Composition and Some Biological Activities of the Essential Oil of *Callicarpa americana* (L.)

Mario R. Tellez,* Franck E. Dayan, Kevin K. Schrader, David E. Wedge, and Stephen O. Duke

Natural Products Utilization Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Box 8048, Oxford, Mississippi 38677

The essential oil profile of *Callicarpa americana* was examined. Samples were collected from Lafayette county in north central Mississippi, and GC-MS data and retention indices were used to identify 67 oil components. Humulene epoxide II (13.9%), α-humulene (10.0%), 7-*epi*-α-eudesmol (9.4%), β-pinene (8.8%), and 1-octen-3-ol (8.5%) were the major components of the steam-distilled oil. The oil was selectively toxic toward the cyanobacterium *Oscillatoria perornata* compared to *Oscillatoria agardhii* and the green alga *Selenastrum capricornutum*, with complete growth inhibition at 28.5 μg/mL. The oil was only mildly phytotoxic and antifungal.

Keywords: *Callicarpa americana; essential oil composition; fungi; algae; plant; bioactivity*

INTRODUCTION

Part of our mission is to discover plant constituents that can be used in pest management. *Callicarpa americana* (L.) (beautyberry, French mulberry) is a shrub (typical height of 0.7−3.3 m) native to the southeastern United States from southern Maryland to Oklahoma south to Texas and Florida (Dean, 1968; Martin and Sick, 1995). It is used as forage by many wildlife species and as an ornamental shrub and has shown value for borrow pit reclamation following mining (Martin and Sick, 1995). Little is known of the secondary constituents of *C. americana* and their biological activity. Aqueous extracts from the leaves have been reported to act as oviposition deterrents for *Heliothis virescens* (Tingle and Mitchell, 1984). Methanol extracts of the leaves showed no piscicidal activity in contrast to some species of the same genus (Nishino et al., 1971). To our knowledge, the composition and herbicidal, algicidal, and antifungal activities of the essential oil of *C. americana* have not been previously reported. In this paper we report on the composition and selected pesticidal activity of the essential oil of *C. americana*.

EXPERIMENTAL PROCEDURES

Plant Material. Samples of *C. americana* were collected in mid-October from five plants growing in Lafayette County, at latitude 34° 20′ north and longitude 89° 40′ west, ~9.2 mi east of Oxford, MS. Fresh leaf samples were collected by removing ~50% of the leaves of plants. A composite sample was made and stored in a labeled plastic bag at −20 °C until steam distillations were performed. A voucher specimen of *C. americana* was placed in the University of Mississippi herbarium located in Oxford, MS.

Essential Oil Isolation and Chemical Characterization. Steam distillation and analyses of the oil were conducted as previously described (Tellez et al., 1999; Adams, 1995) on 50.6 g of plant material. Analyses were performed by GC-MSD (EI, 70 eV) with a DB-5 column (30 m × 0.25 mm fused silica capillary column, film thickness = 0.25 μm) using He as carrier gas (1 mL/min), 1 μL injection size, and a programmed (injector temperature = 220 °C, transfer line temperature = 240 °C, initial column temperature = 60 °C, final column temperature = 240 °C, 3 °C/min) temperature run (Tellez et al., 1999; Adams, 1995). Identifications of oil components were performed by a comparison of mass spectra with literature data and by a comparison of their relative retention times with those of authentic compounds or by comparison of their retention indices with those in the literature (Adams, 1995). The relative amounts (RA) of individual components of the oil are expressed as percent peak area relative to total peak area. A clear yellow oil was obtained in a yield of 57 mg (0.11% of fresh weight).

Phytotoxicity Assays. Bioassays for phytotoxic activity were carried out as previously reported for lettuce (*Lactuca sativa* cv. Iceberg) and bentgrass (*Agrostis stolonifera* cv. Pencross) in 24-well plates (Dayan et al., 1999) except that *n*-pentane was used as the transfer solvent. An added control with *n*-pentane was used to account for possible solvent effects.

Fungicidal Assays. Pathogen production and inoculum preparation for *Botrytis cinerea* Pers.:Fr., *Colletotrichum accutatum* Simmonds, *Colletotrichum fragariae* Brooks, *Colletotrichum gloeosporioides* Penz. & Sacc., and *Fusarium oxysporum* Schlechtend:Fr were performed according to procedures of Wedge and Kuhajek (1998). Conidia concentrations were determined photometrically (Espinel-Ingroff and Kerkering, 1991; Wedge and Kuhajek, 1998) and suspensions adjusted with sterile distilled water to 1.0×10^6 conidia/mL.

Inhibition of fungal growth on thin layer chromatographic (TLC) plates was evaluated by modifications of bioautographic assays (Homans and Fuchs, 1970) using *C. fragariae* and *C. gloeosporioides*. The pentane solution of the oil was spotted on TLC plates (250 μm, silica gel GF Uniplate, Analtech, Inc., Newark, DE) using disposable glass micropipets. Each plate received three spots of 114.0, 57.0, and 5.7 μg of oil, respectively. Plates were sprayed with one of either spore suspension (~3×10^5 conidia/mL) in potato dextrose broth containing 24 g/L potato dextrose, 0.1% bacto agar, and 0.1% Tween 80. Each plate was sprayed lightly (to a damp appearance) three times. Inoculated plates were placed in a 30 × 13 × 7.5 cm moisture chamber (398-C, Pioneer Plastics, Inc., Dixon, KY) and incubated in a growth chamber at 24 ± 1 °C and with a 12/12 h photoperiod under 60 ± 5 μmol/m^2/s light. Inhibition of fungal growth for each test fungus was measured 4 days after treatment. Sensitivity of the fungal species to the oil was determined by comparing the sizes of inhibitory zones.

* Author to whom correspondence should be addressed [telephone (662) 915-5898; fax (662) 915-1035; e-mail matellez@olemiss.edu].

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Table 1. Constituents of the Oil of *C. americana*[a]

compound	RI	RT	% RA	compound	RI	RT	% RA
hexanal	800	175	0.5	α-terpineol	1190	849	0.2
(*E*)-2-hexenal	853	218	4.6	myrtenal	1194	860	0.2
(*Z*)-3-hexenol	858	222	0.8	myrtenol	1195	864	t
2-hexen-1-ol	868	232	0.2	α-ylangene	1372	1304	t
n-hexanol	867	233	0.3	α-copaene	1377	1316	0.2
α-thujene	932	311	0.1	(*E*)-*β*-damascenone	1383	1332	t
α-pinene	939	322	2.5	*β*-bourbonene	1385	1336	0.3
camphene	954	344	t	(*E*)-*β*-caryophyllene	1419	1420	0.6
benzaldehyde	962	357	t	*β*-gurjunene	1429	1444	t
1-octen-3-ol	980	387	8.5	α-humulene	1454	1502	10.1
β-pinene	981	389	8.8	*γ*-muurolene	1477	1559	0.7
3-octanone	987	400	0.1	unknown sesquiterpene $C_{15}H_{24}$	1483	1574	1.1
3-octanol	995	416	t	*β*-selinene	1485	1580	2.2
(*Z*)-3-hexenol acetate	1006	437	t	valencene	1492	1597	3.5
(*E*,*E*)-2,4-heptadienal	1010	442	t	α-selinene	1494	1602	0.5
α-terpinene	1019	460	t	α-muurolene	1499	1616	0.2
p-cymene	1027	474	0.6	*γ*-cadinene	1513	1647	0.3
limonene	1032	483	0.4	7-*epi*-α-selinene	1516	1654	1.3
benzyl alcohol	1033	486	0.2	*cis*-calamene	1522	1666	t
1,8-cineole	1034	487	0.1	*δ*-cadinene	1523	1669	0.5
benzene acetaldehyde	1044	507	0.7	cadina-1,4-diene	1532	1688	t
γ-terpinene	1062	544	0.1	α-cadinene	1537	1701	t
trans-arbusculone	1072	566	t	α-calacorene	1542	1712	t
cis-linalool oxide	1074	572	t	caryophyllene oxide	1581	1802	1.0
terpinolene	1090	609	t	unknown sesquiterpene $C_{15}H_{24}O$	1596	1839	1.6
linalool	1099	632	0.3	humulene epoxide II	1606	1862	13.9
n-nonanal	1103	641	t	unknown sesquiterpene $C_{15}H_{24}O$	1614	1881	1.2
endo-fenchol	1114	664	t	unknown sesquiterpene $C_{15}H_{24}O$	1627	1906	2.0
cis-*p*-menth-2-en-1-ol	1122	682	t	unknown sesquiterpene $C_{15}H_{24}O$	1630	1913	5.8
α-campholenal	1127	692	t	*epi*-α-cadinol	1640	1933	0.6
nopinone	1137	716	0.1	cubenol	1641	1936	0.6
trans-sabinol	1140	723	0.2	α-muurolol	1645	1945	0.5
4-ketoisophorone	1143	729	t	α-cadinol	1653	1963	2.2
pinocarvone	1163	778	0.2	7-*epi*-α-eudesmol	1657	1970	9.5
borneol	1167	787	t	khusinol	1677	2016	0.3
p-mentha-1,5-dien-8-ol	1168	790	t	unknown. *m*/*z* 234 [M^+], 175 (100)	1804	2288	4.3
terpin-4-ol	1178	817	0.3				

[a] Abbreviations: RI, retention index as determined on a DB-5 column using the homologous series of *n*-hydrocarbons; RT, retention time on a DB-5 column in seconds; RA, relative area (peak area relative to total peak area); t, trace (<0.05%).

A 96-well microtiter assay was used to determine sensitivity of all five pathogens to the essential oil in comparison to known fungicidal standards (Wedge and Kuhajek, 1998). Vinclozolin, chlorothalonil, and thiabendazole (ChemService, West Chester, PA), representing three different modes of action, were used. Each fungus was challenged with 0.3, 3.0, and 30.0 μM of each standard. *C. americana* essential oil was tested in a similar dose–response format at concentrations of 0.14, 1.42, and 14.25 μg/mL. Fungal growth was evaluated after 48 h by measuring the absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL). Mean absorbance values were used to evaluate sensitivity to particular concentrations of compound.

Algaecidal Assays. A rapid bioassay (Schrader et al., 1997) was used to determine the lowest observed effect concentration (LOEC) and the lowest complete inhibition concentration (LCIC) of *C. americana* oil toward isolates of the cyanobacteria *Oscillatoria perornata* and *Oscillatoria agardhii* and the green algae *Selenastrum capricornutum*. A 96-well quartz microplate (Hellma Cells, Inc., Forest Hills, NY) was used to perform the bioassay because the pentane loading solvent is incompatible with polystyrene microplates.

RESULTS AND DISCUSSION

The identity, retention index, retention time, and percent composition of the oil of *C. americana* are presented in Table 1. Sixty-seven compounds were identified in the oil of beautyberry, accounting for >78% of the composition of the oil. Among the identified compounds were 9 monoterpene hydrocarbons (12.4%), 18 sesquiterpene hydrocarbons (20.3%), 14 oxygenated monoterpenes (1.6%), and 8 oxygenated sesquiterpenes (28.6%). Unidentified were 1 sesquiterpene hydrocarbon ($C_{15}H_{24}$) at 1483 (RI) accounting for 1.1% (RA), and 4 oxygenated sesquiterpenes ($C_{15}H_{24}O$) at 1596, 1614, 1627, and 1630 (RI) accounting for 1.6, 1.2, 2.0, and 5.8% (RA), respectively. Also unidentified was one compound at 1804 (RI) showing *m*/*z* (relative intensity) 234 [M^+] (5), 190 (41), 175 (100), 147 (16), 121 (26), 105 (25), 91 (26), 79 (21), 55 (23), and 41 (34) and accounting for 4.3% (RA). None of the remaining unidentified compounds accounted for >1.0% of the total area. The dominant compounds in the oil were α-humulene and humulene epoxide II. α-Humulene is an important biogenetic precursor for a large number of sesquiterpenoids (Yang and Deinzer, 1992). The presence of large amounts (30–40% of the essential oil) of α-humulene in hop cultivars appears to be a desirable trait among brewers (Yang and Deinzer, 1992). Humulene epoxide II is also found in hops, where it originates either from biogenesis or postharvest oxidation of α-humulene (Yang and Deinzer, 1992). The origin of humulene epoxide II in *C. americana* is unknown.

The oil of *C. americana* was only mildly phytotoxic. Its activity on lettuce (dicot) and bentgrass (monocot) at 1.0 mg per well was ranked at 3 on a scale of 0–5, where 0 indicates no effect and 5 no germination (Dayan et al., 1999). A rank of 0 was assigned at 0.1 mg. Weighing lettuce seedlings after 1 week and measuring bentgrass shoot length after 2 weeks demonstrated that

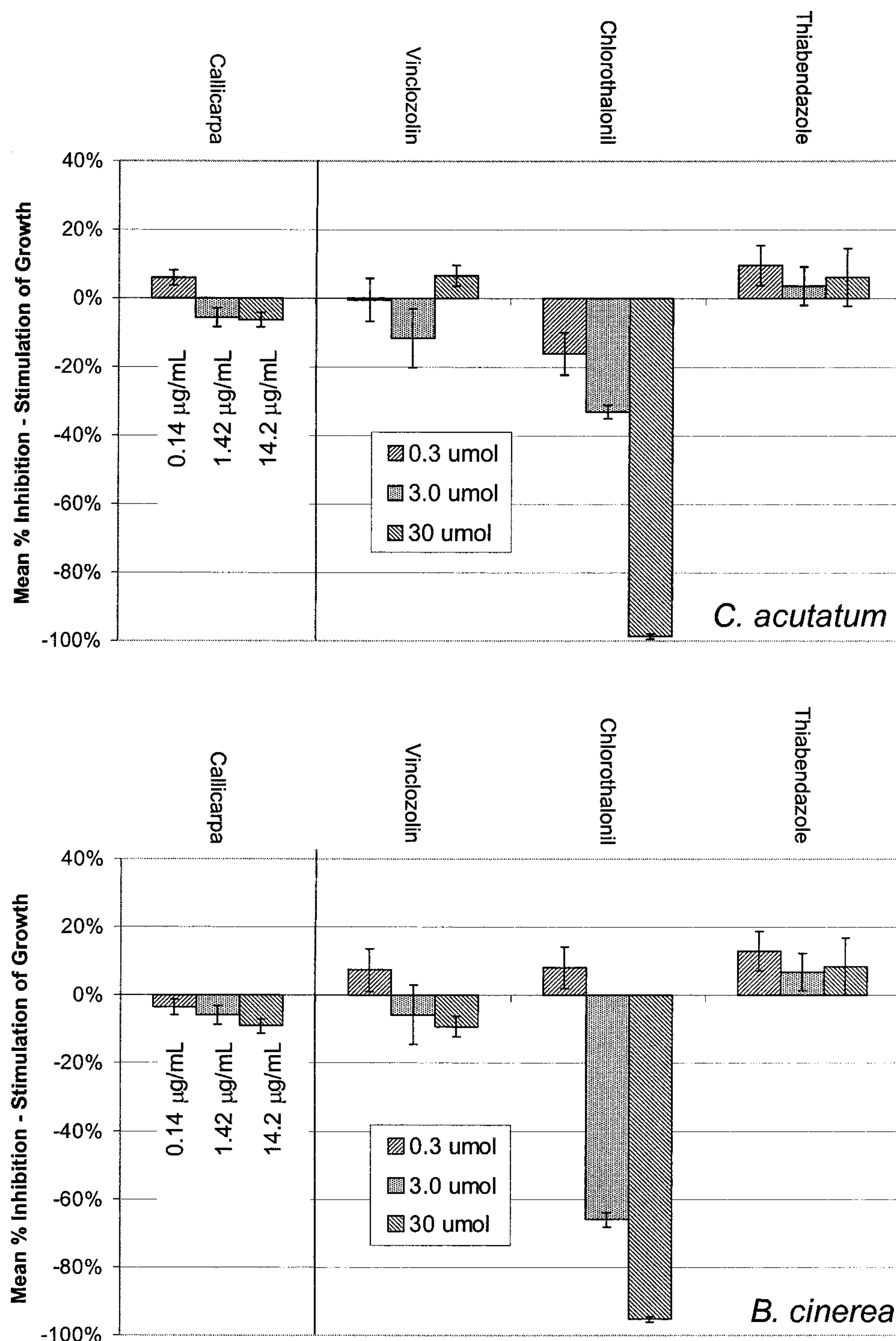

Figure 1. Effect of *C. americana* oil and three commercial fungicides on *C. acutatum* and *B. cinerea.*

Table 2. Seedling Weights (Lettuce) after 1 Week and Shoot Length (Bentgrass) after 2 Weeks

	mean wt (mg)		mean length (mm)
	replicate 1	replicate 2	$N = 50$
control A[a]	0.020	0.023	14.38
control B[a]	0.023	0.017	13.40
1000 µg	0.012	0.011	7.82
100 µg	0.019	0.020	13.22

[a] Control A, no solvent added; control B, *n*-pentane added.

the oil inhibits growth by 40–50% at 1.0 mg per well and loses all effect at 0.1 mg (Table 2).

The oil of *C. americana* showed mild activity against some fungi. Small zones of inhibition were observed in the bioautography assays for *C. fragariae* and *B. cinerea* where 114 or 57 µg of *C. americana* essential oil was spotted. No inhibition was observed at 5.7 µg per spot. Mild activity was observed in the 96-well microbioassay against *B. cinerea* at a concentration as low as 0.14 µg/mL (3.5% inhibition) or against *C. acutatum* at 1.42 µg/mL (5.6% inhibition) (Figure 1). Activity against *C. gloeosporioides* was negligible (5.0% inhibition at 14.2 µg/mL), and no significant activity was observed against *C. fragariae* or *F. oxysporum.* Even though there seems to be some selectivity against *B. cinerea* and *C. acutatum,* two economically important fruit-rot pathogens, the highest concentrations tested (14.2 µg/mL) remained only mildly active (9.0 and 6.3% inhibition, respectively).

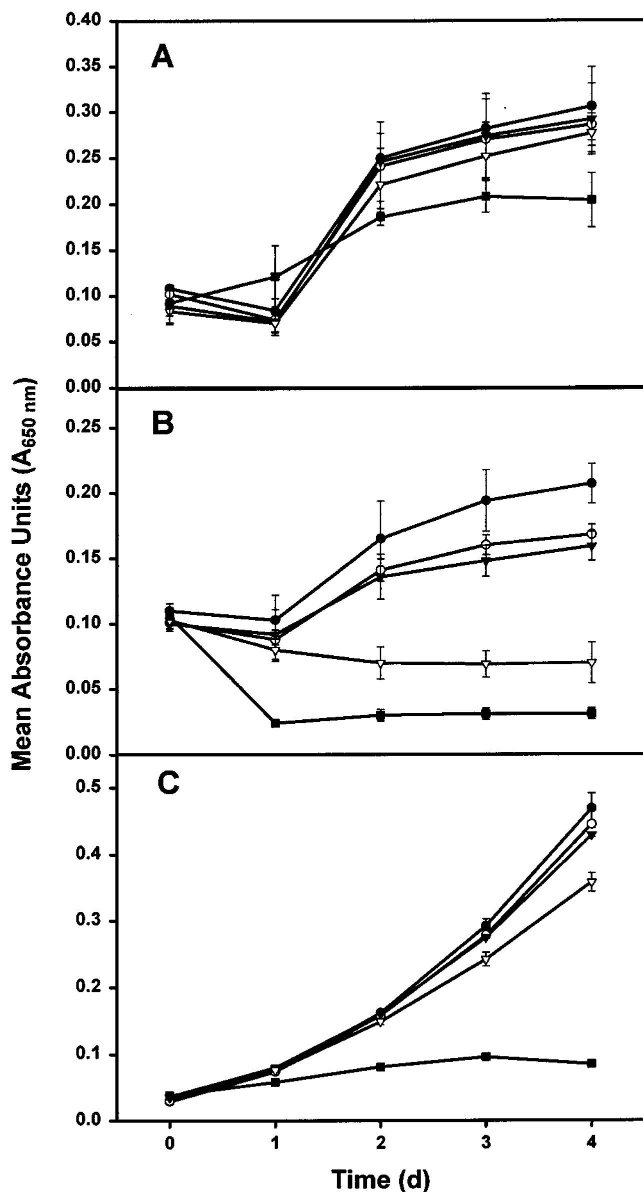

Figure 2. Effect of different concentrations of *C. americana* essential oil on the growth of (A) *O. agardhii*, (B) *O. peornata*, and (C) *S. capricornutum*: (●) control; (○) 0.285, (▼) 2.85, (▽) 28.5, and (■) 285 μg/mL *C. americana* oil.

These results compare favorably against the two commercial fungicides vinclozolin and thiabendazole, for which *B. cinerea* and *C. acutatum* have developed resistance, but not with the commercial fungicide chlorothalonil.

C. americana oil was screened against two species of cyanobacteria (blue-green algae) and one species of green alga to determine its potential as a selective cyanobactericide. Off-flavor in channel catfish (*Ictalurus punctatus*) raised in the southeastern United States creates an unpalatable and, therefore, unmarketable product that results in large economic losses to the industry. Most off-flavor episodes in catfish are attributed to their absorption of earthy/musty compounds produced by certain species of cyanobacteria that grow in the catfish production ponds. In western Mississippi, the cyanobacterium *Oscillatoria perornata* (Skuja), a producer of the musty-odor compound 2-methylisoborneol (MIB), is attributed with being the major cause of musty off-flavor in farm-raised catfish (van der Ploeg et al., 1995). Green algae are not associated with such undesirable metabolites and are preferable to cyanobacteria in catfish production ponds because they are better oxygenators of the water and a better base for aquatic food chains (Paerl and Tucker, 1995). The discovery of safe compounds that selectively kill cyanobacteria would greatly benefit the channel catfish industry. Toxic activity of *C. americana* oil toward *O. agardhii* was observed at 285 μg/mL (LOEC), with complete inhibition at >285 μg/mL (LCIC) (Figure 2A). For *O. perornata*, LOEC is 0.285 μg/mL and LCIC is 28.5 μg/mL (Figure 2B). For *S. capricornutum*, LOEC is 28.5 μg/mL and LCIC is 285 μg/mL (Figure 2C). The oil constituents of *C. americana* do not appear to be useful as a broad-spectrum cyanobactericide due to observed lower toxicity toward *O. agardhii* than that shown toward the green alga *S. capricornutum*. These results do indicate selective toxicity of *C. americana* oil toward *O. perornata*.

In conclusion, *C. americana* has a low oil content showing a prevalence (39.2%) of oxygenated sesquiterpenes. The oil of *C. americana* or one or more of its components hold promise as a possible selective cyanobactericide against the cyanobacterium responsible for musty off-flavor problems in catfish. The relevance of this finding is heightened by the previously reported lack of piscicidal activity (Nishino et al., 1971) and presently reported low phytotoxic activity. We are currently carrying out the bioassay-guided isolation and identification of the active components in *C. americana*.

LITERATURE CITED

Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allured Publishing: Carol Stream, IL, 1995.

Dayan, F. E.; Hernandez, A.; Allen, N. S.; Moraes, R. M.; Vroman, J. A.; Avery, M. A.; Duke, S. O. Comparative phytotoxicity of artemisinin and several sesquiterpene analogues. *Phytochemistry* **1999**, *50*, 607−614.

Dean, B. E. *Trees and Shrubs in the Heart of Dixie*, revised ed.; Southern University Press: Birmingham, AL, 1968.

Espinel-Ingroff, A.; Kerkering, T. M. Spectrophotometric method of inoculum preparation for the in vitro susceptibility testing of filamentous fungi. *J. Clin. Microbiol.* **1991**, *29*, 393−394.

Homans, A. L.; Fuchs, A. Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. *J. Chromatogr.* **1970**, *51*, 327−329.

Martin H.; Sick, G. American Beautyberry for Borrow Pit Reclamation in South Carolina: Trials Produced Good Results after Three Years. *Restoration Manag. Notes* **1995**, *13*, 90−97.

Nishino, V.; Kawazu, K.; Mitsui, T. Isolation and Structure of Maingayic Acid, a Piscicidal Constituent of *Callicarpa maingayi*. *Agric. Biol. Chem.* **1971**, *35*, 1921−1930.

Paerl, H. W.; Tucker, C. S. Ecology of Blue-green Algae in Aquaculture Ponds. *J. World Aquacult. Soc.* **1995**, *26*, 109−131.

Schrader, K. K.; De Regt, M. Q.; Tucker, C. S.; Duke, S. O. A rapid bioassay for selective algicides. *Weed Technol.* **1997**, *11*, 767−774.

Tellez, M. R.; Canel, C.; Rimando, A. M.; Duke, S. O. Differential Accumulation of Isoprenoids in Glanded and Glandless *Artemisia annua*. *Phytochemistry* **1999**, *52*, 1035−1040.

Tingle F. C.; Mitchell, E. R. Aqueous Extracts from Indigenous Plants as Oviposition Deterrents for *Heliothis virescens* (F.). *J. Chem. Ecol.* **1984**, *10*, 101−113.

van der Ploeg, M.; Dennis, M. E.; de Regt, M. Q. Biology of *Oscillatoria cf. chalybea*, a 2-Methylisoborneol Producing Blue-green Alga of Mississippi Catfish Ponds. *Water Sci. Technol.* **1995**, *31*, 173−180.

Wedge D. E.; Kuhajek, J. M. A Microbioassay for Fungicide Discovery. *SAAS Bull. Biochem. Biotechnol.* **1998**, *11*, 1–7.

Yang, X.; Deinzer, M. L. Hydrolisis and Reversible Isomerization of Humulene Epoxides II and III. *J. Org. Chem.* **1992**, *57*, 4717–4722.

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