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A chemical ecological investigation of the allelopathic potential of *Lamium amplexicaule* and *Lamium purpureum*

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ABSTRACT

The overall goal of the project was to test the hypothesis that *Lamium amplexicaule* and *Lamium purpureum*, weedy invasive species to North America, use phytotoxic allelochemicals in interplant competition. The chemical compositions of the essential oils from the aerial parts of *L. amplexicaule* and *L. purpureum* have been obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The essential oils and several essential oil components have been screened for phytotoxic activity on lettuce (*Lactuca sativa*) and perennial ryegrass (*Lolium perenne*) as well as nematocidal activity against *Caenorhabditis elegans*, brine shrimp (*Artemia salina*) lethality, and insecticidal activity against the red imported fire ant (*Solenopsis invicta* × *richteri*). *L. amplexicaule* essential oil was composed largely of *α*-pinene, *β*-pinene, 1-octen-3-ol, (*E*)-caryophyllene, and germacrene D, while *L. purpureum* oil was dominated by *α*-pinene, *β*-pinene, 1-octen-3-ol, *β*-elemene, and germacrene D. Neither essential oil exhibited notable phytotoxicity or lethality against nematodes, brine shrimp, or fire ants. It is unlikely, therefore, that the allelopathy observed in these *Lamium* species is due to volatile phytochemical constituents.

Keywords: Allelopathy; Essential Oil Composition; *Lamium amplexicaule*; *Lamium purpureum*

1. INTRODUCTION

Both *Lamium amplexicaule* L. (henbit deadnettle) and *Lamium purpureum* L. (purple deadnettle) are non-native annual, weedy, invasive species, originally from Eurasia [1]. Invasion of exotic plant species can present a substantial problem to natural ecosystems as well as croplands and pastures [2,3]. Both *L. amplexicaule* and *L. purpureum* can aggressively spread through fields, pastures, and gardens. *L. amplexicaule* has been shown to reduce the yield of wheat (*Triticum aestivum*) [4], and *L. purpureum* has been shown to reduce the growth of soybeans (*Glycine max*) [5]. In this work, we test the hypothesis that volatile phytochemical components from these members of the mint family (Lamiaceae) are responsible for the reported allelopathic effects; we have determined the chemical compositions and biological activities of the essential oils from the aerial parts of *L. amplexicaule* and *L. purpureum* growing in fields in and near Huntsville, Alabama.

2. MATERIALS AND METHODS

2.1. Plant Material

For each species, plant materials were collected from different sites in and near Huntsville, Alabama. The aerial parts were harvested in the early morning, and the fresh plant materials (aerial parts) were chopped and hydrodistilled using a Likens-Nickerson apparatus to give clear, pale-yellow essential oils (**Table 1**). Plants were identified by Robert O. Lawton, Department of Biological Sciences, University of Alabama in Huntsville.

2.2. Gas Chromatography—Mass Spectrometry

The essential oils of the *Lamium* spp. were analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 40 - 400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Inlet temperature was

Table 1. *Lamium amplexicaule* and *Lamium purpureum* essential oil collection and yields.

L. amplexicaule	#1	#2	#3	#4	#5
Collection site	34˚38'46.44"N, 86˚33'27.00"W, 191 m asl	34˚38'46.41"N, 86˚33'26.30"W, 191 m asl	34˚38'46.44"N, 86˚33'27.00"W, 191 m asl	34°23'53.43"N, 86°59'12.98"W, 403 m asl	34˚43'12.66"N, 86˚38'12.81"W, 196 m asl
Collection date	2 - 18 - 12	2 - 18 - 12	2 - 19 - 11	2 - 26 - 12	3 - 7 - 12
Mass of plant	67.11 g	45.87 g	104.03g	134.07 g	227.83 g
Yield of oil	19.2 mg	33.5 mg	20.1 mg	20.7 mg	34.5 mg
L. purpureum	#1	#2	#3	#4	#5
Collection site	34˚38'46.44"N, 86˚33'27.00"W, 191 m asl	34˚38'46.41"N, 86˚33'26.30"W, 191 m asl	34˚38'46.44"N, 86˚33'27.00"W, 191 m asl	34°23'53.43"N, 86°59'12.98"W, 403 m asl	34°43'16.15"N, 86°38'37.58"W, 194 m asl
Collection date	2 - 18 - 12	2 - 18 - 12	2 - 19 - 11	2 - 26 - 12	3 - 7 - 12
Mass of plant	56.89 g	46.40 g	100.40 g	110.86 g	135.80 g
Yield of oil	27.3 mg	37.2 mg	18.2 mg	25.3 mg	19.3 mg

200˚C and interface temperature was 280˚C. The GC oven temperature program was used as follows: 40˚C initial temperature, hold for 10 min; increased at 3˚C/min to 200˚C; increased 2˚/min to 220˚C. A 1% w/v solution of each sample in dichloromethane was prepared and 1 µL was injected using a 10:1 split ratio.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [6] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The essential oil compositions of the *L. amplexicaule* and *L. purpureum* are summarized in **Tables 2** and **3**, respectively.

2.3. Allelopathic Activity Assays

An allelopathic bioassay based on lettuce (*Lactuca sativa*) and perennial rye grass (*Lolium perenne*) germination and subsequent radicle and hypocotyl growth [7] was measured to study the effects of the *L. amplexicaule* and *L. purpureum* essential oils and essential oil components. Stock solutions of each essential oil (4.0 g/L essential oil and 1.0 g/L Tween-80 in water) were prepared and used for the assays. Two-fold serial dilutions of stock test solutions were prepared to give test concentrations of 4000, 2000, 1000, 500, and 250 µg/mL with the control being 1.0 g/L aqueous Tween-80. Seeds were placed in Petri dishes (60 seeds per dish) each dish lined with two layers of Whatman No. 1 filter paper moistened with test solution and the Petri dishes were sealed with Parafilm®. The dishes were incubated at room temperature in the dark for 5 days, after which the number of germinated seeds was determined and the root (radicle) and shoot (hypocotyl) lengths were measured. The allelopathic assay results are summarized in **Table 4**.

2.4. Brine Shrimp Lethality Assay

The brine shrimp (*Artemia salina*) lethality test was carried out using a modification of the procedure by McLaughlin [8]. *Artemia salina* eggs were hatched in a sea salt solution (Instant Ocean®, 38 g/L) with an incandescent light bulb as the heat source. After 48 hours, the newly hatched nauplii were counted using a micropipette and transferred to 20-mL vials. Nine vials each containing 10 *A. salina* nauplii in 10 mL of sea salt solution (same as the hatching solution) were prepared. Three vials were labeled as controls with first one containing no DMSO, another with 10 µL, and the last one with 100 µL DMSO. Three replicate vials contained 10 µL of 1% essential oil solution in DMSO, and the other three were prepared by adding 100 µL of 1% essential oil solution in DMSO. Surviving *A. salina* were counted after 24 hours.

2.5. Nematocidal Assay

A nematocidal assay using *Caenorhabditis elegans* was carried out using a modification of the procedure of Park and co-workers [9]. Stock solutions of each essential oil (2.0 g/L essential oil and 1.0 g/L Tween-80 in water) were prepared and used for the assays. Dilutions

Table 2. Chemical compositions of *Lamium amplexicaule* essential oils.

RI[a]	Compound	Percent Composition				
		#1	#2	#3	#4	#5
799	Hexanal			0.1		
834	2 - Furaldehyde	tr[b]	0.2	0.2	tr	0.2
856	(2*E*) - Hexenal	0.8		0.3	1.3	1.3
857	(3*Z*) - Hexenol		1.4	0.6		
866	(2*Z*) - Hexenol			0.1		
868	1 - Hexanol			0.1		
900	Nonane			tr		
935	*α*-Thujene	0.9	1.3	6.8	0.2	0.9
941	*α*-Pinene	8.5	11.7	16.2	2.2	8.8
966	Benzaldehyde	0.5	0.5	0.1	0.2	tr
976	Sabinene	tr	tr	2.6	0.1	tr
978	*β*-Pinene	6.2	7.9	10.6	2.0	4.8
982	1 - Octen - 3 - ol	8.0	6.6	3.9	3.5	4.6
992	Myrcene		tr	0.3		
1004	*α*-Phellandrene			0.1		
1028	Limonene	0.3	0.3	0.7	0.3	0.7
1033	Benzyl alcohol	1.3	0.9	1.5	1.4	1.9
1039	(*Z*)-*β*-Ocimene	1.3	1.9	4.2	1.9	2.5
1043	Phenylacetaldehyde	0.6	0.4	0.2	0.5	0.6
1049	(*E*)-*β*-Ocimene	1.1	1.2	1.9	2.2	4.9
1100	Undecane	0.9	0.5	0.5	0.4	0.5
1105	Nonanal	2.1	0.6	0.1	0.3	0.3
1113	2 - Phenylethyl alcohol	1.1	0.6	1.7	1.8	2.2
1131	Alloocimene	0.5	1.1	tr	1.0	1.9
1193	Methyl salicylate	0.4	tr	0.3	0.4	0.5
1200	Dodecane	tr	tr	tr	tr	tr
1206	*n*-Decanal	tr	tr		tr	tr
1292	(2*E*,4*Z*) - Decadienal	tr	tr		0.1	tr
1300	Tridecane	1.0	0.4	0.3	0.3	0.3
1304	Undecanal	tr	tr		tr	tr
1312	*p*-Vinylguaiacol	0.2	tr		tr	tr
1315	(2*E*, 4*E*) - Decadienal	tr	tr		0.2	0.2
1337	*δ*-Elemene	tr	tr		0.2	tr
1350	*α*-Cubebene	tr	tr		tr	tr
1364	(2*E*)-Undecenal	tr	tr		tr	tr

Continued

1371	*α*-Ylangene	tr	tr		tr	tr
1376	*α*-Copaene	0.7	0.6	0.3	0.6	0.6
1384	*β*-Bourbonene	0.9	0.9	0.3	1.3	1.7
1390	*β*-Cubebene	0.3	0.3	0.1	0.3	tr
1393	*β*-Elemene	2.2	1.6	0.4	2.1	2.3
1400	Tetradecane		tr			tr
1411	Dodecanal	tr	tr		tr	tr
1419	(*E*) - Caryophyllene	8.6	8.9	2.5	11.9	9.6
1430	*β*-Copaene	3.3	3.1		4.1	4.2
1435	*γ*-Elemene	5.6	5.0	0.1	1.5	1.8
1445	Aromadendrene	1.3	1.2		1.5	1.6
1454	*α*-Humulene	3.5	3.6	1.4	4.8	5.0
1458	(*E*)-*β*-Farnesene	0.4	0.3	0.2	1.8	1.4
1461	Alloaromadendrene	tr	0.4	0.3	4.8	1.3
1465	*cis*-Muurola - 4 (14), 5 - diene	1.1	1.2		0.1	tr
1483	Germacrene D	26.7	28.0	33.0	34.9	18.5
1493	*trans*-Muurola - 4(14), 5 - diene	tr	tr		tr	tr
1496	*cis*-Cadina - 1, 4 - diene		tr		tr	tr
1498	Bicyclogermacrene			0.2		
1500	*γ*-Amorphene	1.8	1.8		2.2	2.4
1500	Pentadecane	1.4	tr	0.1		
1502	*α*-Muurolene		tr		tr	
1505	Germacrene A			tr		
1511	(*E*,*E*)-*α*-Farnesene	1.2	1.6	0.8	1.8	1.6
1515	*γ*-Cadinene	tr	tr		tr	0.4
1525	*δ*-Cadinene	0.7	0.8	0.3	1.2	1.2
1533	*trans*-Cadina - 1, 4 - diene	tr	tr		tr	tr
1538	*α*-Cadinene	tr	tr		tr	0.1
1557	Germacrene B	0.5	tr	3.7	tr	0.2
1562	1 - *nor*-Bourbananone	tr	tr		tr	tr
1577	Germacrene D - 4 - ol	tr	tr	0.7	0.7	0.8
1643	*τ*-Muurolol	tr	tr		tr	0.6
1646	*α*-Muurolol (=Torreyol)	tr	tr		tr	tr
1656	*α*-Cadinol	tr	0.6	0.3	0.9	1.2
1689	Shyobunol			0.1		
1700	Heptadecane				tr	0.1
1716	Pentadecanal	tr	tr		tr	tr

Continued

1738	Mint sulfide	tr	tr		tr	tr
1845	Phytone	0.7	0.7	0.3	0.6	0.8
1900	Nonadecane	tr	tr		tr	0.2
1921	Methyl palmitate	tr	tr		tr	tr
1958	Palmitic acid		tr		tr	1.0
2100	Heneicosane					0.2
2106	(*E*) - Phytol		tr	0.3		0.5
2300	Tricosane	tr	tr	0.2	0.6	0.8
2400	Tetracosane	tr				tr
2500	Pentacosane	0.6	0.4		0.6	1.0
2600	Hexacosane	tr	tr		tr	tr
2700	Heptacosane	0.6	0.5	0.3	tr	0.6
2800	Octacosane					tr
2900	Nonacosane	1.5	0.8	0.6	0.8	0.8
	Monoterpene hydrocarbons	18.8	25.4	43.4	9.9	24.5
	Sesquiterpene hydrocarbons	58.8	59.5	43.6	75.3	53.7
	Oxygenated sesquiterpenoids	tr	0.6	1.0	1.7	2.6
	Diterpenoids	0.7	0.7	0.5	0.6	1.3
	Benzenoid Aromatics	4.0	2.5	3.8	4.4	5.2
	Fatty-acid-derived Compounds	17.0	11.2	7.1	8.1	11.7
	Others	tr	0.2	0.2	tr	0.2
	Total Identified (86)	99.3	100.0	99.7	100.0	99.3

[a]RI = "Retention Index" determined with respect to a homologous series of normal alkanes on an HP - 5 ms column; [b]tr = "trace" (<0.05%).

of the sample solution were prepared in sterile water solution beginning with 50 µL of the 1% essential oil solution plus 50 µL sterile water. The sample solution was serially diluted (1:1) with sterile water in a 96-well plate. Into each well, 10 - 30 *C. elegans* (mixtures of juvenile and adult nematodes, male: female: juvenile ~1:1:2) per 50 µL of sample solution. Sterile water and serially diluted DMSO were used as controls. The dead and living nematodes were counted after 24 h under a microscope. Dead nematodes were identified by their immobility, and straight body, even after transfer to clean water.

2.6. Fire Ant Insecticidal Assay

Worker red imported fire ants, probably *Solenopsis invicta* × *richteri* hybrid [10], were collected from the University of Alabama in Huntsville. Sample solutions of 2000, 1000, 500, and 250 µg/mL were prepared in 1% aqueous Tween-80® solution. The control was 1% Tween solution. Each assay was carried out in triplicate using a 40-mL vial, fitted with a filter paper disk on the bottom. The filter paper was sprayed with 100 µL of sample solution, the walls of each vial were dusted with talcum powder to keep the ants from climbing out, and then 10 fire ant workers were transferred into each. The mortality of fire ants was recorded after 24 h. The bioassay was carried out in room temperature.

2.7. Statistical Analysis

Calculations were carried out using Excel. Student's *t*-test [11] was used to compare radicle and hypocotyl test means with controls. Seed germination IC_{50} and *A. salina* LC_{50} values were determined using the Reed-Muench method [12].

3. RESULTS AND DISCUSSION

The essential oils of *L. amplexicaule* were dominated by sesquiterpene hydrocarbons, notably germacrene D (18.5% - 34.9%) and (*E*)-caryophyllene (2.5% - 11.9%),

Table 3. Chemical compositions of *Lamium purpureum* essential oils.

RI[a]	Compound	Percent Composition				
		#1	#2	#3	#4	#5
835	2 - Furaldehyde	tr[b]	tr	tr	0.1	0.1
855	(2*E*) - Hexenal					tr
859	(3*Z*) - Hexenol	0.9	1.7	0.9	0.7	0.3
904	Heptanal				0.1	tr
936	*α*-Thujene					tr
941	*α*-Pinene	4.1	9.2	15.3	10.6	4.4
963	Benzaldehyde					tr
975	Sabinene					tr
978	*β*-Pinene	5.8	9.6	16.3	9.2	6.3
981	1 - Octen - 3 - ol	4.2	12.0	15.1	15.3	4.8
990	Myrcene	tr				0.1
995	3 - Octanol					tr
1022	*p*-Cymene			tr		
1025	Limonene	tr	0.2	0.4	tr	0.2
1032	Benzyl alcohol	tr	0.2	0.3	0.4	0.4
1036	(*Z*)-*β*-Ocimene	0.9	0.8	0.7	0.8	0.8
1040	Phenylacetaldehyde	tr	0.3	tr	0.3	0.1
1046	(*E*)-*β*-Ocimene	0.2	0.6	tr	0.4	0.2
1056	*γ*-Terpinene			tr	tr	
1067	(2*E*) - Octen - 1 - ol		tr	tr	tr	tr
1070	1 - Octanol			0.9	0.4	0.1
1089	*o*-Guaiacol					tr
1090	Terpinolene				tr	
1098	Linalool		tr	tr		tr
1102	Nonanal	tr	0.7	0.3	tr	tr
1109	2 - Phenylethyl alcohol	0.3	1.2	1.8	2.0	1.4
1128	Alloocimene		0.6	0.4	0.5	tr
1152	(2*E*,6*Z*) - Nonadienal				tr	
1177	Terpinen - 4 - ol				tr	tr
1189	*α*-Terpineol			tr	tr	tr
1192	Methyl salicylate	tr	tr	0.5	0.2	0.2
1205	Decanal		tr	tr	tr	tr
1213	(2*E*,4*E*) - Nonadienal				tr	
1219	2,3 - Dihydrobenzofuran				tr	tr
1229	*m*-Cumenol		tr	tr		

Continued

1239	Cuminaldehyde			2.2		
1283	*α*-Terpinen - 7 - al			1.7		
1290	*γ*-Terpinen - 7 - al			0.5		
1291	*p*-Cymen - 7 - ol			0.9		
1292	Indole + 1 - Tridecene		tr		tr	tr
1293	(2*E*,4*Z*) - Decadienal				tr	
1300	Tridecane	tr	tr	tr	tr	tr
1306	Undecanal		tr	tr	tr	
1312	*p*-Vinylguaiacol		tr	tr	0.2	0.2
1316	(2*E*,4*E*) - Decadienal				tr	
1324	Myrtenyl acetate			tr		
1326	2 - Methoxy - 5 - vinylphenol				tr	0.2
1327	*p*-Mentha - 1, 4 - dien - 7 - ol			0.4		
1339	*δ*-Elemene	tr	0.4	0.4	0.3	0.2
1358	Eugenol			tr	tr	tr
1375	*α*-Copaene	0.5	0.7	0.5	0.5	0.5
1385	*β*-Bourbonene	0.7	1.4	0.6	1.3	1.0
1390	*β*-Cubebene	tr	tr	tr	tr	tr
1393	*β*-Elemene	10.5	16.0	3.7	13.1	10.2
1400	Geosmin	tr	tr	tr	tr	tr
1410	Dodecanal		tr	tr	tr	
1418	*β*-Ylangene	0.3	5.7	4.2	5.7	0.6
1428	*β*-Copaene	tr	2.9	2.2	3.1	0.2
1433	*γ*-Elemene		tr	tr		
1436	Iridomyrmecin					1.5
1443	Aromadendrene	tr	1.1	0.9	1.2	tr
1452	Unidentified sesquiterpene[c]	tr	1.8	1.6	2.0	tr
1457	(*E*)-*β*-Farnesene	1.1	1.0	1.3	1.5	1.5
1463	*cis*-Cadina - 1(6), 4 - diene		0.9	tr	0.3	
1482	Germacrene D	45.0	27.2	15.0	17.4	46.3
1488	*β*-Selinene	tr	tr	tr	0.2	tr
1491	*trans*-Muurola - 4(14), 5 - diene		tr	tr	tr	tr
1493	*δ*-Selinene					tr
1495	Bicyclogermacrene	tr				tr
1495	*epi*-Cubebol			tr		
1495	*cis*-*β*-Guaiene				0.1	
1498	*γ*-Amorphene		1.5	1.3	2.3	

Continued

1500	Pentadecane	5.5	0.6	tr		0.5
1507	Germacrene A	2.1			0.2	
1508	(*E*,*E*)-*α*-Farnesene	tr	tr	0.5	0.4	0.4
1513	*γ*-Cadinene		tr	tr	tr	tr
1522	*δ*-Cadinene	tr	tr	0.2	0.3	9.0
1539	*α*-Cadinene				tr	tr
1577	Germacrene D - 4 - ol	tr			tr	tr
1644	*τ*-Muurolol				tr	tr
1655	*α*-Cadinol	tr	tr	0.3	0.3	tr
1686	Unidentified sesquiterpenoid[d]	6.5	1.7		3.8	4.4
1701	Heptadecane					tr
1738	Mint sulfide	tr	tr	0.2	tr	tr
1761	Cyclocolorenone			tr		
1766	Tetradecanoic acid				tr	tr
1767	Dodecanamide			0.3		
1845	Neophytadiene		tr	0.2	tr	
1850	2 - Pentadecanone	tr	tr	0.6	0.3	tr
1918	Methyl palmitate			0.1	tr	
1955	Palmitic acid	0.6			0.4	1.2
2100	Heneicosane				tr	tr
2108	(*E*) - Phytol	3.5		3.6	0.8	1.1
2300	Tricosane	tr	tr	0.2	tr	tr
2355	(9*Z*) - Octadecenamide			0.2		
2500	Pentacosane	0.7	tr	0.5	0.2	tr
2600	Hexacosane				tr	
2700	Heptacosane	1.9	tr	1.0	0.3	tr
2800	Octacosane	tr			tr	tr
2900	Nonacosane	4.6	tr	1.5	0.7	1.6
	Monoterpene hydrocarbons	11.0	21.0	33.1	21.5	12.0
	Oxygenated monoterpenoids		tr	5.7	tr	1.5
	Sesquiterpene hydrocarbons	60.2	60.5	32.4	50.0	70.1
	Oxygenated sesquiterpenoids	6.5	1.7	0.3	4.1	4.4
	Diterpenoids	3.5	tr	3.8	0.8	1.1
	Aromatics	0.3	1.7	2.8	3.1	2.4
	Fatty-acid derived	18.5	15.1	21.5	18.4	8.4
	Others	tr	tr	tr	0.1	0.1
	Total Identified (99)	93.5	96.5	98.0	92.3	95.6

[a]RI = "Retention Index" determined with respect to a homologous series of normal alkanes on an HP - 5 ms column; [b]tr = "trace" (<0.05%); [c]MS, m/e (%): 204 (12), 189 (5), 176 (4), 162 (22), 161 (100), 148 (13), 133 (25), 119 (30), 105 (44), 93 (39), 91 (52), 81 (44), 79 (36), 77 (24), 67 (21), 55 (15), 43 (14), 41 (31); [d]MS, m/e (%): 220 (2), 202 (33), 177 (19), 159 (100), 131 (29), 121 (61), 119 (89), 107 (35), 105 (41), 97 (28), 93 (61), 91 (79), 79 (65), 77 (53), 69 (32), 55 (40), 43 (38), 41 (50).

Table 4. Allelopathic activity of *Lamium amplexicaule* and *Lamium purpureum* essential oils on lettuce (*Lactuca sativa*) and perennial ryegrass (*Lolium perenne*).

Sample (Concentration, μg/mL)	Germination Inhibition (%)		Seedling Growth (% of Controls)			
	Lactuca sativa	*Lolium perenne*	*Lactuca sativa*		*Lolium perenne*	
			radicle	hypocotyl	radicle	hypocotyl
L. amplexicaule EO (4000)	23.33	13.33	77.6[a]	57.4[a]	70.3[a]	88.3[c]
L. amplexicaule EO (2000)	8.33	13.33	87.4[b]	97.8[c]	98.8[c]	129[c]
L. purpureum EO (4000)	8.33	28.33	78.6[a]	55.9[a]	51.1[a]	26.4[a]
L. purpureum EO (2000)	5.00	11.67	93.7[c]	94.1[c]	85.0[b]	142[c]
α-Pinene (4000)	6.67	13.33	121[c]	99.6[c]	90.0[c]	102[c]
β-Pinene (4000)	13.33	18.33	84.1[b]	58.8[a]	77.0[b]	11.5[a]
β-Pinene (2000)	1.67	25.00	98.6[c]	85.1[c]	82.5[c]	39.4[a]
β-Ocimenes (4000)	23.33	28.33	71.6[a]	43.2[a]	39.5[a]	24.1[a]
β-Ocimenes (2000)	23.33	16.67	85.7[b]	43.5[a]	46.5[a]	76.7[c]
(*E*) - Caryophyllene (4000)	3.33	13.33	80.2[a]	71.9[a]	57.1[a]	53.4[b]
(*E*) - Caryophyllene (2000)	8.33	11.67	90.5[c]	83.1[b]	90.9[c]	104[c]
α-Humulene (4000)	28.33	15.00	34.8[a]	18.8[a]	48.4[a]	33.2[a]
α-Humulene (2000)	13.33	13.33	59.1[a]	25.6[a]	66.7[a]	224[c]

[a]Significantly inhibited compared to control ($P < 0.001$); [b]Significantly inhibited compared to control ($0.001 < P < 0.1$); [c]Not significantly inhibitory ($P > 0.1$)

and monoterpene hydrocarbons, *α*-pinene (2.2% - 16.2%), *β*-pinene (2.0% - 10.6%), as well as 1-octen-3-ol (3.5% - 8.0%). *L. purpureum* essential oil also had large concentrations of germacrene D (15.0% - 46.3%), *α*-pinene (4.1% - 15.3%), *β*-pinene (6.3% - 16.3%), and 1-octen-3-ol (4.2% - 15.3%). *L. purpureum* oil was also rich in *β*-elemene (3.7% - 16.0%), but devoid of (*E*)-caryophyllene. *L. amplexicaule* and *L. purpureum* essential oils from Italy have been reported [13], and while there are similarities, there are also some notable differences in compositions. The *L. amplexicaule* essential oil from Italy had no 1-octen-3-ol and no benzyl alcohol; *trans*-chrysanthenyl acetate was abundant (41.1%) in the Italian sample, but was not observed in the Alabama samples. Neither *β*-copaene nor *γ*-elemene was observed in the Italian *L. amplexicaule* sample. No 1-octen-3-ol was found in the Italian *L. purpureum* oil, but rather 1-octen-3-one, which was not detected in the Alabama samples. The Alabama samples did contain *β*-ylangene but not (*E*)-caryophyllene.

The essential oil of *L. amplexicaule* was only marginally allelopathic to lettuce (*Lactuca sativa*) or perennial ryegrass (*Lolium perenne*). At 4000 μg/mL, the essential oil showed only 23.3% and 13.3% germination inhibition of *L. sativa* and *L. perenne*, respectively. Radicle and hypocotyl elongation of these test species were only slightly inhibited at 4000 μg/mL, but largely unaffected at 2000 μg/mL.

Some of the essential oil components that were screened for allelopathic activity did demonstrate notable activity in terms of growth inhibition, but not germination inhibition. The monoterpenes *α*-pinene and *β*-pinene were inactive against *L. sativa* at 2000 μg/mL, but *β*-ocimene (mixture of isomers) inhibited growth of both *L. sativa* and *L. perenne*. The sesquiterpene (*E*)-caryophyllene was not notably active at 2000 μg/mL, but *α*-humulene did exhibit significant radicle elongation inhibition. Unfortunately, neither *β*-elemene nor germacrene D are commercially available and were not tested.

L. amplexicaule has been shown to reduce the yield of wheat (*Triticum aestivum*) [4], and has demonstrated allelopathic activity against lettuce (*Lactuca sativa*) [14]. Similarly, *L. purpureum* has been shown to reduce the growth of soybeans (*Glycine max*) [5], and this plant has also demonstrated allelopathic effects on lettuce [16]. Some plant species have demonstrated allelopathic effects toward *L. amplexicaule* and *L. purpureum*. For example, a field plot of rye (*Secale cereale*) reduced the biomass of weeds including *L. amplexicaule* [16]. Jerusalem artichoke (*Helianthus tuberosus*) residues reduced weed density, including *L. purpureum* [17].

L. amplexicaule and *L. purpureum* essential oils were screened for biological activity against the nematode *Caenorhabditis elegans*, the brine shrimp *Artemia salina*,

and the red imported fire ant *Solenopsis invicta* × *richteri*. Neither oil showed activity against these organisms: *C. elegans* (LC_{50} > 2500 µg/mL), *A. salina* (LC_{50} > 100 µg/mL), nor *Solenopsis* (LC_{50} > 4000 µg/mL). The absence of nematocidal activity on *C. elegans* is perhaps not surprising; both *L. amplexicaule* and *L. purpureum* are alternative and excellent hosts for the soybean cyst nematode, *Heterodera glycines* [18,19]. Likewise, insecticidal activity of *L. amplexicaule* or *L. purpureum* essential oils should not be expected as these plants are hosts to several generalist phytophagous insects [20]. Thus, for example, *L. amplexicaule* is an excellent host plant for the tarnished plant bug, *Lygus lineolaris* [21], the western flower thrip, *Frankliniella occidentalis* [22], and the silverleaf whitefly, *Bemisia tabaci* [23], while *L. purpureum* has been identified as a host for the black bean aphid, *Aphis fabae* [24]. These *Lamium* spp. are utilized by the insects as both feeding and reproductive hosts as well as overwintering hosts.

4. CONCLUSION

The allelopathic effects of *Lamium amplexicaule* and *L. purpureum* are not likely due to volatile phytochemical components; the essential oils of these plants do not exhibit notable phytotoxic effects. Previously observed allelopathy may have been due to non-volatile chemical components or due to biotic factors such as nematode infestations, phytophagous insect assaults, or plant viral diseases borne by infected plants and spread by insect vectors. *L. amplexicaule* is known to be a reservoir of tomato spotted wilt virus, which is spread by viruliferous *Frankliniella* thrips [25,26] as well as melon yellow spot virus, spread by *Thrips palmi* [27]. Similarly *L. purpureum* can be infected with cucumber mosaic virus [28] and potato virus Y [29], which are transmitted by the aphid *Myzus persicae* [30]. Additionally, the silverleaf whitefly, *B. tabaci*, is known to transmit several economically and ecologically important plant viruses [31].

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REFERENCES

[1] Radford, A.E., Ahles, H.E. and Bell, C.R. (1968) Manual of the Vascular Flora of the Carolinas. University of North Carolina Press, Chapel Hill.

[2] Pimentel, D., Lach, L., Zuniga, R. and Morrison, D. (2000) Environmental and economic costs of nonindigenous species in the United States. *BioScience*, **50**, 53-65. doi:10.1641/0006-3568(2000)050[0053:EAECON]2.3.CO;2

[3] Olliff, T., Renkin, R., McClure, C., Miller, P., Price, D., Reinhard, D. and Whipple, J. (2001) Managing a complex exotic vegetation program in Yellowstone National Park. *Western North American Naturalist*, **61**, 347-358. https://ojs.lib.byu.edu/ojs/index.php/wnan/article/viewArticle/1012

[4] Conley, S.P. and Bradley, K.W. (2005) Wheat (Triticum aestivum) yield response to henbit (Lamium amplexicaule) interference and simulated winterkill. *Weed Technology*, **19**, 902-906. doi:10.1614/WT-04-252R.1

[5] Mock, V.A., Creech, E., Davis, V.M. and Johnson, W.G. (2009) Plant growth and soybean cyst nematode response to purple deadnettle (Lamium purpureum), annual ryegrass, and soybean combinations. *Weed Science*, **57**, 489-493. doi:10.1614/WS-09-005.1

[6] Adams, R.P. (2007) Identification of essential oil components by gas chromatography/mass spectrometry. 4th Edition Allured Publishing Corp., Carol Stream.

[7] Kennedy, J.E., Davé, P.C., Harbin, L.N. and Setzer, W.N. (2011) Allelopathic potential of Sassafras albidum and Pinus taeda essential oils. *Allelopathy Journal*, **27**, 111-122. http://indianjournals.com/ijor.aspx?target=ijor:aj&volume=27&issue=1&article=011

[8] McLaughlin, J.L. (1991) Bench-top bioassays for the discovery of bioactive compounds in higher plants. *Brenesia*, **34**, 1-14.

[9] Park, I.K., Kim, J., Lee, S.G. and Shin, S.C. (2007) Nematicidal activity of plant essential oils and components from ajowan (Trachyspermum ammi), allspice (Pimenta dioica) and litsea (Litsea cubeba) essential oils against pine wood nematode (Bursaphelenchus xylophilus). *Journal of Nematology*, **39**, 257-279. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2586506/

[10] Chen, L., Lu, Y.Y., Hu, Q.B. and Fadamiro, H.Y. (2012) Similarity in venom alkaloid chemistry of alate queens of imported fire ants: Implication for hybridization between Solenopsis richteri and S. invicta in the southern United States. *Chemistry & Biodiversity*, **9**, 702-713. doi:10.1002/cbdv.201100109

[11] Zar, J.H. (1996) Biostatistical analysis. 3rd Edition, Prentice Hall, New Jersey.

[12] Reed, L.J. and Muench, H. (1938) A simple method of estimating fifty per cent endpoints. *American Journal of Hygiene*, **27**, 493-497.

[13] Glamini, G., Cioni, P.L. and Morelli, I. (2005) Composition of the essential oils and *in vivo* emission of volatiles of four Lamium species from Italy: L. purpureum, L. hybridum, L. bifidum, and L. amplexicaule. *Food Chemistry*, **91**, 63-68. doi:10.1016/j.foodchem.2004.05.047

[14] Fujii, Y., Shibuya, T. and Yasuda, T. (1992) Allelopathy of velvetbean: Its discrimination and identification of L-DOPA as a candidate of allelopathic substances. *Japan*

Agricultural Research Quarterly, **25**, 238-247.

[15] Shiraishi, S., Watanabe, I., Kuno, K. and Fujii, Y. (2002) Allelopathic activity of leaching from dry leaves and exudate from roots of ground cover plants assayed on agar. *Weed Biology and Management*, **2**, 133-142. doi:10.1046/j.1445-6664.2002.00063.x

[16] Pérez, F.J. and Ormeño-Núñez, J. (1993) Weed growth interference from temperate cereals: The effect of hydroxamic-acids-exuding rye (Secale cereale L.) cultivar. *Weed Research*, **33**, 115-119. doi:10.1111/j.1365-3180.1993.tb01924.x

[17] Tesio, F., Vidotto, F. and Ferrero, A. (2012) Allelopathic persistence of Helianthus tuberosus L. residues in the soil. *Scientia Horticulturae*, **125**, 98-105. doi:10.1016/j.scienta.2011.12.008

[18] Creech, J.E., Faghihi, J., Ferris, V.R., Westphal, A., and Johnson, W.G. (2007) Influence of intraspecific henbit (Lamium amplexicaule) and purple deadnettle (Lamium purpureum) competition on soybean cyst nematode reproduction. *Weed Science*, **55**, 665-670. doi:10.1614/WS-07-051.1

[19] Creech, J.E., Webb, J.S., Young, B.G., Bond, J.P., Harrison, S.K., Ferris, V.R., Faghihi, J., Westphal, A. and Johnson, W.G. (2007) Development of soybean cyst nematode on henbit (Lamium amplexicaule) and purple deadnettle (Lamium purpureum). *Weed Technology*, **21**, 1064-1070. doi:10.1614/WT-07-079.1

[20] Marshall, E.J.P., Brown, V.K., Boatman, N.D., Lutman, P.J.W., Squire, G.R. and Ward, L.K. (2003) The role of weeds in supporting biological diversity within crop fields. *Weed Research*, **43**, 77-89. doi:10.1046/j.1365-3180.2003.00326.x

[21] Esquivel, J.F. and Mowery, S.V. (2007) Host plants of the tarnished plant bug (Heteroptera: Miridae) in central Texas. *Environmental Entomology*, **36**, 725-730. doi:10.1603/0046-225X(2007)36[725:HPOTTP]2.0.CO;2

[22] Atakan, E. (2010) Influence of weedy field margins on abundance patterns of the predatory bugs Orius spp. and their prey, the western flower thrips (Frankliniella occidentalis), on faba bean. *Phytoparasitica*, **38**, 313-325. doi:10.1007/s12600-010-0105-9

[23] Bayhan, E., Ulusoy, M.R. and Brown, J.K. (2006) Host range, distribution, and natural enemies of Bemisia tabaci "B biotype" (Hemiptera: Aleyrodidae) in Turkey. *Journal of Pesticide Science*, **79**, 233-240. doi:10.1007/s10340-006-0139-4

[24] Wilkinson, T.L. and Douglas, A.E. (2003) Phloem amino acids and the host plant range of the polyphagous aphid, Aphis fabae. *Entomologia Experimentalis et Applicata*, **106**, 103-113. doi:10.1046/j.1570-7458.2003.00014.x

[25] Groves, R.L., Walgenbach, J.F., Moyer, J.W., and Kennedy, G.G. (2002) The role of weed hosts and tobacco thrips, Frankliniella fusca, in the epidemiology of tomato spotted wilt virus. *Plant Disease*, **86**, 573-582. doi:10.1094/PDIS.2002.86.6.573

[26] Okazaki, S., Okuda, M., Komi, K., Yoshimatsu, H. and Iwanami, T. (2007) Overwintering viruliferous Frankliniella occidentalis (Thysanoptera: Thripidae) as an infection source of tomato spotted wilt virus in green pepper fields. *Plant Disease*, **91**, 842-846. doi:10.1094/PDIS-91-7-0842

[27] Yamasaki, S., Okazaki, S. and Okuda, M. (2012) Temporal and spatial dispersal of melon yellow spot virus in cucumber greenhouses and evaluation of weeds as infection sources. *European Journal of Plant Pathology*, **132**, 169-177. doi:10.1007/s10658-011-9860-9

[28] Tomlinson, J.A., Carter, A.L., Dale, W.T. and Simpson, C.J. (1970) Weed plants as sources of cucumber mosaic virus. *Annals of Applied Biology*, **66**, 11-16. doi:10.1111/j.1744-7348.1970.tb04597.x

[29] Kaliciak, A. and Syller, J. (2009) Aphid transmissibility of genetically different isolates of potato virus Y and susceptibility of weeds to virus infection. *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin*, **253**, 285-295. http://www.cabdirect.org/abstracts/20103094750.html

[30] Tomlinson, J.A. and Carter, A.L. (1970) Studies on the seed transmission of cucumber mosaic virus in chickweed (Stellaria media) in relation to the ecology of the virus. *Annals of Applied Biology*, **66**, 381-386. doi:10.1111/j.1744-7348.1970.tb04617.x

[31] Oliveira, M.R.V., Henneberry, T.J. and Anderson, P. (2001) History, current status, and collaborative research projects for Bemisia tabaci. *Crop Protection*, **20**, 709-723. doi:10.1016/S0261-2194(01)00108-9

