The two-way ANOVA revealed that the concentration factor had a highly significant effect on the inhibition rate ($F(1,12) = 2506.71$, $p < 0.001$). The partial eta squared (η^2) value for the concentration factor was 0.995, indicating that the concentration levels accounted for approximately 99.5% of the total variance in the inhibition rate. This finding clearly demonstrates that the concentration level is the most influential factor affecting the inhibition rate. In contrast, the main effect of the species factor was not statistically significant ($F(1,12) = 2.41$, $p = 0.147$, partial $\eta^2 = 0.001$), suggesting that there was no significant difference in the mean inhibition rates between *Fusarium oxysporum* and *Verticillium dahliae*. Furthermore, the interaction effect between species and concentration was also not significant ($F(1,12) = 3.67$, $p = 0.075$, partial $\eta^2 = 0.003$), indicating that both species responded similarly to different concentration levels (*Table 2*). Since the concentration factor had a statistically significant effect on the inhibition rate, pairwise comparisons are presented in *Table 3*.

Table 2. *ANOVA results for inhibition rate*

Source of Variation	df	SS	MS	F	P-value	Partial η^2
Species	1	2.56	2.56	2.41	0.147	0.001
Concentration	1	2663.08	2663.08	2506.71	0.001 **	0.995
Species × Concentration	1	7.97	7.97	3.67	0.075	0.003
Error	12	12.75	1.06			
Total	15	2686.36				

Table 3. *Pairwise comparison results*

Sample	Application concentrations (v/v)	N	Inhibition rate (mm) (Mean±SE Mean)	CoefVar (%)	Min (mm)	Max (mm)	*Signifiance
Fusarium oxysporum	1% PL	4	57.50±0.51	1.77	56.25	58.75	b
	Control (Positive)	4	85.00±0.51	1.20	83.75	86.25	a
Verticillium dahliae	1% PL	4	60.00±0.58	1.94	58.57	61.42	b
	Control (Positive)	4	84.10±0.45	1.07	82.85	85.00	a

*Means ± SE; different superscripts indicate significant differences, $P < 0.05$

According to the pairwise comparison results, statistically significant differences were observed between the 1% PL treatment and the positive control groups for both *Fusarium oxysporum* and *Verticillium dahliae* species ($p < 0.05$). In both species, the inhibition rates measured at the 1% concentration were found to be significantly lower compared to the control group (*Table 3, Figure 3*).

Antibacterial activity

No antibacterial activity of the PL was observed against the tested bacteria (*Clavibacter michiganensis* subsp. michiganensis, *Pseudomonas syringae* pv. syringae, *Erwinia amylovora*, and *Xanthomonas euvesicatoria*) at different concentrations (v/v). The inhibition zones formed by the positive control (Ciprofloxacin, 5 µg), negative control (Tryptic Soy Broth), and the 10%, 15%, 20%, and 25% (v/v) PL solutions are shown in *Figure 4*.

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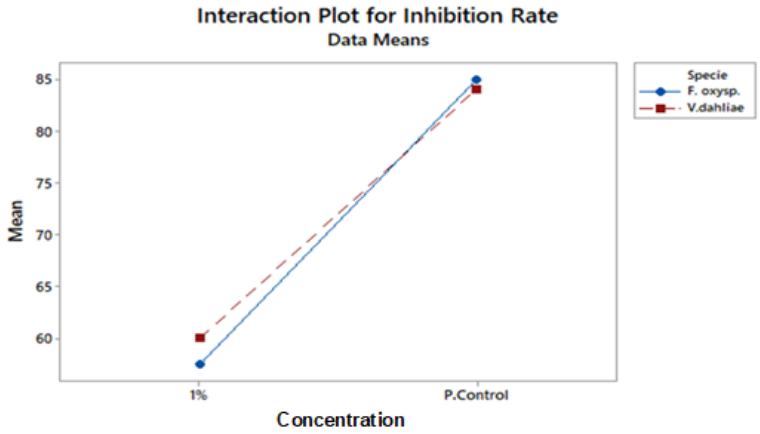

Figure 3. *Interaction plot of species and concentration levels on inhibition rate*

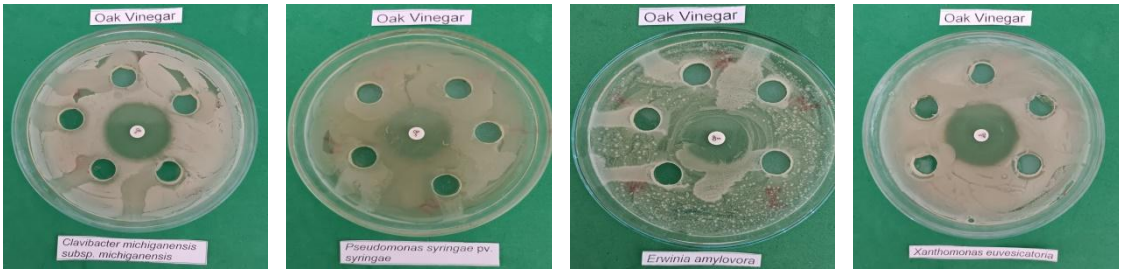

Figure 4. *Sample images of antibacterial activity of PL at different concentrations*

Discussion

The GC-MS (gas chromatography-mass spectrometry) analysis of crude PL produced from the pyrolysis of oak residues identified a total of 146 components, among which Propanoic acid, methyl ester (CAS) (10.07%), Benzene, 1-methoxy-3-methyl- (6.75%), and Phenol, 2-methoxy- (3.56%) were the major ones. Literature reviews related to content analysis have shown that various compounds at different concentrations have been identified in PL (wood vinegar) analyzed by GC-MS. For instance, one study identified 48 organic compounds, including phenols at a concentration of 2.0% (Liu et al., 2018). In a study by Yang et al. (2016), a total of 17 chemical compounds representing 83.96% of the compositions in PL produced from L. chinensis was identified via GC-MS analysis. Yin et al. (2023) reported that PL contains over 70 different oxygenated organic compounds. Ouattara et al. (2023) stated that PL primarily consists of aliphatic, aromatic, and naphthenic hydrocarbons, as well as other oxygenated compounds such as alcohols, aldehydes, ketones, furans, acids, phenols, and ethers. Li et al. (2017) identified 32 major components in PL produced from the shells of Spina date seeds (HSDS) and pea nuts (PS) using GC/MS. With the 100% of the components detection, the content analysis of PL in the current study appears to be richer compared to those of obtained in similar studies.

The crude PL produced from the pyrolysis of oak residues showed no antibacterial activity. However, a literature searches regarding the antibacterial activity of PL revealed numerous studies with both positive (Yang et al., 2016; Öğün and Koç, 2019; Xue et al., 2022; Feng et al., 2023; Chang et al., 2024) and negative (Yang et al., 2016; Öğün and Koç, 2019) results. Öğün and Koç (2019), which investigated the effects of PL on certain

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plant pathogenic bacteria (*Erwinia amylovora, Pseudomonas syringae* pv. lachrymans, and *Xanthomonas axonopodis* pv. phaseoli) indicated that two types of vinegar produced from hazelnut shells and broiler chicken bedding yielded different results at various concentrations and types. Plant pathogenic bacteria were found to be sensitive to the vinegar produced from hazelnut shells but resistant to the vinegar produced from broiler chicken bedding. It has been determined that the findings obtained in the current study are consistent with the effects of vinegar produced from broiler chicken bedding. In another study, Yang et al. (2016) predicted that antibiotic-resistant strains, except for the ornithine-resistant *Staphylococcus aureus* strain, were more sensitive to PL compared to the antibiotic-sensitive strain, which was attributed to its high phenolic composition based on the chemical profile. Xue et al. (2022) reported that PL (WV_{FT}) produced from *Eucommia ulmoides* oliver branches through pyrolysis at 650 °C exhibited antibacterial activity against *Bacterium prodigiosum* (83.33%). The antibacterial activity of crude PL (WVC) produced from the same raw material was fount to be related to its aldehyde and ester content (Feng et al., 2023). In the current study, the lack of activity of PL against bacteria is attributed to factors such as bacterial resistance, differences in cell structure, and the concentration of active components. Such studies are important for the development of biopesticides and the formulation of new strategies in the fight against plant pathogens.

In the present study, the crude PL produced from the pyrolysis of oak residues exhibited excellent antifungal activity by halting the growth and reproduction of fungi at concentrations of 2% and above. Previous studies have mostly reported the antifungal activity of PL rather than its antibacterial activity. For instance, Koç et al. (2017) reported that the application of 10 mL of PL (wv) produced from broiler chicken bedding showed fungicidal effects against *P. Digitatum*, while (Koç et al., 2018) indicated that PL (wv) produced from hazelnut shells exhibited antifungal activity against the same pathogens. Yin et al. (2023) found that PL *from Coptis chinensis* 400 had high total phenolic content and possessed antifungal properties against fungi such as *Penicillium sp., Aspergillus niger*, and *Trichoderma viride*. In this regard, Guo et al. (2019) found that the isolate 11-07D of *Setosphaeria turcica* showed no hyphal growth, and the inhibition rate reached 100% at a PL concentration of 25.25 mg/mL. The pyrolysis temperature and concentration of PL produced from empty fruit bunches (EFB) were found to be significant for inhibiting fungal growth (Oramahi et al., 2021). The highest inhibition for EFB was at a pyrolysis temperature of 450 °C and an average value of 1% PL concentration, resulting in 100% inhibition. The results of similar studies indicated that coconut shell pyrolytic oil inhibited the growth of *Polyporus sanguineus* (L) G Mey by 81.5% and *Tyromyces palustris* (Berk M & A Curtis) by 90% at a concentration of 0.25% (Shiny et al., 2014). This phenomenon was attributed to the high phenolic content of coconut shell pyrolytic oil.

The PL from oak residues exhibited antifungal activity against *F. oxysporum* and *V.* but showed no inhibitory effect on the bacterial pathogens tested. GC–MS analysis identified various organic acids, esters, ketones, aldehydes, furan derivatives, and phenolic compounds (e.g., phenol, cresols, methoxyphenols). The antifungal effect can be attributed mainly to phenolics and organic acids, which disrupt fungal cell membranes and enzymes. In contrast, the resistance of Gram-negative bacteria is likely due to their outer membrane barrier and detoxification systems, combined with the relatively low abundance of active phenolic constituents. These results align with earlier studies

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reporting stronger antifungal than antibacterial effects of PLs, suggesting their potential use as natural fungicidal agents.

The results of current study appear well aligned with those of similar studies reported in the literature. The reason for both overlapping and diverging points can be attributed to the complex structure of the pyrolysis liquid. The pyrolysis liquid is influenced by various factors, including the type and structure of the raw material, the conditions of the pyrolysis process (such as device, temperature, duration, and pressure), as well as experimental factors and the test organisms used. The results of this study and the ones of similar studies reported in the literature clearly indicate that PL has a potential for use in agriculture, health, and food sectors. The utilization of biomass resources based on a circular economy through pyrolysis is expected to yield sustainable (Lu et al., 2020; Xue et al., 2022; Cândido et al., 2023; Yin et al., 2023; Derbali et al., 2024; Chang et al., 2024; Sivaram et al., 2024), high-value-added (Yang et al., 2016; Li et al., 2017; Lu et al., 2020; Yin et al., 2023; Cândido et al., 2023; Derbali et al., 2024; Sivaram et al., 2024), and environmentally friendly products (Koç et al., 2017, 2018; Guo et al., 2019; Lu et al., 2020; Ouattara et al., 2023; Cândido et al., 2023; Sivaram et al., 2024; Derbali et al., 2024) in the future.

Conclusion

In the context of a circular economy, this study has yielded valuable findings regarding the conversion of pyrolysis liquid (PL, wood vinegar), which is produced as a waste by-product during the pyrolysis of oak residues into a value-added product (antimicrobial). In terms of antifungal activity, concentrations of 2% and above (v/v) completely inhibited the growth of pathogens such as *F. oxysporum* and *V. dahliae*. Furthermore, the lack of antibacterial activity in PL indicates the need for a more in-depth investigation of the factors affecting the content and effectiveness of the pyrolysis process of plant waste. The findings of this study highlight the potential of oak residues as a biopesticide and demonstrate that such natural products can be used as alternatives to chemical pesticides in agriculture. Additionally, it has been shown that PL produced from the pyrolysis of oak residues based on a circular economy can be transformed into sustainable, environmentally friendly, and value-added products. Future research is recommended to further investigate the antimicrobial activities of pyrolysis liquids obtained from different raw material sources. Moreover, the optimization of the pyrolysis process should aim to enhance the effectiveness of the products obtained. In this context, the use of biopesticides should be promoted to contribute to the development of sustainable agricultural practices.

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