ANALYSIS OF PYROLYSIS LIQUID FROM GREEN WALNUT SHELLS (*JUGLANS REGIA* L.) AND ITS ANTIFUNGAL ACTIVITY AGAINST PATHOGENIC FUNGI IN ORNAMENTAL PLANTS

KOÇ, İ

Department of Medical Services and Techniques, Vocational School of Health Services, Mardin Artuklu University, Mardin 47200, Türkiye
(e-mail: ibrahimkoc@artuklu.edu.tr; ORCID: 0000-0003-0803-6801)

(Received 16th Apr 2025; accepted 29th Jul 2025)

Abstract. Pathogenic fungi damage ornamental plant production in both quality and quantity. Organic fungicides are significantly needed for the control of these pathogens. This study was carried out to analyze the content of pyrolysis liquid (PL) obtained from green walnut shells (*Juglans regia* L.) and to evaluate its antifungal activity against *Fusarium oxysporum* and *Verticillium dahliae*. This research was carried out under *in vitro* conditions with 5%, 10%, 15%, 20% and 25% (v/v) PL solution applications in a Randomized Plot Design with four replications. The Poisoned Food Method was used to determine the antifungal activity of PL. As a result of chemical content analysis of PL, 53 components were detected by GC-MC, and the major components were propanoic acid, 2-hydroxy- (CAS) (21.69%), e-2-undecenal (7.11%), and nonanal (5.76%). Twelve components were detected by LC-MS/MS, with vanillic acid (393.28 µg/L), vanillin (334.34 µg/L), and syringic acid (222.80 µg/L) identified as the major components. Total phenolic content (201.76 ± 12.50 mg GAE/mL) and total flavonoid content (114.74 ± 4.50 mg QE/mL) were determined. Therefore, the effect of concentration on colony size varied depending on the species. It was determined that PL showed stronger antifungal activity at higher concentrations, with the highest activity observed at the 25% (v/v) solution.
Keywords: *biomass, GC-MC, LC-MS/MS, total phenolic, total flavonoid*

Introduction

Ornamental plant production is a thriving industry in the United States, Canada, Australia, Europe, and South America. This sector is also supported by the plant industries in many developing countries. Quality is paramount in this industry, as it is essential not only for ensuring productivity but also for meeting high aesthetic standards (Gullino et al., 2015). However, during production and storage, these plants can become infected (Lecomte et al., 2016; Kowalska, 2021). Among the most destructive pathogenic microorganisms are the soil-borne fungi *Fusarium oxysporum* (Lecomte et al., 2016) and *Verticillium dahliae* (Kowalska, 2021). Unfortunately, there are currently no effective protection methods to control these pathogens (Lecomte et al., 2016; Kowalska, 2021). Once a disease outbreak occurs, the affected plants are rarely suitable for commercialization (Lecomte et al., 2016). Chemical fungicides are commonly used to control these pathogens (Babcock et al., 1992; Kowalska, 2021). However, many studies have indicated that fungicides can have detrimental effects on the environment and non-target organisms (White, 1984; Potter et al., 1990; Tosun et al., 2001; Arora and Sahni, 2016; Koç et al., 2019; Kowalska, 2021). In summary, great care must be taken in the management of diseases and pests in this sector (Gullino et al., 2015). Therefore, there is a pressing need for human- and environmentally-friendly organic fungicides that consider factors such as quality, efficiency, sustainability, and environmental impact. Wood vinegar (WV) emerges as a promising organic product in the fight against pathogenic fungi (Mela et al., 2013; Koç et al., 2017, 2018, 2019; Guo

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_88818894

et al., 2019; Oramahi et al., 2021; Yin et al., 2023; Sivaram et al., 2024). WV, produced through the pyrolysis of biomass, is also referred to as pyrolysis liquid (PL), bio-oil, and wood distillate (Zhang et al., 2007; Uddin et al., 2018). This product is characterized as a dark brown, almost black liquid with a distinctive smoky odor (Jahirul et al., 2012; Kan et al., 2016; Uddin et al., 2018; Jerzak et al., 2024). Koç et al. (2017) reported that WV produced from broiler chicken litter inhibited the mycelial development of *Aspergillus niger* and *Penicillium digitatum*. In another study, Koç et al. (2018) found that WV from hazelnut shells effectively prevented mold growth. Additionally, research indicated that WV produced from *Coptis chinensis* at various temperatures exhibited antifungal effects against fungi such as *Penicillium spp., Aspergillus niger,* and *Trichoderma viride* (Yin et al., 2023). Guo et al. (2019) identified WV as an effective fungistatic agent against *Setosphaeria turcica*. Lee et al. (2010) demonstrated that WV from *Pinus densiflora* had greater antifungal activity compared to that from *Quercus spp*. Oramahi et al. (2021) evaluated the efficacy of WV produced from empty fruit bunches at different pyrolysis temperatures (350, 400, and 450°C) against *Phytophthora citrophthora* at varying concentrations (0, 0.5, 1.0, and 1.5%, v/v), finding the highest inhibition effect at 450°C. Koç et al. (2017) suggested that testing WV against disease agents could be beneficial. In conclusion, there is a critical need for environmentally friendly, sustainable, economic, and organic fungicides in the production, storage, and protection of ornamental plants. This research was determined to determine the content analysis of PL obtained from green walnut shells (*Juglans regia* L.) and to investigate the antifungal activity of different concentrations (5%, 10%, 15%, 20%, and 25% v/v) against *F. oxysporum* and *V. dahliae.*

Materials and methods

Pyrolysis liquid production (PL)

In this research, the from green walnut shells (*Juglans regia* L.) was pyrolysis in a double cooling PID controlled system (*Fig. 1a*). The pyrolysis process (slow pyrolysis method) was carried out in an inert environment (nitrogen gas supplied system) at nearly 475°C for 2 h. *Figure 1b* shows pyrolysis liquid (PL) produced from green walnut shells.

(a) (b)

Figure 1. *(a) Double condensing PID controlled biomass pyrolysis system. (b) Pyrolysis liquid (PL)*

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DOI: http://dx.doi.org/10.15666/aeer/2305_88818894

Volatile aromatic compound analysis by GC-MS

In the identification of aroma compounds, "Shimadzu GC-2010 Plus" brand gas chromatography and the attached "Shimadzu GCMS-QP2020" mass spectrophotometer were used. The separation of aroma compounds was carried out using DB-HeavyWax column (60 m × 0.25 mm × 0.25 µm). The injection temperature was set at 250 °C and the oven temperature at 40°C, then it was increased by 3°C per min to 80°C and held at this temperature for 1 min, then increased by 5°C per minute to 240°C and held at this temperature for 6 min. Helium was used as the carrier gas (gas flow rate was 1.05 mL/min). 2 mL of the sample was taken into a 20 mL vial. The vial was placed on the fiber at 45°C and adsorption was provided from the headspace for 50 min. The selected fiber was 50/30 µm 2 cm long divinylbenzene carboxene polydimethylsiloxene (DVB/CAR/PDMS, Supelco Inc., USA) (Genovese et al., 2015).

Content analysis by LC-MS/MS

50 mg of sample was taken into a 2 mL Eppendorf tube. It was dissolved by adding 1 mL (mixed solution; acetonitrile-methanol-water (1-1-1)). The samples were vortexed until dissolved. An ultrasonic bath was used for the insoluble sample. Extraction was performed by adding 0.8 mL of hexane to the obtained extract. It was then centrifuged at 7000 rpm for 5 min. It was then taken from the lower phase and diluted 1: 4. In the last stage, LC-MS/MS analysis was performed after filtering with a 0.25 filter. In this process, the Agilent 6460 Triple Quad LC-MS/MS system was used (Atalar et al., 2021).

Total phenolic analysis

Total phenolic content of the extracts was calculated as gallic acid equivalent. TPC values of the samples were determined using the Folin-Ciocalteu method in accordance with the literature. 900 µL of deionized water and 5 mL of 0.2 N Folin-Ciocalteu reagent were added to 100 µL of extract. After waiting for 8 min, 5 mL of Na_2CO_3 solution was added to the mixture, vortexed and kept in the dark for 2 h. UV absorbance values of the mixture were measured at 765 nm and compared with the UV absorbance values of gallic acid standard at different concentrations (Spanos and Wrolstad, 1990; Chlopicka et al., 2012; Capanoglu et al., 2013).

Total flavonoid analysis

Total flavonoid contents were calculated as quercetin equivalents. The extract was placed in a 10 mL flask of known volume. Distilled water was added to make up to 5 mL and 0.3 mL of $NaNO_2$ (1:20) was added. After 5 min, 3 mL of $AlCl_3$ (1:10) was added. After 6 min, 2 mL of 1 mol liter-1 NaOH was added and the total was made up to 10 mL with distilled water. The solution was mixed well again and the absorbance was measured at 510 nm with a UV device (Zhishen et al., 1999; Dewanto et al., 2002).

Antifungal assay

The plant pathogenic fungal isolates used to determine antifungal activity (*Fusarium oxysporum* and *Verticillium dahliae*) were obtained from the Department of Plant Protection, Faculty of Agriculture, Van Yüzüncü Yıl University, Türkiye. The Poisoned Food Method was used to determine the antifungal activities of PL (Balouiri et al., 2016). For this purpose, after the autoclave stage, PL was added to Malt Extract Agar (MERCK)

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at concentrations of 5%, 10%, 15%, 20%, and 25% (v/v) and distributed into Petri dishes. Malt Extract Agar plates containing 50 µL of cycloheximide (stock solution prepared by dissolving at a concentration of 35 mg/mL in ethanol) were prepared as positive controls, while Malt Extract Agar plates without cycloheximide and PL served as negative controls. Circular sections with a diameter of 10 mm taken from *F. oxysporum* and *V. dahliae* cultures were transferred to all Petri dishes. After inoculation, petri dishes were incubated at 27°C for 7 days. The study was carried out in a Randomized Plot Design with 4 replications. Following incubation, fungi in the petri dishes were checked and colony diameters were measured and recorded. The percentage of inhibition of mycelium development was calculated using the following formula (Daouk et al., 1995; Yahyazadeh et al., 2008):

$$MGI\ (\%) = [(C - T) / C] \times 100$$

MGI (%) is the percent mycelial growth inhibition, C the colony radius of the pathogen when growing (negative control); and T the colony radius of the pathogen for each PL concentration.

Statistical analysis

Two-way analysis of means (ANOM) and two-way analysis of variance (ANOVA) were used in analyzing data (Mendeş and Yiğit, 2013; Genç and Mendeş, 2022). All statistical analyses were permed by using Minitab (ver. 17) statistical package program.

Results

Aroma compounds identified by GC-MS

In the GC-MS analysis of PL, 100% was elucidated and 53 components were detected. Among the detected components, propanoic acid, 2-hydroxy-(CAS) (21.69%), e-2-undecenal (7.11%) and nonanal (5.76%) were found to be the major components (*Table 1*; *Fig. 2*).

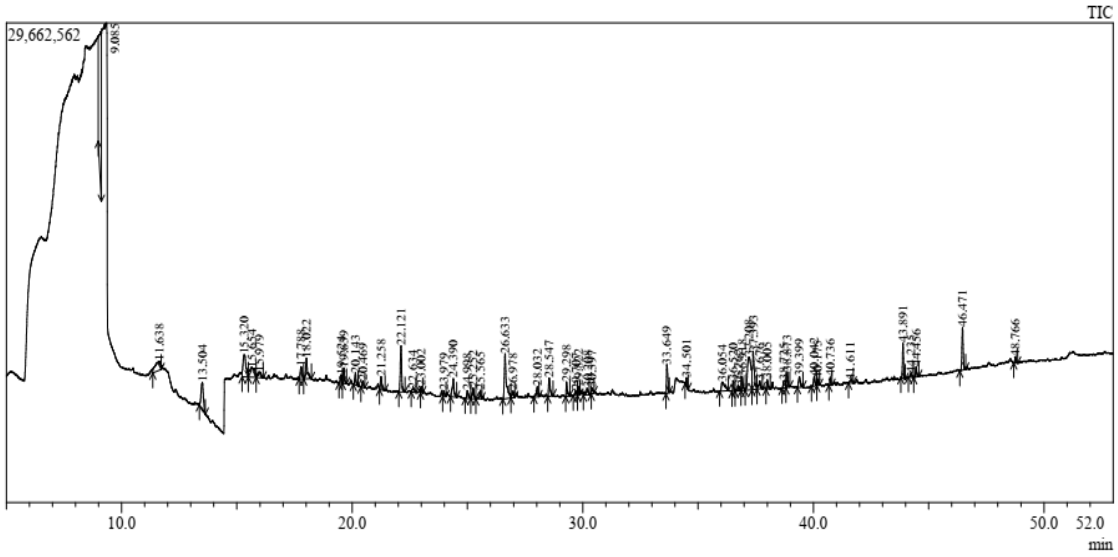

Figure 2. *Volatile components chromatogram of PL*

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Table 1. *Volatile components of PL*

No	Retention time	Retention index	Name	Area (%)
1	7.950	969	Hydrazine	-
2	8.095	974	Hydrazinecarboxylic acid, ethyl ester (CAS)	-
3	8.985	1005	Ethanol, 2-methoxy-	-
4	9.085	1007	Propanoic acid, 2-hydroxy- (CAS)	**21.69**
5	9.155	1009	Dimethyl ether	-
6	9.315	1013	Ethanol	-
7	11.305	1059	Benzene, methyl- (CAS)	-
8	11.640	1067	1-butyl-2-methylcyclopentene	2.96
9	13.505	1109	Undecane	3.69
10	15.115	1139	1-Decene, 2-methyl-	-
11	15.320	1143	1-Dodecene	4.13
12	15.655	1150	o-Xylene	2.90
13	15.980	1156	1-Hexanol,5-methyl-2-(1-methylethyl)-(CAS)	1.00
14	17.785	1190	Benzene, 1,2-dimethyl- (CAS)	1.38
15	18.020	1195	Dodecane (CAS)	2.27
16	19.525	1226	Benzene, 1-ethyl-2-methyl-	0.81
17	19.640	1228	Benzene, 1-ethyl-3-methyl-	1.41
18	20.145	1239	1-Tridecene	0.97
19	20.470	1246	Benzene, 1,2,3-trimethyl- (CAS)	0.53
20	21.260	1262	Benzene, 1-ethyl-2-methyl-	1.12
21	22.120	1280	Benzene, 1,2,3-trimethyl- (CAS)	3.92
22	22.635	1291	Tridecane	0.49
23	23.000	1299	Benzene, 1-methyl-4-propyl-	0.40
24	23.980	1323	Cyclohexasiloxane, dodecamethyl-	0.42
25	24.390	1334	Benzene, 1,2,3-trimethyl- (CAS)	2.02
26	25.000	1349	Benzene, 2-ethyl-1,4-dimethyl-	0.71
27	25.250	1355	Benzene, methyl(1-methylethyl)- (CAS)	1.11
28	25.565	1363	Benzene,1-methyl-3-(1-methylethyl)-	0.71
29	26.630	1390	Nonanal	**5.76**
30	26.980	1399	Ethanol, 2-butoxy-	0.72
31	28.030	1430	Benzene, 1-ethyl-2,3-dimethyl-	1.01
32	28.545	1445	Acetic acid	1.74
33	29.300	1467	Cycloheptasiloxane, tetradecamethyl-	0.98
34	29.705	1479	1H-Indene, 1-chloro-2,3-dihydro-	0.44
35	29.820	1482	Benzene, 1-ethyl-3,5-dimethyl-	0.62
36	30.210	1494	Benzene, 1-methyl-2-(2-propenyl)-	0.88
37	30.395	1499	Formic acid	0.47
39	33.650	1607	Cyclooctasiloxane, hexadecamethyl-	2.21
40	34.500	1638	2-Decenal, (E)- (CAS)	0.64
41	36.055	1695	1-Heptadecene	1.82
42	36.520	1713	Heptasiloxane, hexadecamethyl- (CAS)	0.66
43	36.760	1722	Pentanoic acid	0.47
44	36.920	1729	Oxime-, methoxy-phenyl-_	1.09
45	37.210	1740	E-2-undecenal	**7.11**
46	37.395	1747	Cyclononasiloxane, octadecamethyl-	4.25
47	37.675	1758	E,E-2,4-dodecadienal	0.89
48	38.005	1771	Cyclotrisiloxane, hexamethyl- (CAS)	0.70
49	38.725	1799	2,4-Decadienal, (E,E)-	1.07
50	38.875	1806	2,4-Decadienal, (E,E)-	1.24
51	39.400	1828	Hexanoic acid	1.13
52	40.040	1854	Phenol, 2-methoxy- (CAS)	0.89
53	40.175	1860	Heptasiloxane, hexadecamethyl-	0.48
54	40.735	1883	Octadecamethylcyclononasiloxane	0.41
55	41.610	1921	2(3H)-Furanone, 5-butyldihydro-	0.40
56	43.890	2022	Isopropyl tetradecanoate	2.27
57	44.235	2037	Octanoic acid	0.39
58	44.455	2047	Octanoic acid, decyl ester	0.75
59	46.470	2136	Nonanoic acid (CAS)	3.43
60	48.765	2237	cis-9-Hexadecenal	0.47
Total				**100**

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DOI: http://dx.doi.org/10.15666/aeer/2305_88818894

Compounds identified by LC-MS/MS

In the LC-MS/MS analysis of PL, 33 components were studied and 12 of them were identified. Vanillic acid (393.28 µg/L), vanillin (334.34 µg/L) and syringic acid (222.80 µg/L) were identified as the major components (*Table 2*; *Fig. 3*).

Table 2. *LC-MS/MS analysis of phenolic compounds (µg/L)*

No	Standards	Retention time (min)	Amount
1	Gallic acid	2.227	*ND
2	Epigallocatechin	2.576	ND
3	Chlorogenic acid	2.572	ND
4	Catechin	2.826	ND
5	Gentisic acid	3.082	**107.09**
6	Caffeic Acid	3.321	ND
7	Syringic acid	3.495	**222.80**
8	Vanillic acid	3.628	**393.28**
9	Rutin	2.885	ND
10	Isoquercitrin	4.486	ND
11	Polydatin	4.601	ND
12	Hydroxybenzaldehyde	4.802	**8.67**
13	p-coumaric acid	4.737	**105.13**
14	Sinapic acid	5.406	ND
15	Vanillin	5.375	**334.34**
16	trans-ferulic acid	5.635	**30.62**
17	Taxifolin	5.930	ND
18	Salicylic acid	7.589	ND
19	o-coumaric acid	7.824	ND
20	Baicalin	8.079	ND
21	Protocatehuic ethyl ester	8.353	ND
22	Protocatechuic acid	8.529	**42.62**
23	Kaempferol	9.975	ND
24	Trans-cinnamic acid	10.995	**174.62**
25	Naringenin	11.735	**38.55**
26	Morin	12.077	**35.34**
27	Quercetin	11.839	**33.06**
28	7-Hydroxyflavone	12.113	ND
29	Chrysin	13.619	ND
30	Luteolin	14.042	ND
31	Biochanin A	13.719	ND
32	5-Hydroxyflavone	15.762	ND
33	Diosgenin	20.976	ND

ND: Not detected

Total phenolic and total flavonoid content

The rich phenolic content of PL was calculated as gallic acid equivalent. The flavonoid content was calculated as quercetin equivalent. When the results were examined, it was

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DOI: http://dx.doi.org/10.15666/aeer/2305_88818894

determined that PL had rich phenolic and flavonoid content (*Table 3*). In addition, the phenolic content of PL (201.76 ± 12.50 mg GAE/mL) was found to be higher than the flavonoid content (114.74 ± 4.50 mg QE/mL).

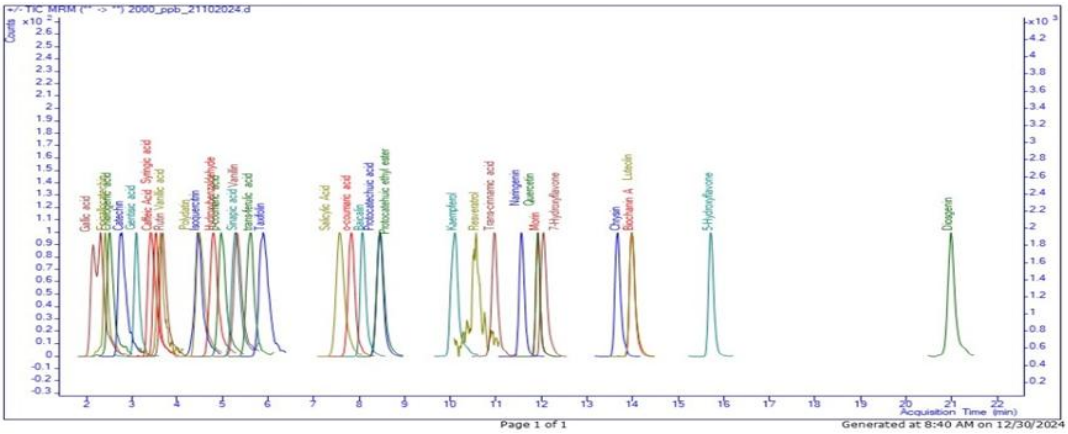

Figure 3. *Chromatogram of the components of PL determined by LC-MS/MS*

Table 3. *Total phenolic and flavonoid content results of PL*

Sample[a]	Total phenolic content[b]	Total flavonoid content[c]
PL	201.76 ± 12.50	114.74 ± 4.50

a: Values are given as the mean and standard deviation of 3 parallel measurements
b: Gallic Acid equivalent phenolic content (y = 0.8391x-0.0145 (R^2 = 0.9945)
c: Flavonoid content equivalent to quercetin (y = 2.2931x-0.0185 (R^2 = 0.9968)

Antifungal activity analysis

After 1 week of incubation, fungal growth in petri dishes was visually checked and the diameters of the colonies were measured in millimeters. It was determined that there was no growth in the positive control and antifungal activity increased with increasing PL concentration. It has been determined that the best activity occurs at a concentration of 25% (*Table 4*; *Figs. 4–6*).

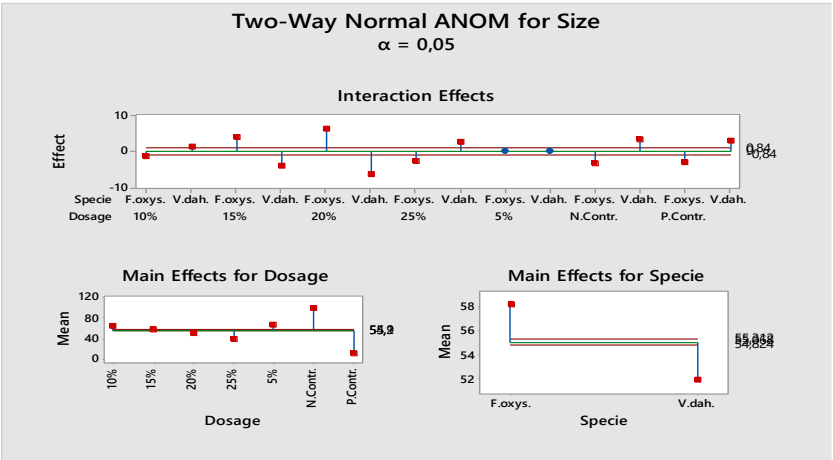

Figure 4. *Results of ANOM for determining the effect of dosage and species on the size*

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DOI: http://dx.doi.org/10.15666/aeer/2305_88818894

Table 4. *Descriptive statistics results in terms of colony diameter*

Sample	Application concentrations (v/v)	$\bar{X} \mp SX$	Inhibition rate (mm)	Min (mm)	Max (mm)
Fusarium oxysporum	5%	69.82 ± 0.73	30.18	68.50	71.80
	10%	66.73 ± 0.61	33.25	65.40	68.00
	15%	63.70 ± 0.51	36.30	62.80	65.00
	20%	59.03 ± 0.34	40.98	58.50	60.00
	25%	38.75 ± 0.78	61.25	37.00	40.50
	Control (positive)	10.00 ± 0.00	100	10.00	10.00
	Control (negative)	99.55 ± 0.31	0.45	98.70	100.00
Verticillium dahliae	5%	63.58 ± 0.41	36.43	62.80	64.50
	10%	62.98 ± 0.41	37.03	62.00	64.00
	15%	49.38 ± 0.26	50.63	48.90	50.00
	20%	39.88 ± 0.35	60.13	39.00	40.70
	25%	37.95 ± 0.55	62.05	37.00	39.00
	Control (positive)	10.00 ± 0.00	100	10.00	10.00
	Control (negative)	99.63 ± 0.26	0.38	98.90	100.00

Two-way analysis of means (ANOM) technique was used in investigating the effect of species and dosage on size values and the results of ANOM was presented in *Figures 4* and *5*.

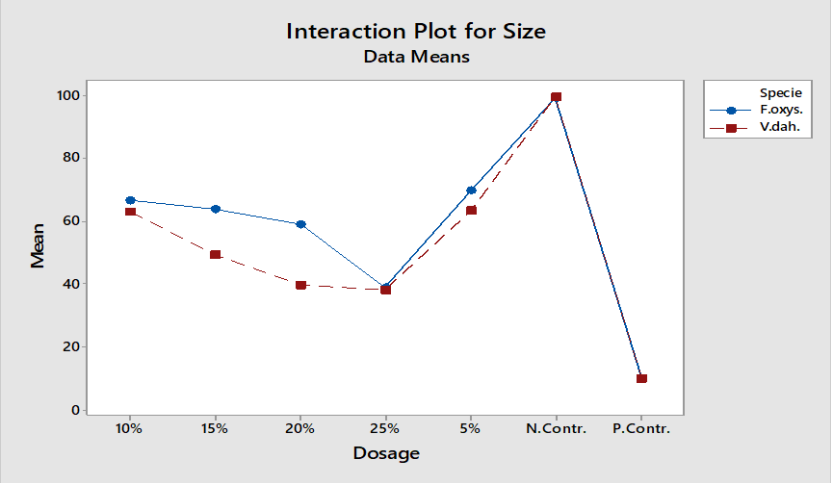

Figure 5. *Dose by species interaction graph*

Results of ANOVA for determining the effect of dosage and species on the size were given in *Tables 5* and *6*, respectively.

When analysis of variance is examined it can be seen that the interaction effect is significant (P = 0.000). As it is expected, the same results have been produced by both ANOM and ANOVA. *Table 6* shows the how much variation in the size can be explained by the two-way ANOVA model. As it can be seen from *Table 6,* almost whole variation can be explained by the model. That means the two-way ANOVA model is a good one for analyzing data set.

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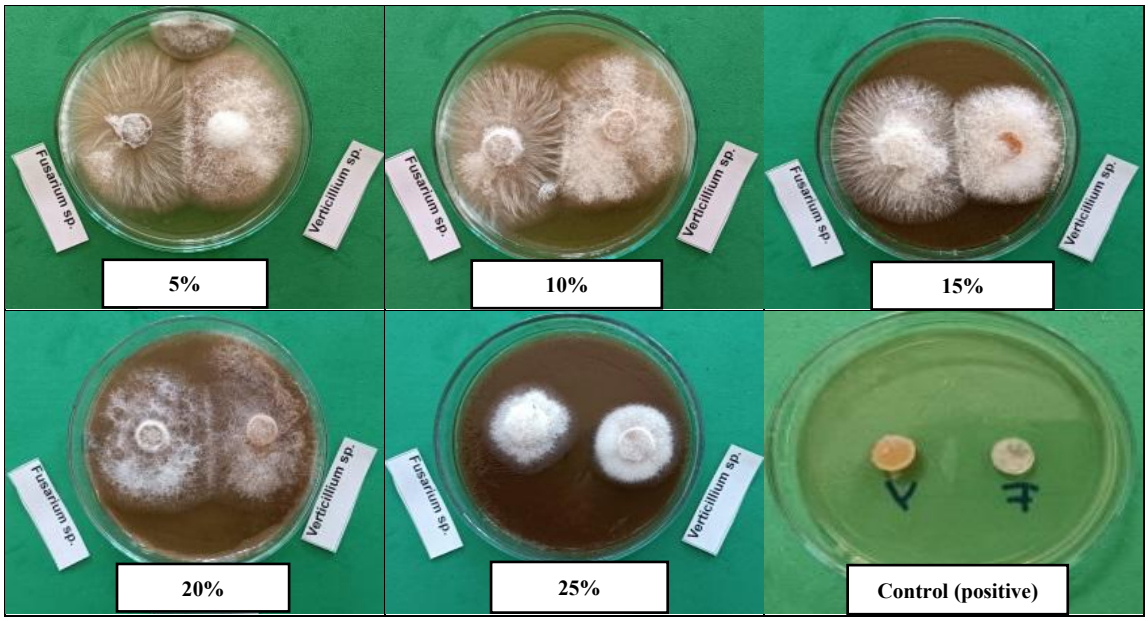

Figure 6. *Sample images showing the effects of PL on F. oxysporum and V. dahliae*

Table 5. *Analysis of variance table*

Source	DF	Adj SS	Adj MS	F-value	P-value
Concentration	7	50676.3	7239.47	10092.79	0.000
Species	1	488.4	488.41	680.91	0.000
Concentration * species	7	763.0	109.00	151.96	0.000
Error	48	34.4	0.72		
Total	63	51962.1			

Table 6. *Model summary*

S	R-sq	R-sq(adj)	R-sq(pred)
0.846931	99.93%	99.91%	99.88%

Discussion

In this study, pyrolysis liquid (PL) produced from green walnut shells through slow pyrolysis (approximately 2 h at 475°C) was analyzed using GC-MS, revealing 53 components. Among these, the major components identified were propanoic acid, 2-hydroxy- (CAS) (%21.69), e-2-undecenal (%7.11), and nonanal (%5.76). Literature searches revealed studies that conducted content analyses of PL produced from various raw materials and temperatures using GC-MS (Yang et al., 2016; Liu et al., 2018; Feng et al., 2023; Başar et al., 2024). One of these studies found high amounts of methyl esters of stearic acid (18.97%), palmitic acid (18.10%), o-xylene (12.17%), and o-ethyl toluene (8.14%) in the PL of walnut shells (Başar et al., 2024). Another study (Feng et al., 2023) reported that wood vinegar (WV) produced at 650°C was refined using various methods, resulting in differing WV compositions based on the refinement method. Yang et al. (2016) identified 17 chemical compounds in the WV obtained from *Litchi chinensis*.

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Among these, 2,6-dimethoxyphenol (syringol, 29.54%), 2-methoxyphenol (guaiacol, 12.36%), and 3,5-dimethoxy-4-hydroxytoluene (11.07%) were expressed as major components. In the research conducted by Liu et al. (2018), 48 organic compounds were identified. In the context of the LC-MS/MS analysis of PL, this study found 12 out of 33 standard compounds investigated. Among these, vanillic acid (393.28 µg/L), vanillin (334.34 µg/L), and syringic acid (222.80 µg/L) were identified as major components. In a similar study (Başar et al., 2024), 2 out of 41 standard compounds investigated (naringenin, diosgenin) were identified. The total phenolic and flavonoid contents of PL were found to be high in the current study. In a similar investigation (Liu et al., 2018), phenolic compounds were identified at a rate of 2.0% in the pyrolysis liquid. Another study (Feng et al., 2023) reported that different distillation methods affected the phenolic content of PL. As a result, it was determined that walnut shell pyrolysis liquid contains a wide variety of phenolic, acid, and ester compounds (Başar et al., 2024). In summary, it was observed that many parameters such as raw material, production temperature, reactor, and pressure significantly affect the composition of the produced PL (Mela et al., 2013; Yang et al., 2016; Feng et al., 2023; Yin et al., 2023; Ouattara et al., 2023; El-Fawy et al., 2023; Başar et al., 2024). In terms of antifungal activity, it was determined that the PL was more effective against *F. oxysporum* and *V. dahliae* with increasing concentrations, with the best activity observed at a concentration of 25 mL (v/v). Literature searches indicated that PL produced from different raw materials, temperatures, and processes has been tested against various plant pathogenic fungi and has shown activity (Koç et al., 2017, 2018; Guo et al., 2019; Faisal et al., 2021; Chairudin et al., 2022; Kara et al., 2024). One of these studies reported that PL produced at various pyrolysis temperatures affected the inhibition of *F. oxysporum* (Faisal et al., 2021). Another study indicated that PL produced from coconut husk exhibited activity against *F. oxysporum* F. sp. lycopersici (Chairudin et al., 2022). PL produced from the pyrolysis of apricot seeds (AKPA), hazelnut shells (HSPA), and kermes oak (OPA) was determined to be effective against *F. proliferatum* (Kara et al., 2024). In a study conducted by Guo et al. (2019), it was reported that WV showed activity against *Setosphaeria turcica* at different concentrations, with a 100% inhibition rate at a concentration of 25.25 mg/mL in the 11-07D isolate. In another study (Lee et al., 2010), it was found that unrefined WV had a more effective inhibition effect compared to refined WV; the highest inhibition effect was observed in *Libertella betulina*, and WV produced from *Pinus densiflora* exhibited higher antifungal activity than that produced from *Quercus spp.* In a study by Oramahi et al. (2021), it was reported that WVs produced from empty fruit clusters (at 350, 400, and 450°C) were effective against *Phytophthora citrophthora*, and that pyrolysis temperature and concentrations were significant for antifungal activity. Yin et al. (2023) stated that WV (CCR 400) had a high total phenolic content and possessed antifungal properties against fungi such as *Penicillium spp., Aspergillus niger,* and *Trichoderma viride*. Rabbi et al. (2017) noted that two types of WV were effective against *A. flavus*, *A. niger*, and *Fusarium spp.*, but *Trichoderma spp.* showed resistance to WV. Feng et al. (2023) stated that although crude WV exhibited weak inhibition against fungi, it showed excellent inhibition in antioxidant and antibacterial tests. In another study (Lee et al., 1992), it was reported that WV did not show activity against *Tyromyces palustris* and *Coriolus versicolor*. Yang et al. (2016) suggested that the strong antioxidant and antibacterial activities of WV, as indicated by chemical analyses, are derived from high phenolic compounds. In conclusion, many studies have indicated that WV possesses antimicrobial, antioxidant, and antifungal effects, and due to these effects, it has potential applications as a biopesticide (Lee et al.,

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2010; Koç et al., 2017, 2018, 2019; Guo et al., 2019; Ayhan and Ayaz, 2022; Sivaram et al., 2024; Kara et al., 2024), as a food preservative (Lee et al., 2010; Mela et al., 2013; Yang et al., 2016), and in chemistry, pharmacy, cosmetics (Mela et al., 2013).

Conclusion

In this study, the pyrolysis liquid (PL) derived from the slow pyrolysis of green walnut shells was subjected to analysis using gas chromatography-mass spectrometry (GC-MS), which identified a total of 53 distinct components. Among the 33 standard compounds analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS), 12 were successfully identified. The findings indicate that PL is characterized by a rich content of phenolic and flavonoid compounds. Furthermore, PL demonstrated significantly enhanced antifungal activity against the plant pathogens *F. oxysporum* and *V. dahliae* at elevated concentrations. Solutions containing 25% or more (v/v) are proposed to be effective against these pathogens and exhibit potential as organic fungicides.

Acknowledgements. I would like to thank Prof. Dr. Mehmet MENDEŞ, Assoc. Prof. Dr. Erdal Öğün, and Assoc. Prof. Dr. Ercan AYDOĞMUŞ for their contributions.

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