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Phytotoxicity of Secondary Metabolites Isolated from *Flourensia oolepis* S.F.BLAKE

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The aim of this study was to isolate the active principles of *Flourensia oolepis* S.F.BLAKE (Asteraceae), which completely inhibited the germination of *Raphanus sativus* seeds at 10 mg/ml. Flavanone pinocembrin and sesquiterpene ilicol, were isolated by bioassay-guided fractionation. They were active both against monocot and dicot seeds. Pinocembrin was the most active compound, with an IC_{50}(germination) value of 0.24, 3.40, 3.28, and 3.55 mM against *Panicum miliaceum, Avena sativa, Lactuca sativa*, and *R. sativus*, respectively; ilicol, however, exhibited IC_{50}(germination) values of 0.67, 2.73, 5.25, and 9.66 mM for the same species, respectively. Pinocembrin and ilicol inhibited root growth and showed IC_{50}(root growth) values of 0.199, 14.68, 8.05, 7.69 mM, and 1.22, 2.90, 7.35, 8.07 mM, against *P. miliaceum, A. sativa, L. sativa*, and *R. sativus*, respectively. Pinocembrin and ilicol reduced *Allium cepa* cell division without chromosome aberrations.

Introduction. – In the last decade, the use of land for organic agricultural purposes has continuously grown all over the world, reaching 37 million hectares cultivated under this system at the end of 2010 [1]. Latin America accounts for 6.4% of this land (8.4 million hectares), and 85% of its organic production is exported to the USA, Europe, and Japan [1].

In organic agriculture, the problem of weed control is often cited as one of the major constraints to production, despite the huge research efforts undertaken on weed management [2]. Weed control within organic agriculture is based primarily on the use of agricultural techniques and crop rotation [3]. However, a more rational way of controlling weeds is needed [4], which may result from identifying natural substances that can control the emergence and growth of weeds [5][6]. The discovery of new natural herbicides could positively contribute to satisfying the need of natural pesticides required for the continuous development of organic agriculture.

In a research program aimed at identifying natural herbicides, over 170 different extracts representing 100 plant species from Central Argentina were evaluated [7][8] with *Flourensia oolepis* EtOH extract being the one with the highest phytotoxic activity. *F. oolepis* (common name: *chilca*) is a ligneous and very resinous bush widely spread in the hilly areas of the provinces of Córdoba and San Luis (Central Argentina).

Following bioguide techniques, herein we report the isolation of two phytotoxic compounds from *F. oolepis*, together with the evaluation of their potential as natural herbicides by determining germination, seedling growth, and mitosis inhibition.

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Results and Discussion. – 1. *Isolation of* F. oolepis *Active Principle.* The EtOH extract of the aerial parts of *F. oolepis* inhibited *R. sativus* and *P. miliaceum* germination with *GI*% values of 99.1 and 100% at 10 mg/ml concentration, respectively. Using this activity against both *R. sativus* and *P. miliaceum* as a guide, bioassay-directed chromatographic fractionation of the extract yielded two active fractions, which, after further purifications, led to the isolation of compounds **1** and **2** (*Fig. 1*) as the most active principles against both species. The structure of **1**, deduced from ^{1}H- and ^{13}C-NMR, and mass spectra, was identified as pinocembrin [9].

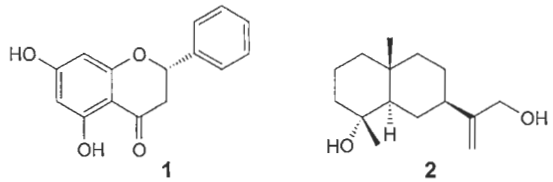

Fig. 1. *Chemical structures of pinocembrin* (**1**) *and ilicol* (**2**)

Pinocembrin (**1**) is a dihydroxyflavanone previously isolated from different plant species. It has also been reported as one of the main components of propolis of different geographical origins [9].

A broad spectrum of antifeedant [9][10], antifungal [11], antiviral [12], antioxidant [13], anti-inflammatory [14], and vasorelaxant [15] activities, among others, has been reported for **1**. However, there is no report about its phytotoxic effects. To our knowledge, this article may, for the first time, shed some light on this matter.

In the case of **2**, its NMR data were in good agreement with the literature values for compounds from *F. oolepis* species [16], which allowed us to assign the structure of ilicol (ilicic alcohol), a sesquiterpene with β-selinene-type structure, to **2**.

As far as we know, **2** has been isolated only from *F. oolepis* and *Gonospermum fruticosum* [17], without any information about its biological activity or phytotoxicity.

2. *Pre-emergent Activity of Compounds* **1**, **2**, *and 2,4-D.* The germination inhibition of **1** and **2** on both two monocot and two dicot species was evaluated at doses ranging from 0.125 to 20 mM (*Fig. 2*). Identical experiments were carried out with the commercial herbicide 2,4-D as positive control in order to obtain a comparative evaluation of the phytotoxicity of the isolated compounds. Compound **1** completely inhibited the germination of *P. miliaceum* at 1 mM, while **2** and 2,4-D showed, at that concentration, 81 and 100% germination inhibition, respectively (*Fig. 2,a*). The other monocot species, *A. sativa*, was more sensitive to **2**, which showed 77% germination inhibition at 4 mM, while **1** and 2,4-D caused, at the same concentration, 45 and 35% inhibition, respectively (*Fig. 2,b*). Both **1** and **2** were less effective than 2,4-D as inhibitors of *L. sativa* germination, with 82 and 72% inhibition at 20 mM, respectively (*Fig. 2,c*). Compound **1** was more effective than **2** at inhibiting the germination of *R. sativus* seeds. Both were more effective than 2,4-D at doses below 8 mM (*Fig. 2,d*).

The IC_{50} (germination) value of **1** was 2.8, 1.6, and 2.7 times more active than **2** for *P. miliaceum, L. sativa*, and *R. sativus*, respectively. However, IC_{50}(germination) value of **1** for *A. sativa* was 1.2 times less active than **2** (*Table 1*).

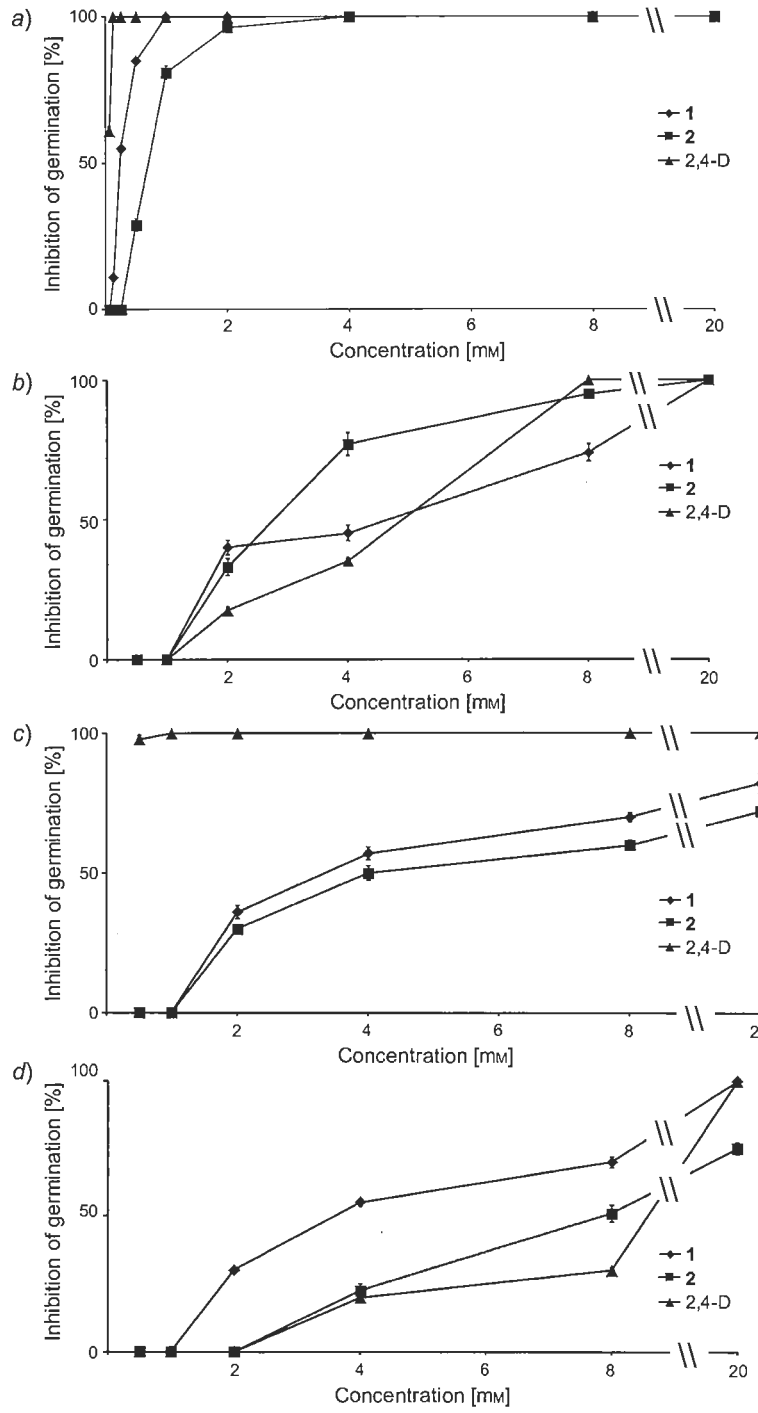

Fig. 2. *Inhibitory effects of* **1**, **2**, *and positive control 2,4-D on seed germination of* a) Panicum miliaceum, b) Avena sativa, c) Lactuca sativa, *and* d) Raphanus sativus, *7 days after seeding on filter paper wetted with the solutions* (water/ketone 3%) *of the compounds.* Error bars show standard deviation, error cannot be detected when it is smaller than the symbol.

Table 1. *Germination Inhibitory Activity* (IC_{50}(germination) [mM]; with 95% confidence intervals) *of* **1** *and* **2** *against* Panicum miliaceum, Avena sativa, Lactuca sativa, *and* Raphanus sativus

Compound	*P. miliaceum*	*A. sativa*	*L. sativa*	*R. sativus*
1	0.24 (0.21–0.28)	3.40 (2.15–5.08)	3.28 (1.80–5.60)	3.55 (2.58–5.08)
2	0.67 (0.59–0.80)	2.73 (2.10–3.57)	5.25 (2.86–9.66)	9.66 (6.72–13.15)

There are some reports on flavonoids with phytotoxic properties; *e.g.*, the flavone formononetin, isolated from *Melilotus messanensis*, showed 31% germination inhibition against *A. cepa*, but exhibited no effect against *L. sativa*, *Lycopersicum esculentum*, and *Hortodeum vulgare* at 10^{-1} mM [18]. Another flavone, cyclosin, was inactive at 10^{-1} mM against the same species [18]. The flavonol quercetin was inactive at 10^{-1} mM against *L. sativa* and *A. cepa* [19].

Structures related to **2** were less frequently encountered as phytotoxins. Some sesquiterpenes with phytotoxic activities present chemical structures bearing α,β-unsaturated lactone moieties [20][21], open structures [22] and aromatic moieties [23]. No phytotoxic activity for sesquiterpenes with β-selinene-type structure was found. For example, parthenin, an α,β-unsaturated lactone sesquiterpene, completely inhibited the germination of *Ageratum conyzoides* at 0.4 mM concentration [21]. In the same way, amarantholidoside I inhibited the germination of *Taraxacum officinale* by 24% at 10^{-1} mM [24]. The well-known artemisinin, a sesquiterpene endoperoxide lactone isolated from the shoots of *Artemisia annua* [25], displayed no germination inhibition of *L. sativa* at 0.35 mM [26]. The sesquiterpene **2** also showed no effect on *L. sativa*, at the same concentration.

It could be seen that the activity of **2** is quite similar to the phytotoxicity exhibited by the above mentioned sesquiterpenes.

3. *Growth-Inhibititory Activity of Compounds* **1** *and* **2**. The growth-inhibition effect was evaluated by determining root length at germination sub-inhibitory concentrations that allow the growth of some seedlings. Both **1** and **2** inhibited root growth of the four species, with *P. miliaceum* being the most affected one (*Table 2*). Flavanone **1** was six times more active than **2** against *P. miliaceum*, but five times less active against *A. sativa*, while the activities of **1** and **2** against the two dicot seeds were similar. The isolated compounds were highly effective as germination inhibitors but less effective as root-growth inhibitors.

Table 2. *Root-Growth-Inhibitory Activity* (IC_{50}(root growth) [mM]; with 95% confidence intervals) *of* **1** *and* **2** *against* Panicum miliaceum, Avena sativa, Lactuca sativa, *and* Raphanus sativus

Compound	*P. miliaceum*	*A. sativa*	*L. sativa*	*R. sativus*
1	0.19 (0.16–0.24)	14.68 (8.67–24.76)	8.05 (5.97–10.82)	7.69 (5.86–10.12)
2	1.22 (1.09–1.43)	2.90 (2.23–3.74)	7.35 (4.24–8.78)	8.07 (4.05–16.09)

The effect of **1** on root growth turned out to be of about the same order of magnitude as the activity of other flavonoids; *e.g.*, tricin or 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone inhibited cress root elongation with IC_{50} values of 0.37 and 0.17 mM, respectively [27]. Some sesquiterpenes, such as podolactones isolated from *Acrosta-*

lagmus sp. fungus, were reported as strong root-growth inhibitors against *L. sativa* and *A. cepa*, with a *ca.* 50% inhibition at 10^{-2} mM [28].

4. *Post-Emergent Effect of Compounds* **1**, **2**, *and Atrazine.* After treating the leaves of 14-day-old seedlings of *P. miliaceum*, *A. sativa*, *L. sativa*, and *R. sativus* with **1** and **2** at different concentrations, significant inhibitions of seedling dry weights were observed for all treatments (*Table 3*). When seedlings of *P. miliaceum* were treated with **1** at 20 mM, 50% growth inhibition was observed. At the same concentration, *A. sativa* showed 32% inhibition. There were similar effects when *L. sativa* and *R. sativus* seedlings were treated with **1** at 20 mM, showing 79 and 45% inhibitions, respectively (*Table 3*). The synthetic herbicide atrazine, assayed as positive control, produced similar inhibition at the same concentrations (*Table 3*). The growth of the mentioned species seedlings treated with **2** at 20 mM, showed 31, 31, 60, and 24% inhibition, respectively (*Table 3*). These post-emergent results in the test species indicate that the potency shown by **1** was similar to that of atrazine and higher than that of **2** as growth inhibitors.

Table 3. *Effects of* **1**, **2**, *and Atrazine on the Growth of 14-Day-Old Seedlings*

Compound	Concentration [mM]	Dry weight[a]) (Growth inhibition [%][b]))[c])			
		P. miliaceum	*A. sativa*	*L. sativa*	*R. sativus*
Control		149 ± 1.6^{f}	554 ± 8.4^{f}	62 ± 1.6^{g}	263 ± 2.5^{h}
1	20	77 ± 1.2^{a} (50)	376 ± 2.5^{b} (32)	15 ± 0.9^{b} (79)	154 ± 0.6^{b} (45)
	8	133 ± 2.6^{d} (11)	390 ± 4.0^{b} (30)	17 ± 1.4^{c} (73)	156 ± 1.5^{bc} (41)
	4	138 ± 1.0^{e} (8)	524 ± 1.8^{e} (5)	27 ± 2.0^{e} (56)	135 ± 1.0^{a} (49)
2	20	103 ± 2.1^{e} (31)	383 ± 3.5^{b} (31)	25 ± 1.7^{de} (60)	199 ± 3.0^{g} (24)
	8	105 ± 1.9^{e} (30)	413 ± 3.0^{e} (25)	35 ± 1.2^{f} (44)	160 ± 2.5^{d} (39)
	4	101 ± 2.0^{e} (32)	446 ± 2.4^{d} (19)	34 ± 1.8^{f} (45)	160 ± 1.0^{d} (39)
Atrazine	20	83 ± 3.2^{b} (45)	326 ± 2.5^{a} (41)	12 ± 1.0^{a} (81)	157 ± 1.2^{e} (40)
	8	104 ± 2.4^{e} (30)	449 ± 1.7^{d} (19)	23 ± 1.2^{d} (63)	162 ± 0.8^{e} (38)
	4	100 ± 3.8^{e} (31)	550 ± 1.0^{f} (0.1)	34 ± 0.6^{f} (45)	175 ± 1.2^{f} (33)

[a]) Dry weight was determined 7 days after the topical treatment of 14-day-old seedlings; mean values of three replicated (100 plants per replicate) expressed in mg. [b]) Values in parentheses indicate growth inhibition compared to control. [c]) Within columns, different superscript letters indicate significant differences (*Kruskal–Wallis*, followed by *Dunn*; $P < 0.05$).

5. Allium cepa *Test.* The phytotoxic effects of **1** and **2** were also evaluated by the *A. cepa* test, and a mitotic index (*MI*), a parameter that estimates the frequency of cellular division of *Allium* root tips, was determined for each compound. The *MI* of the control assay was 5.1, while treatments with **1** at 20, 8, 4 and 2 mM showed *MI* values of 0, 0, 0 and 1.3, respectively (*Table 4*). Onion bulbs treated with **2** exhibited *MI* values of 0, 0.5, 1.1, and 4.1 at 20, 8, 4 and 2 mM, respectively (*Table 4*). Atrazine inhibited *A. cepa* mitosis, with *MI* values of 0, 0, 0, and 1.5 at 20, 8, 4 and 2 mM, respectively (*Table 4*). A reduction in the *MI* indicated that the number of cells entering mitotic division decreased, indicating that the test compounds produced cell cycle disturbances [29].

Table 4. *Mitotic Index and Mitotic Phases of Onion Root Meristem Cells Exposed to Different Concentrations of* **1**, **2**, *and Atrazine*

Compound	Concentration [mM]	Cells in mitosis[a])	Mitotic index (mean ± SE)	Mitotic phase index		
				Prophase [%]	Metaphase [%]	Ana-Telophase [%]
Control		153	5.1 ± 1.6	41.2 ± 3.2	27.45 ± 3.5	31.37 ± 2.08
1	20	0	0	0	0	0
	8	0	0	0	0	0
	4	0	0	0	0	0
	2	39	1.3 ± 1.12	46.15 ± 2.12	15.38 ± 0.70	38.46 ± 1.41
	1	144	4.8 ± 2.82	47.9 ± 0.70	18.75 ± 0.85	33.3 ± 1.2
2	20	0	0	0	0	0
	8	15	0.50 ± 0.19	40 ± 1	33.4 ± 0.58	26.7 ± 0.5
	4	33	1.1 ± 0.83	39.4 ± 1.15	30.3 ± 0.60	30.3 ± 1.18
	2	123	4.1 ± 1.20	43.9 ± 2.0	17.07 ± 1.57	39.02 ± 2.64
	1	138	4.6 ± 1.76	47.82 ± 1.41	16.30 ± 5.65	35.86 ± 3.53
Atrazine	20	0	0	0	0	0
	8	0	0	0	0	0
	4	0	0	0	0	0
	2	45	1.5 ± 0.38	53.3 ± 1.0	40 ± 0.8	6.67 ± 0.2
	1	75	2.5 ± 0.43	56.0 ± 0.71	24.0 ± 0.9	28.0 ± 1.41
	0.5	105	3.5 ± 1.1	62.86 ± 1.5	30.0 ± 1.06	24.3 ± 0.36

[a]) Total number of examined cells, *ca.* 3000.

Bulbs treated with atrazine at 2 mM showed chromosome abnormalities, mainly in C-metaphase, 26% of the cells presented this abnormality; however, onions treated with **1** and **2** showed no chromosome aberrations. These results were similar to those reported by *Srivastava* and *Mishra* for atrazine [30]. Previous studies about Amaicha del Valle propolis containing **1** revealed that propolis was not able to induce chromosomal damage [31]. In the case of artemisinin, mitotic inhibition was observed at 100 μM but *ca.* 0.8% of those cells showed chromosomal aberrations, while **1** and **2** produced inhibitory effects without any chromosomal abnormalities whatsoever [26].

Conclusions. – In conclusion, **1** and **2** were identified as inhibitors of germination of mono- and dicot species. They also caused a reduction of root length in pre-emergence treatment and a substantial reduction in plant weight in the post-emergence assay. The *A. cepa* test revealed that **1** and **2** are capable of inhibiting cell division with a potency comparable to that of atrazine and without the chromosomal aberrations produced by the latter.

These findings indicate that pinocembrin (**1**) and ilicol, (**2**) or *F. oolepis* aerial parts could be used as phytotoxic material for weed control and might offer new and effective tools in organic agriculture.

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Experimental Part

Plant Material. Aerial parts of *F. oolepis* were collected in Traslasierra Valley, Córdoba, Argentina, in March 2006. A voucher specimen (UCC 135) was deposited with the Herbarium Marcelino Sayago of the School of Agricultural Sciences, Catholic University of Córdoba, and identified by the agronomist *Gustavo Ruiz*.

General Exper. Procedures and Apparatus. Pinocembrin (**1**; used as a chromatographic standard) was purchased from *Sigma Chemical Co. Inc.* (St. Louis, MO). Anal. TLC: silica gel *60 F_{254} Merck* plates (D-Darmstadt). Silica gel, grade 200–400 mesh, 60 Å, for column chromatography (CC) and (D_6)DMSO were purchased from *Sigma Chemical Co., Inc.* (St. Louis, MO). All other solvents were purchased from *Merck* (D-Darmstadt) and *Fischer Scientific* (New Jersey, NJ). Atrazine and 2,4-D were gifts from Prof. *A. Santiago* (National University of Córdoba) and the firm *Atanor S. A.*, resp. For quantifying the pure compound, HPLC was performed on a *Phenomenex Prodigy 5 μ ODS* (4.6 mm i.d. × 250 mm) reversed-phase (RP) column, and it was eluted with 80% MeOH in H_2O with 1% CF_3COOH (TFA) as mobile phase and UV detection at 280 nm. The optical rotation angle was measured in a *JASCO DIP-370* spectropolarimeter. UV Spectra: in EtOH on a *Hewlett Packard 8452 A* diode array spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: in $CDCl_3$ with the *Bruker AVANCE II 400* spectrometer operated at 400 (^{1}H) and at 100 MHz (^{13}C), TMS as internal reference (δ 0.00 ppm) for the spectra recorded in (D_6)DMSO; *J* in Hz. Electron-impact mass spectra (EI-MS): at 70 eV by GC/MS on a *Hewlett-Packard 5970 Series* mass spectrometer, interfaced with a *Hewlett-Packard 5890* gas chromatograph fitted with a column (*HP-5MS*, 15 m × 0.25 mm i.d., temp. from 100° to 200°, 10°/min); in *m/z* (rel. int. in %).

Extraction and Isolation of the Active Compounds. To isolate the compound responsible for the germination-inhibitory activity of *F. oolepis*, a bio-guided fractionation of the EtOH extract against *R. sativus* and *P. miliaceum* was carried out. Air-dried aerial parts of *F. oolepis* (97 g) were extracted with EtOH for 24 h at r.t. After removal of the solvent (reduced pressure), extract was obtained (7.8 g, 8.04% yield). The extract (1.8 g) was fractionated by CC (silica gel), and 19 fractions were eluted with a

gradient of hexane/Et_2O and MeOH. Two fractions showed activity. The active *Frs. 7–9* (*GI*% of 97 at 10 mg/ml), which were eluted with hexane/Et_2O 50:50, were then rechromatographed on silica gel with a gradient of hexane/Et_2O from 100% hexane to 100% Et_2O. A crystalline solid was isolated (258 mg, 1.11% yield with respect to plant material) and identified as *pinocembrin* (**1**) by the ^1H and ^{13}C-NMR, DEPT, and MS techniques [9][10]. The *Frs. 16–19* (*GI*% 80 at 10 mg/ml), eluted with hexane/Et_2O 25:75, were then rechromatographed on silica gel with a gradient of hexane/Et_2O from 100% hexane to 100% Et_2O, yielding 42 mg of a crystalline solid (0.19% yield with respect to plant material), which was identified as *ilicol* (**2**) [16] by ^1H-NMR, ^{13}C-NMR, DEPT, and COSY.

Pinocembrin (*=(2S)-2,3-Dihydro-5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one*; **1**). White powder. M.p. 194–195°. $[\alpha]_D^{20} = -22.81$ (*c* = 0.86, EtOH). ^1H NMR: 2.77 (*dd*, *J* = 17.2, 3.2, H_a–C(3)); 3.06 (*dd*, *J* = 12.8, 17.2, H_b–C(3)); 5.44 (*dd*, *J* = 3.2, 12.8, H–C(2)); 5.52 (*d*, *J* = 2.2, H–C(6)); 6.01 (*d*, *J* = 2.2, H–C(8)); 7.39–7.45 (*m*, 5 arom. H). ^{13}C-NMR: 40.45 (C(3)); 80.17 (C(2)); 95.94 (C(8)); 96.84 (C(6)); 102.69 (C(10)); 127.47 (C(2′,6′)); 129.39 (C(4′)); 129.46 (C(3′,5′)); 139.59 (C(1′)); 163.59 (C(9)); 164.41 (C(5)); 167.62 (C(7)); 196.75 (C(4)). EI-MS: 256 (100, M^+), 179 (82), 152 (67), 124 (52), 96 (31), 69 (34). The spectral data were identical to previously published data for pinocembrin [32–34].

Ilicol (*=(1R,4aR,7R,8aR)-Decahydro-7-(3-hydroxyprop-1-en-2-yl)-1,4a-dimethylnaphthalen-1-ol*; **2**). White powder. M.p. 130–131°. $[\alpha]_D^{20} = -28$ (*c* = 0.80, EtOH). ^1H-NMR: 0.84 (*s*, Me–C(4a)); 0.95 (*s*, Me–C(1)); 1.00–1.18 (*m*, CH_2(3), CH_2(4)); 1.24–1.50 (*m*, CH_2(2), CH_2(5), CH_2(6), H–C(8a)); 1.61 (*dd*, *J* = 1.2 , 11.2, CH_2(8)); 1.85–1.92 (*m*, H–C(7)); 3.91 (*s*, OH)); 3.92 (*s*, OH)); 3.99 (*s*, CH_2OH); 4.80, 4.95 (2*s*, 1 H each, $=CH_2$). ^{13}C-NMR: 19.04 (*Me*–C(4a)); 20.29 (C(3)); 22.96 (*Me*–C(1)); 26.48 (C(6)); 27.56 (C(8)); 34.59 (C(4a)); 41.38 (C(5) 42.09 (C(7)); 43.52 (C(4)); 44.98 (C(2)); 54.70 (C(8a)); 63.44 (CH_2OH)); 70.46 (C(1)); 106.47 (C=CH_2); 155.33 (*C*=CH_2). The spectral data were identical to previously published data for ilicol [16].

Bioassays against P. miliaceum, A. sativa, L. sativa, *and* R. sativus. A filter paper (*Whatman N° 1*), and 12, 9, and 6 seeds of *P. miliaceum*, *L. sativa*, and *R. sativus*, resp., were placed in each well of a 12-multiwell plate (*Corning Inc.*, Corning, NY). Seven *A. sativa* seeds were placed in each well of a 6-multiwell plate (*Corning Inc.*, Corning, NY). Test fractions or pure compounds were dissolved in acetone and mixed with dist. H_2O. The final concentration of acetone in H_2O was 3%. This soln. (500 µl) was added to each test well. Only acetone and H_2O were added to each control well. Pure compounds were evaluated at 0.125, 0.25, 0.5, 1, 2, 4, 8, and 20 mM. Plates were covered, sealed with *Parafilm*, and incubated in the dark at 26° in a growth chamber. Phytotoxicity was expressed as $GI\% = [1 - (G_t/G_c)] \times 100$, where G_t and G_c are the number of germinated seeds in treatment and control, resp. IC_{50} (germination) values were calculated by the *Probit* method based on *GI*% values.

Treatment and control assays were performed in triplicate. The phytotoxicity for *P. miliaceum*, *A. sativa*, *L. sativa*, and *R. sativus* was also evaluated by measuring root lengths, and $IL\% = [1 - (L_t/L_c)] \times 100$, was estimated, where L_t and L_c were the root length in treatment and control, resp. IC_{50} (root growth) values were calculated by the *Probit* method based on *IL*% values.

Effect of **1** *and* **2** *against* P. miliaceum, A. sativa, L. sativa, *and* R. sativus *in Post-Emergent Assay.* Seedlings ($n = 100$) were grown in plastic containers (50 ml) filled with vermiculite as substrate, which were watered twice a week with a soln. of 13 mg of $NaNO_3$, 33 mg of K_2PO_4/ml. Foliar applications were made when seedlings were *ca.* 2 weeks old, with 10 µl of solvent per leaf for the control and 10 µl of soln. per leaf (2, 4, 8, and 20 mM) of **1** and **2**. Treatment and control were conducted in triplicate. All pots were stored in a growth chamber under fluorescent lighting, at 28° and 60% humidity. Plants were kept there for 7 d. Dry weights of plants were measured after drying until constant weight was reached. Mean dry weights and average growth inhibition were calculated [35].

Mitotic Indexing. Onion bulbs were placed in water for a week. An appropriate amount of **1**, **2**, or atrazine, dissolved in acetone, was mixed with H_2O for a final concentration of 0.5, 1, 2, 4, 8, and 20 mM. A mixture of acetone and H_2O was used for the control. The final concentration of acetone was 3% in H_2O in the test as well in the control soln. The bulbs were put over the appropriate containers in contact with the solns. at r.t. for 24 h. The roots were cut and placed in *Carnoy* fixative soln. for 2 h. They were then changed to EtOH at 40 and 70%. The root tips were hydrolyzed in 1N HCl for 15 min and then stained in the dark for 1 h using *Schiff*'s reagent, and fragmented with a glass rod in 45% AcOH with carmine red. Mitotic stages were observed in at least 1,000 cells per slide at a magnification of 40× with an optical

microscope. Three replicates for treatment and control were performed. The mitotic index (*MI*) was calculated as the ratio between dividing cells and total examined cells. The frequency of each mitotic phase was calculated as the percentage in relation to dividing cells counted in mitosis, whereas chromosomal abnormalities were calculated as the percentage in relation to examined total cells [36].

Statistical Analyses. The results of inhibition of germination were analyzed by the *t*-test ($P<0.05$), and IC_{50} (germination and root growth) values were calculated by the *Probit* method based on the percentage of inhibition obtained at each concentration of **1** and **2**. For each compound, results from the post-emergent assay were compared between concentrations by ANOVA followed by the *Kruskal–Wallis* test. Differences were considered significant at $p \leq 0.05$.

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