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A N I N T E R N A T I O N A L J O U R N A L

Phytotoxicity of Perlatolic Acid and Derivatives

Marize Terezinha Peres[1]; Ana Carina S. Cândido[1]; Odival Faccenda[2]; Aline Siqueira Gianini[3]; Neli Kika Honda[3*].

[1]Universidade Federal de Mato Grosso do Sul, Laboratory of Natural Insecticides, Campo Grande, Mato Grosso do Sul, Brasil; [2] Universidade Federal de Mato Grosso do Sul, Department of Computing and Information Sciences, Dourados, Mato Grosso do Sul, Brasil; [3] Universidade Federal de Mato Grosso do Sul, Institute of Chemistry, Campo Grande, Mato Grosso do Sul, Brasil.

ABSTRACT

*Perlatolic acid (**1**), methyl perlatolate (**2**), and the products of perlatolic acid alcoholysis—namely, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, n-pentyl, and n-hexyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**3-11**)—were evaluated for their herbicidal potential on Lactuca sativa L. (lettuce) and Allium cepa L. (onion) seeds. The compounds exhibited low phytotoxicity on L. sativa germination. Perlatolic acid (**1**) proved the most active compound (20%). Activity was lowest for n-hexyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**11**) (3.5%). The esters iso-propyl and sec-butyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**6** and **8**, respectively) inhibited root* (*35% e 43%, respectively*) *and hypocotyl growth* (*59% e 56%, respectively*)*. The esters n-butyl, n-pentyl, and n-hexyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**7**, **10**, and **11**, respectively) proved phytotoxic to A. cepa, delaying and reducing seed germination (27%), while n-pentyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**10**) was the most inhibitory for root (42%) and coleoptile growth (24%). The behaviors of iso-propyl, sec-butyl, and n-pentyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**6**, **8**,* and *__10__, respectively) suggest the potential utility of these esters as natural herbicides. The esters iso-propyl and sec-butyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**6** and **8**,* respectively*) may serve as model molecules in the investigation of potential herbicides for dicotyledon control, while n-pentyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (**10**) may serve the same function for monocotyledon species.*

Key words: Perlatolic acid, 2-hydroxy-4-methoxy-6-*n*-pentylbenzoates, *Lactuca sativa*, *Allium cepa*, allelopathy.

[*]Authors for correspondence: nelihonda8@gmail.com

INTRODUCTION

Pesticide consumption in Brazil ranks among the highest in the world, comprising 366 active ingredients from 200 chemical groups, in the form of 1458 formulations, including fungicides, herbicides, nematicides, insecticides, and other products. Herbicides account for 48% of this market [1,2]. Despite the significant effect of these products on agricultural quality and productivity, the indiscriminate use of synthetic herbicides has led to increased weed resistance, environmental pollution, and health risks for individuals exposed to these formulations [3,4].

The search for novel products providing the same functionality as traditional pesticides and herbicides, while posing no risks to the environment or human health, has engaged a great number of laboratories. For this purpose, natural products, as well as those obtained by structural modification, have been viewed as suitable candidates, given their low toxicity to a wide range of life forms and their potential ability to avert soil and plant contamination [5-7].

Amongst naturally synthesized compounds, phenols constitute a class characterized by ample structural diversity and numerous biological activities. Irrespective of their complexity, phenols share a common feature: the presence of at least one aromatic ring containing one or more hydroxyls. In addition to hydroxyls, other substituents—*e.g.*, alkyl chains, carboxyl groups, aldehyde groups—are found in a large number of natural substances, such as alkylphenols, alkylresorcinols, anacardic acids, and alkylcatechols. Of these, alkylresorcinols are by far the most prevalent in nature, occurring in higher plants, fungi, and bacteria [8-10]. The potent antimicrobial activity demonstrated *in vitro* for many naturally occurring alkylresorcinols and their derivatives has led to the widely held view that these compounds play a primarily defensive role in plants [11].

Lichens, symbiotic association of a fungus and one or more algae produce secondary metabolites via three routes. The acetate-polimalonate is the main pathway of biosynthesis of phenolic compounds, such as, depsides, depsidones, dibenzofuranes, xanthones, anthraquinones, chromones and usnic acid, among others. Pulvinic acid derivatives and terphenylquinones are produced through shikimate pathway, while terpenes, sterols and carotenoids are synthesized via mevalonate pathway [12].

Because of their broad spectrum of activities, phenolic compounds have been the focus of growing interest. Substances produced by lichens have noteworthy properties, including antimicrobial and antitumoral activities and inhibition of enzymes such as lipoxygenases and phenolases, as well as viral enzymes such as HIV-1 integrase and HSV-1 [12-14]. In addition to these activities of pharmacological interest, phenolic substances produced by lichens can inhibit germination and growth in several plant species [15,16]. Our group has been investigating the effect of orsellinates on the germination and growth of *Lactuca sativa* L. (lettuce, dicotyledon) and *Allium cepa* L. (onion, monocotyledon), and has found that these esters do not affect germination in *L. sativa* ($p > 0.05$), but ethyl, *n*-propyl, *iso*-propyl, and *n*-butyl orsellinates influence this process in *A. cepa*. In both species, radicle growth was significantly inhibited by orsellinates, with the exception of methyl orsellinate, whereas in *A. cepa* all the orsellinates tested inhibited radicle growth ($p > 0.05$) [17]. Following on from our studies to obtain compounds with potential herbicide activity, this paper reports the results of bioassays conducted with perlatolic acid (**1**), methyl perlatolate (**2**), and products resulting from perlatolic acid alcoholysis (**3-11**) (Fig. 1) on germination and growth of *L. sativa* and *A. cepa*.

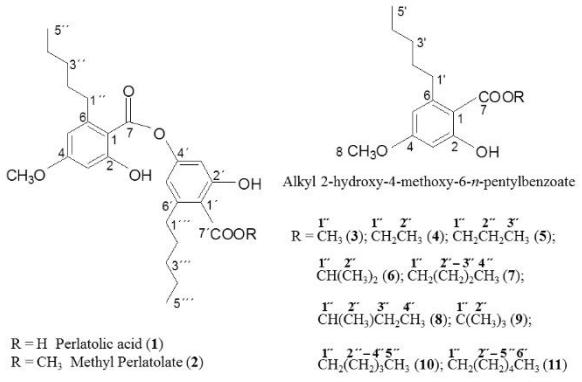

Figure 1. Compounds evaluated for herbicidal activity against *Lactuca sativa* and *Allium cepa* seeds.

MATERIAL AND METHODS

General Experimental Procedures

^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz, and ^{13}C NMR were acquired at 75 MHz in $CDCl_3$ on a Bruker DPX300 spectrometer. Solvent

resonances were used as the internal references. Column chromatography was carried out in a flash silica gel column (230-400 mesh). Sample purity was checked by TLC on pre-coated silica gel GF_{254} plates (0.25 mm thickness, Merck) under UV radiation (254 nm), while methanol : sulfuric acid (10%) and *p*-anisaldehyde : sulfuric acid were used as spraying reagents. Mass spectra (EI, 70 eV) were obtained on a Shimadzu CGMS QP2010 Plus gas chromatography mass spectrometer in direct injection mode. Melting points were acquired using a Uniscience do Brasil apparatus, model 498.

Extraction and Isolation of Perlatolic Acid

The lichen *Cladina confusa* (Sant.) Folmm. & Ahti was obtained from a shop of decoration products. The identification was conducted by Prof. Dr. Mariana Fleig from UFRGS and Prof. Dr. Marcelo P. Marcelli from the IBt – São Paulo. Voucher specimen were deposited at the Campo Grande Herbarium of the Universidade Federal de Mato Grosso do Sul (CGMS 40953). The dried lichen *C. confusa* (240.0 g) was extracted with hexane at room temperature, for four times. The extracts were concentrated and the residue was fractionated by silica gel CC, eluted with hexane/CH_2Cl_2 mixtures in gradient. Perlatolic acid (**1**) was eluted with hexane/CH_2Cl_2 1:9 v/v [18].

Perlatolic acid (**1**), 1% yield, white solid, mp 108–110 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.88 (m, CH_3-5″, CH_3-5‴), 1.30–1.65 (m, CH_2-4″, CH_2-4‴, CH_2-3″, CH_2-3‴, CH_2-2″, CH_2-2‴), 2.97 (m, CH_2-1″), 2.98 (m, CH_2-1‴), 3.83 (s, OCH_3), 6.38 (s, H-3, H-5), 6.62 (d, *J* = 2.4 Hz, H-5′), 6.74 (d, *J* = 2.4 Hz, H-3′), 11.34 (OH-2), 11.45 (s, OH-2′). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 14.0 (CH_3-5″, CH_3-5‴), 22.5 (C-4″*), 22.6 (C-4‴*), 31.4 (C-3″, C-3‴**), 31.9 (C-2″, C-2‴**), 36.6 (C-1‴), 37.2 (C-1″), 55.4 (OCH_3), 99.0 (C-3), 103.6 (C-1), 108.6 (C-1′), 109.0 (C-3′), 111.5 (C-5), 116.2 (C-5′), 148.4 (C-6), 150.0 (C-6′), 155.0 (C-4′), 164.9 (C-4) 165.3 (C-2′), 166.6 (C-2), 169.4 (C-7), 174.8 (C-7′) [19]. *,** signals may be interchanged.

Derivatives

Methyl Perlatolate (**2**)

Perlatolic acid (**1**) (0.54 mmol) was dissolved in acetone and 0.29 mmol of potassium carbonate was added. The mixture was cooled and stirred and 5.7 mmol of methyl iodide was added after 10 min. After 1 h, upon reaching room temperature, the mixture was stirred until completion of the reaction (TLC control) and then filtered. The solvent was subsequently evaporated and the residue was purified by column chromatography. Elution was conducted with hexane: $CHCl_3$ 1:4 v/v.

Methyl perlatolate (**2**), 57% yield, white amorphous solid, mp 45-47 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.88 (m, CH_3-5″, CH_3-5‴), 1.29 – 1.66 (m, CH_2-4″, CH_2-4‴, CH_2-3″, CH_2-3‴, CH_2-2″, CH_2-2‴), 2.92 (m, CH_2-1″, CH_2-1‴), 3.82 (s, OCH_3), 6.36 (s, H-3, H-5), 6.57 (d, *J* = 2.3 Hz, H-5′), 6.70 (d, *J* = 2.3 Hz, H-3′), 11.35 (OH-2), 11.45 (s, OH-2′). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 14.0 (CH_3-5″, CH_3-5‴), 22.4 (C-4″*), 22.5 (C-4‴*), 31.4(C-3″, C-3‴**), 32.0(C-2″, C-2‴**), 36.7 (C-1‴), 37.2 (C-1″), 52.3 (OCH_3-7′), 55.4 (OCH_3-4), 98.9 (C-3), 103.6 (C-1), 108.7 (C-1′), 110.0 (C-3′), 111.3 (C-5), 115.7 (C-5′), 148.3 (C-6), 148.4 (C-6′), 154.0 (C-4′),164.2 (C-4), 164.8 (C-2′), 166.5 (C-2), 169.5 (C-7), 171.5 (C-7′) [19]. *,** signals may be interchanged.

2-Hydroxy-4-Methoxy-6-n-Pentylbenzoates

Esters (**3** – **11**) were prepared through the reaction of perlatolic acid (**1**) (125 mg, 0.28 mmol) with 50 mL of alcohol (methanol, ethanol, *n*-propanol, *iso*-propanol, *n*-butanol, *sec*-butanol, *tert*-butanol, *n*-pentanol and *n*-hexanol) at 40 °C in a steam bath (TLC control). After completion of each reaction the mixture evaporated and the compounds were separated by silica gel CC with hexane: chloroform gradient. All the esters alkyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate were eluted with chloroform. In all reactions the esters (**3** – **11**) and 1,3-dihydroxy-5-*n*-pentylbenzene (olivetol) were obtained [18] (Fig. 2).

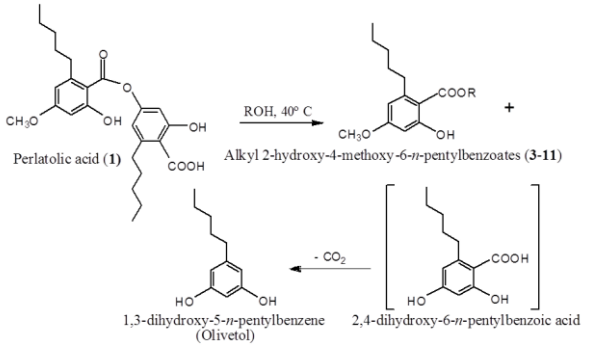

Figure 2. Alcoholysis of perlatolic acid (**1**) producing Alkyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoates(**3**-**11**), 2,4-dihydroxy-6-*n*-pentylbenzoic acid and 1,3-dihydroxy-5-*n*-pentylbenzene (olivetol).

The structures of all compounds were confirmed by ^{1}H, ^{13}C, and DEPT 135 spectra.

Methyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**3**), 30.7% yield, colourless oil. ^{1}H- NMR **(**300 MHz**,** $CDCl_3$**)** δ0.89 (t, *J*= 6.9 Hz, CH_3-5′), 1.32 (m, CH_2-3′, CH_2-4′), 1.51 (m, CH_2-2′), 2.83 (t, *J* = 7.8 Hz, CH_2-1′), 3.79 (s, OCH_3- 8), 3.91 (s, OCH_3-1′′), 6.28 (d, *J* = 2.7 Hz, H-3), 6.32 (d, *J* = 2.7 Hz, H-5), 11.72 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ 14.0 (C-5′), 22.5 (C-4′), 31.5 (C-2′), 32.1 (C-3′), 36.9 (C-1′), 51.8 (C-1′′), 55.3 (C-8), 98.8 (C-3), 104.6 (C-1), 110.6 (C-5), 148.1 (C-6), 164.0 (C-4), 165.5 (C-2), 171.9 (C-7). EI-MS *m*/*z*: 252.

Ethyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**4**), 22.3% yield, colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$**)** δ 0.89 (t, *J* = 6.9 Hz, CH_3-5′), 1.33 (m, CH_2-3′, CH_2-4′),1.40 (t, *J* = 7.2 Hz, CH_3-2′′), 1.55 (m, CH_2-2′), 2.85 (t, *J* = 7.8 Hz, CH_2-1′), 3.79 (s, OCH_3- 8),4.39 (m, *J* = 7.2 Hz, CH_2-1′′), 6.27 (d, *J* = 2.5 Hz, H-3), 6.32 (d, *J* = 2.5 Hz, H-5), 11.83 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ 14.1 (C-5′, C-2′′), 22.6 (C-4′), 31.7 (C-2′), 32.1 (C-3′), 37.0 (C-1′), 55.2 (C-8), 98.8 (C-3), 104.8 (C-1), 110.6 (C-5), 148.0 (C-6), 163.8 (C-4), 165.6 (C-2), 171.6 (C-7). EI-MS *m*/*z*: 266.

n-Propyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**5**), 36.5% yield, colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$**)** δ0.88 (t, *J* = 6.9 Hz, CH_3-5′), 1.03 (t, *J* = 7.5 Hz, CH_3-3′′), 1.32 (m, CH_2-3′, CH_2-4′), 1.54 (m, CH_2-2′), 1.80 (m, CH_2-2′′), 2.86 (t, *J* = 7.8 Hz, CH_2-1′), 3.79 (s, OCH_3- 8), 4.29 (m, *J* = 6.6 Hz, CH_2-1′′), 6.28 (d, *J* = 2.5 Hz, H-3), 6.32 (d, *J* = 2.5 Hz, H-5), 11.84 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ10.7 (C-3′′), 14.1 (C-5′), 21.9 (C-2′ ′), 22.6 (C-4′), 31.6 (C-2′), 32.0 (C-3′), 36.9 (C-1′), 55.3 (C-8), 67.1 (C-1′′), 98.8 (C-3), 104.8 (C-1), 110.6 (C-5), 148.0 (C-6), 163.8 (C-4), 165.6 (C-2), 171.6 (C-7). EI-MS m/z: 280.

iso-Propyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**6**) 20.5% yield, colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$**)** δ0.89 (t, *J* = 6.9 Hz, CH_3-5′) 1.33 (m, CH_2-3′, CH_2-4′), 1.38 (d, *J* = 6.4 Hz, CH_3-2′′), 1.55 (m, CH_2-2′), 2.85 (t, *J* = 7.7 Hz, CH_2-1′), 3.78 (s, OCH_3-8), 5.31 (m CH-1′′), 6.26 (d, *J* = 2.5 Hz, H-3), 6.32 (d, *J* = 2.5 Hz, H-5), 11.93 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ 14.1 (C-5′), 21.9 (C-2′′), 22.7 (C-4′), 31.9 (C-2′), 32.1 (C-3′), 37.1 (C-1′), 55.2 (C-8), 69.1 (C-1′′), 98.8 (C-3), 105.1 (C-1), 110.6 (C-5), 148.0 (C-6), 163.8 (C-4), 165.6 (C-2), 171.1 (C-7). EI-MS *m*/*z*: 280.

n-Butyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**7**) 35.8% yield, colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$**)** δ 0.89 (t, *J* = 6.9 Hz, CH_3-5′), 0.97 (t, *J* = 7.3 Hz, CH_3-4′′), 1.32 (m, CH_2-3′, CH_2-4′), 1.49 (m, CH_2-2′), 1.75 (m, CH_2-2′′), 2.85 (t, *J* = 7.8 Hz, CH_2-1′), 3.79 (s, OCH_3-8), 4.33 (t, *J* = 6.6 Hz, CH_2-1′′), 6.27 (d, *J* = 2.7 Hz, H-3), 6.32 (d, *J* = 2.7 Hz, H-5), 11.86 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ 13.7 (C-4′′*), 14.0 (C-5′*), 19.4 (C-3′′), 22.6 (C-4′), 30.6 (C-2′′), 31.7 (C-2′), 32.0 (C-3′), 36.9 (C-1′), 55.2 (C-8), 65.3 (C-1′′), 98.8 (C-3), 104.8 (C-1), 110.5 (C-5), 147.9 (C-6), 163.8 (C-4), 165.6 (C-2), 171.7 (C-7). *signals may be interchanged. EI-MS *m*/*z*: 294.

sec-Butyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**8**) 24.5% yield, colourless oil.^{1}H-NMR (300 MHz, $CDCl_3$**)** δ0.89 (t, *J* = 7.1 Hz, CH_3-5′), 0.97 (t, *J* = 7.1 Hz, CH_3-4′′), 1.31 (m, CH_2-3′, CH_2-4′), 1.35 (d, *J* = 6.4 Hz, CH_3-2′′), 1.54 (m, CH_2-2′), 1.72 (m, CH_2-3′′), 2.85 (m, CH_2-1′), 3.79 (s, OCH_3- 8), 5.17 (m, CH-1′′), 6.27 (d, *J* = 2.5 Hz, H-3), 6.32 (d, *J* = 2.5 Hz, H-5), 11.97 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ 9.9 (C-4′′), 14.1 (C-5′), 19.5 (C-2′′), 22.7 (C-4′), 28.9 (C-3′′), 31.9 (C-2′), 32.1 (C-3′), 37.0 (C-1′), 55.2 (C-8), 73.8 (C-1′′), 98.8 (C-3), 105.1 (C-1), 110.5 (C-5), 148.0 (C-6), 163.8 (C-4), 165.7 (C-2), 171.3 (C-7). EI-MS *m*/*z*: 294.

tert-Butyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**9**) 22.4% yield, colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$**)** δ0.89 (t, *J* = 6.8 Hz CH_3-5′), 1.32 (m, CH_2-3′, CH_2-4′), 1.52 (m, CH_2-2′), 1.60 (s,CH_3-2′′), 2.84 (t, *J* = 7.8 Hz, CH_2-1′), 3.78 (s, OCH_3- 8), 6.25 (d, *J* = 2.5 Hz, H-3), 6.31 (d, *J* = 2.5 Hz, H-5), 11.97 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ14.0 (C-5′), 22.8 C-4′), 31.8 (C-2′), 31.9 (C-3′), 36.9 (C-1′), 55.2 (C-8), 83.0 (C-1′′), 98.8 (C-3), 106.0 (C-1), 110.4 (C-5), 147.7 (C-6), 163.4 (C-4), 165.5 (C-2), 171.2 (C-7). EI-MS *m*/*z*: 294.

n-Pentyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**10**), 34.7% yield, colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$**)** δ 0.90 (m, CH_3-5′, CH_3-5′′), 1.33 (m, CH_2-3′, CH_2-4′), 1.40 (m, CH_2-4′′), 1.54 (m, CH_2-2′), 1.77 (m, CH_2-2′′, CH_2-3′′), 2.86 (t, *J* = 7.8 Hz, CH_2-1′), 3.79 (s, OCH_3- 8), 4.32 (t, *J* = 6.7 Hz, CH_2-1′′), 6.28 (d, *J* = 2.7 Hz, H-3), 6.32 (d, *J* = 2.7 Hz, H-5), 11.86 (s, OH). ^{13}C-NMR **(**75 MHz, $CDCl_3$) δ 13.9 (C-5′′*), 14.1 (C-5′*), 22.3 (C-4′**), 22.78 (C-5′′**), 28.3 (C-3′′), 31.7 (C-2′), 32.1 (C-3′), 36.9 (C-1′), 55.2 (C-8), 65.6 (C-1′′), 98.8 (C-3), 104.9 (C-1), 110.5 (C-5), 148.0 (C-6), 163.8 (C-4), 165.6

(C-2), 171.7 (C-7). *,** signals may be interchanged. EI-MS *m*/*z*: 308.

n-Hexyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**11**) 40.4% yield, colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.90 (m, CH_3-5′, CH_3-6′′), 1.32 (m, CH_2-3′, CH_2-4′), 1.40 – 1.56 (m, CH_2-2′, CH_2-4′′), 1.76 (m, CH_2-2′′), 2.85 (t, J = 7.9 Hz, CH_2-1′), 3.78 (s, OCH_3- 8), 4.32 (t, J = 6.7 Hz, CH_2-1′′), 6.27 (d, J = 2.5 Hz, H-3), 6.32 (d, J = 2.5 Hz, H-5), 11.86 (s, OH). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 14.0 (m, C-5′, C-6′′), 22.5 (C-4′*), 22.6 (C-5′′*), 25.8 (C-4′′), 28.6 (C-3′′), 31.6 (C-2′), 32.0 (C-3′), 36.9 (C-1′), 55.2 (C-8), 65.6 (C-1′′), 98.7 (C-3), 104.8 (C-1), 110.5 (C-5), 147.9 (C-6), 163.8 (C-4), 165.6 (C-2), 171.7 (C-7). * signals may be interchanged. EI-MS *m*/*z*: 322.

Phytotoxicity Bioassays

Bioassays with *L. sativa* and *A. cepa* were carried out in Petri dishes (90 mm diameter) containing a grade 1 Whatman paper sheet [20]. For germination and growth bioassays, a 10^{-3} M solution was prepared using DMSO (0.1% v:v) [21] and further diluted to 10^{-4}, 10^{-5}, and 10^{-6} M. The solutions were then amended with 2-(N-morpholino)ethanesulfonic acid (MES) (10 mM), and pH values were adjusted to 6.0 with 0.1 M KOH [20]. Fifty seeds of either target species were distributed per dish and four replicates were performed for each treatment. Control dishes were prepared under the same conditions, but in the absence of test substances.

The prepared Petri dishes were placed in a germination chamber (BOD) under constant humidity (approximately 80%), as follows: 25 ± 2 °C under constant 160 W lighting for *L. sativa* and 15 ± 2 °C under a 12 h, 160 W photoperiod for *A. cepa* [22]. The seeds were counted daily (*A. cepa*) or twice daily (*L. sativa*). Germinated seeds were considered those with a radicle protrusion of at least 2.0 mm. The experiment was halted when no further germination had occurred for three consecutive days.

After three days of radicle protrusion, the main roots and hypocotyls/coleoptiles of ten plantules per dish were measured using millimetric paper. These plantules were subsequently dried to a constant weight in an oven at 60 °C for measurement of dried mass. Bioassays with commercial herbicides were performed as positive controls—namely, Glyphosate 480 Agripec (glyphosate), Basagran 600 (bentazon), and Atrazine Nortox 500 SC (atrazine) for *L. sativa* and Glyphosate, Gesagard 500 SC (prometryne), and Poast (sethoxydim) for *A. cepa*. For the bioassays containing herbicides, the concentrations (10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M) and conditions reported by Macías et al. [20] were employed.

The parameters evaluated (germination speed index (*GSI*), germination percentage (*G*%), root and hypocotyl/coleoptile growth, and plantule dried mass) were subjected to analysis of variance, followed by Dunnett's test whenever the effect of treatment differed significantly ($p < 0.05$) from controls. Growth results were expressed as percent differences, relative to controls, with 0 representing controls, and positive and negative values indicating stimulation and inhibition, respectively [23]. *GSI* was calculated as $\Sigma(G_i/N_i)$, where G_i is the number of germinated seeds in a time interval $t_{i-1} \leftrightarrow t_i$ and N_i is the number of days after sowing [24]. *G*% was calculated as $(\Sigma n_i \times N^{-1}) \times 100$, where n_i is the number of germinated seeds in a time interval $t_{i-1} \leftrightarrow t_i$ and N is the number of seeds used in each treatment [25].

RESULTS AND DISCUSSION

Overall, the compounds evaluated did not affect *G*% values, despite reducing *GSI* values for *L. sativa* (Table 1). A similar behavior was observed for the commercial herbicides tested (positive controls).

Table 1. Effects of compounds **1-11** and commercial herbicides on *Lactuca sativa* germination.

	Germination speed index (*GSI*; mean ± SD)				
Compound	**Control**	**10^{-3} M**	**10^{-4} M**	**10^{-5} M**	**10^{-6} M**
1	98.40 ± 1.34	79.00 ± 1.54*	86.00 ± 0.93*	91.60 ± 1.27*	93.50 ± 2.62*
2	98.40 ± 1.34	91.50 ± 2.92*	91.80 ± 1.30*	94.00 ± 2.14*	95.20 ± 1.60^{ns}
3	98.40 ± 1.34	91.70 ± 2.03*	92.70 ± 2.48*	95.00 ± 2.83^{ns}	97.00 ± 0.82^{ns}
4	98.40 ± 1.34	86.60 ± 2.39*	88.20 ± 2.27*	92.20 ± 2.67*	93.30 ± 0.32*
5	98.40 ± 1.34	90.50 ± 2.48*	92.50 ± 0.70*	92.90 ± 1.34*	93.70 ± 2.51*
6	98.40 ± 1.34	91.20 ± 2.70*	91.30 ± 2.36*	92.60 ± 1.52*	94.20 ± 1.57*

7	98.40 ± 1.34	93.40 ± 1.76*	93.80 ± 2.96*	94.30 ± 1.32*	94.50 ± 1.75*
8	98.40 ± 1.34	89.80 ± 2.35*	91.10 ± 1.75*	92.50 ± 1.93*	93.90 ± 1.67*
9	98.40 ± 1.34	87.20 ± 2.38*	88.80 ± 1.73*	92.20 ± 2.76*	92.70 ± 2.52*
10	98.40 ± 1.34	89.30 ± 1.19*	92.40 ± 2.50*	96.00 ± 2.86^{ns}	96.70 ± 2.72^{ns}
11	98.40 ± 1.34	94.90 ± 2.06^{ns}	94.90 ± 2.53^{ns}	95.40 ± 2.08^{ns}	96.10 ± 2.74^{ns}
Glyphosate	98.40 ± 1.34	93.60 ± 2.00*	95.30 ± 0.60^{ns}	96.30 ± 0.50^{ns}	96.50 ± 0.60^{ns}
Basagran	98.40 ± 1.34	85.60 ± 1.20*	91.90 ± 1.90*	92.20 ± 2.20*	93.50 ± 1.50*
Atrazine	98.40 ± 1.34	85.30 ± 2.20*	92.50 ± 1.30*	92.60 ± 1.40*	96.30 ± 1.10^{ns}

Percentage of germination (*G*%; mean ± SD)

Compound	Control	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
1	99.50 ± 1.00	96.59 ± 1.00^{ns}	97.00 ± 2.00^{ns}	97.00 ± 2.00^{ns}	98.50 ± 1.90^{ns}
2	99.50 ± 1.00	96.00 ± 1.60^{ns}	98.00 ± 2.30^{ns}	98.50 ± 1.90^{ns}	98.50 ± 1.00^{ns}
3	99.50 ± 1.00	98.00 ± 2.30^{ns}	98.00 ± 1.60^{ns}	99.00 ± 1.20^{ns}	99.50 ± 1.00^{ns}
4	99.50 ± 1.00	95.50 ± 1.90*	97.00 ± 2.60^{ns}	98.00 ± 1.20^{ns}	99.00 ± 1.60^{ns}
5	99.50 ± 1.00	96.00 ± 1.60^{ns}	97.00 ± 2.60^{ns}	98.00 ± 1.60^{ns}	99.00 ± 1.20^{ns}
6	99.50 ± 1.00	95.00 ± 2.60*	97.50 ± 2.50^{ns}	98.50 ± 1.90^{ns}	99.00 ± 1.20^{ns}
7	99.50 ± 1.00	95.50 ± 1.00*	96.50 ± 2.50^{ns}	97.00 ± 1.00^{ns}	97.50 ± 1.20^{ns}
8	99.50 ± 1.00	97.00 ± 2.00^{ns}	97.50 ± 1.90^{ns}	98.50 ± 1.00^{ns}	99.00 ± 2.00^{ns}
9	99.50 ± 1.00	97.50 ± 1.90^{ns}	97.50 ± 1.90^{ns}	98.00 ± 1.60^{ns}	99.00 ± 1.20^{ns}
10	99.50 ± 1.00	96.00 ± 2.80^{ns}	97.50 ± 1.90^{ns}	98.00 ± 1.60^{ns}	99.00 ± 2.00^{ns}
11	99.50 ± 1.00	96.00 ± 2.30^{ns}	98.00 ± 2.30^{ns}	98.50 ± 1.90^{ns}	98.50 ± 1.90^{ns}
Glyphosate	99.50 ± 1.00	97.50 ± 1.90^{ns}	98.50 ± 1.00^{ns}	99.50 ± 1.00^{ns}	99.50 ± 1.00^{ns}
Basagran	99.50 ± 1.00	96.00 ± 2.80^{ns}	97.50 ± 1.90^{ns}	98.50 ± 1.90^{ns}	98.50 ± 1.90^{ns}
Atrazine	99.50 ± 1.00	96.00 ± 1.60^{ns}	96.50 ± 1.00^{ns}	98.50 ± 1.00^{ns}	99.00 ± 1.20^{ns}

*Significant difference from controls ($p < 0.05$) on Dunnett's test.
ns: non-significant difference from controls.

Growth inhibition was strongest for isopropyl and *sec*-butyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**6** and **8**, respectively), which inhibited root and hypocotyl protrusion and reduced the accumulation of dried biomass in *L. sativa* plantules (Fig. 3), thus behaving similarly to the herbicides Glyphosate and Basagran. Extracts of nine green seaweed species were examined based on the germination and growth of lettuce seedlings, which found that these extracts generally showed low anti-germination activities [26].

In *A. cepa*, *n*-butyl, *n*-pentyl, and *n*-hexyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**7**, **10**, and **11**, respectively) delayed germination and reduced *G*% values, similarly to the commercial herbicides tested on this species (Table 2).

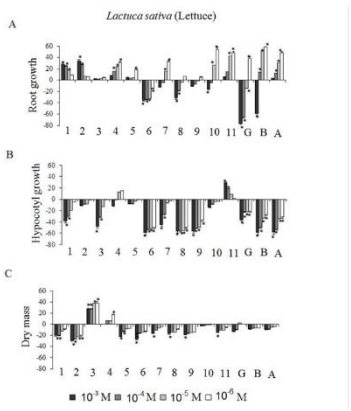

Figure 3. Effects of compounds **1**-**11** and commercial herbicides Glyphosate (G), Basagran (B), and Atrazine (A) on *Lactuca sativa* plantule growth and dried mass accumulation. Values expressed as percentages relative to controls. *Significant differences from controls (Dunnett's test).

Table 2. Effect of compounds **1**-**11** and commercial herbicides on *Allium cepa* germination.

Germination speed index (*GSI*; mean ± SD)

Compounds	Control	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
1	9.00 ± 0.37	8.30 ± 0.51^{ns}	8.60 ± 0.58^{ns}	8.60 ± 0.80^{ns}	9.40 ± 0.70^{ns}
2	9.00 ± 0.37	8.20 ± 0.62^{ns}	8.70 ± 0.20^{ns}	8.80 ± 0.99^{ns}	9.30 ± 0.43^{ns}
3	9.00 ± 0.37	8.50 ± 0.54^{ns}	8.80 ± 0.69^{ns}	8.90 ± 0.05^{ns}	9.10 ± 0.45^{ns}
4	9.00 ± 0.37	8.50 ± 0.25^{ns}	8.20 ± 0.21^{ns}	8.60 ± 0.34^{ns}	8.70 ± 0.45^{ns}

5	9.00 ± 0.37	8.00 ± 0.24^{ns}	8.50 ± 0.57^{ns}	8.60 ± 0.67^{ns}	8.90 ± 0.79^{ns}
6	9.00 ± 0.37	8.70 ± 0.22^{ns}	9.10 ± 0.10^{ns}	9.40 ± 0.39^{ns}	9,40 ± 0.67^{ns}
7	9.00 ± 0.37	6.60 ± 0.55*	6.10 ± 0.48*	6.90 ± 0.34*	7.20 ± 0.57*
8	9.00 ± 0.37	8.60 ± 0.25^{ns}	8.90 ± 0.58^{ns}	9.10 ± 0.48^{ns}	9.10 ± 0.54^{ns}
9	9.00 ± 0.37	8.80 ± 0.17^{ns}	8.80 ± 0.59^{ns}	8.90 ± 0.63^{ns}	9.10 ± 0.29^{ns}
10	9.00 ± 0.37	6.60 ± 0.60*	6.80 ± 0.52*	7.00 ± 0.33*	7.40 ± 0.06*
11	9.00 ± 0.37	6.40 ± 0.67*	6.80 ± 0.20*	6.90 ± 0.41*	7.50 ± 0.48*
Glyphosate	9.00 ± 0.37	6.40 ± 0.80*	7.50 ± 0.30*	7.70 ± 0.70*	7.90 ± 0.30*
Gesagard	9.00 ± 0.37	7.80 ± 0.50*	7.90 ± 0.50*	7.90 ± 0.80*	8.00 ± 0.50^{ns}
Poast	9.00 ± 0.37	6.10 ± 0.70*	7.70 ± 0.30*	8.00 ± 0.60^{ns}	8.30 ± 0.20^{ns}

	Percentage of germination (*G*%; mean ± SD)				
Compounds	**Control**	**10^{-3} M**	**10^{-4} M**	**10^{-5} M**	**10^{-6} M**
1	92.50 ± 3.00	91.00 ± 1.20^{ns}	93.00 ± 2.00^{ns}	94.50 ± 1.00^{ns}	99.50 ± 1.00*
2	92.50 ± 3.00	91.00 ± 2.60^{ns}	93.50 ± 2.50^{ns}	96.00 ± 2.80^{ns}	98.00 ± 1.60^{ns}
3	92.50 ± 3.00	96.50 ± 1.90^{ns}	96.00 ± 1.60^{ns}	95.00 ± 1.20^{ns}	93.00 ± 3.50^{ns}
4	92.50 ± 3.00	84.00 ± 1.60*	88,00 ± 2,80^{ns}	90.50 ± 2.50^{ns}	94.00 ± 1.60^{ns}
5	92.50 ± 3.00	86.50 ± 1.00*	92.00 ± 2.80^{ns}	94.50 ± 2.50^{ns}	96.00 ± 1.60^{ns}
6	92.50 ± 3.00	89.00 ± 2.60^{ns}	94.00 ± 2.80^{ns}	94.50 ± 1.90^{ns}	96.50 ± 2.50^{ns}
7	92.50 ± 3.00	86.50 ± 2.50*	88.00 ± 2.30^{ns}	88.50 ± 1.90^{ns}	95.00 ± 2.60^{ns}
8	92.50 ± 3.00	95.50 ± 3.40^{ns}	93.00 ± 2.60^{ns}	92.50 ± 1.90^{ns}	90.50 ± 1.00^{ns}
9	92.50 ± 3.00	91.00 ± 1.20^{ns}	91.50 ± 3.00^{ns}	91.50 ± 3.00^{ns}	92.50 ± 3.80^{ns}
10	92.50 ± 3.00	80.00 ± 0.00*	86.00 ± 2.80*	87.00 ± 1.20*	93.00 ± 2.60^{ns}
11	92.50 ± 3.00	87.00 ± 2.00*	88.50 ± 1.00^{ns}	87.50 ± 1.90^{ns}	94.50 ± 1.90^{ns}
Glyphosate	92.50 ± 3.00	83.50 ± 2.50*	85.00 ± 2.60*	88.00 ± 2.30^{ns}	88.50 ± 1.90^{ns}
Gesagard	92.50 ± 3.00	84.00 ± 2.80*	87.00 ± 2.60*	89.00 ± 2.00^{ns}	91.00 ± 1.20^{ns}
Poast	92.50 ± 3.00	82.50 ± 1.90*	83.50 ± 2.50*	86.00 ± 2.80*	89.50 ± 1.90^{ns}

*Significant difference from controls ($p < 0.05$) on Dunnett's test.
ns: non-significant difference from controls.

In *A. cepa*, inhibition of root and coleoptile growth was most pronounced for *n*-pentyl 2 hydroxy-4-methoxy-6-*n*-pentylbenzoate (**10**), which did not, however, inhibit dried biomass accumulation (Fig. 4). These effects were similar to those exhibited by Gesagard and Poast.

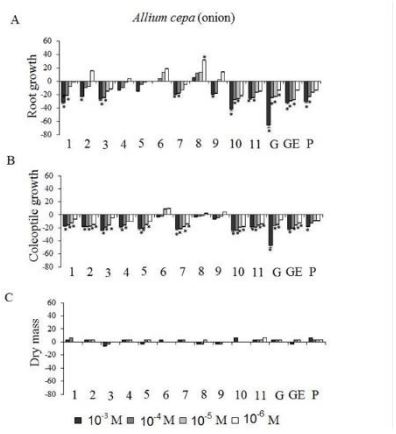

Figure 4. Effects of compounds **1**-**11** and commercial herbicides Glyphosate (G), Gesagard (GE) and Poast (P) on *Allium cepa* plantule growth and dried mass accumulation. Values expressed as percentages relative to controls. *Significant differences from controls (Dunnett's test).

Some allelochemicals are known to behave similarly to commercial herbicides in inhibiting plant germination and growth, in some cases at lower doses than those needed for synthetic herbicides [27], as observed in the present study for *n*-pentyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**10**) tested on *A. cepa*. Agrochemicals developed from natural compounds can also have advantages over their synthetic counterparts, including novel modes of action, high biodegradability, and low environmental impact [28].

In bioassays with sesquiterpene lactones, Macías et al. [23] observed that helivypolide F tested at 5×10^{-4} M inhibited root development by 36% in *L. sativa*, while in *A. cepa* the same concentration inhibited coleoptile growth by 20% and root protrusion by 54%. According Serniak [29] Resveratrol, (-)-epicatechin and emodin isolated *Fallopia japonica* significantly reduced root growth, demonstrating their phytotoxicity effect in radish.

The present results revealed the specificity of *iso*-propyl and *sec*-butyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**6** and **8**, respectively) for *L. sativa*, inhibiting root and hypocotyl growth while also reducing dried biomass. *n*-Pentyl 2-hydroxy-4-

methoxy-6-*n*-pentylbenzoate (**10**) proved the most phytotoxic to *A. cepa*, inhibiting its growth. These results suggest that *iso*-propyl, *sec*-butyl, and *n*-pentyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**6**, **8**, and **10**, respectively) have herbicidal properties. Of these, esters **6** and **8** may be used as model molecules for the study of potential herbicides for control of dicotyledons, while ester **10** may serve the same purpose for monocotyledons, warranting greenhouse and field trials.

CONCLUSIONS

Three of the 11 compounds evaluated exhibited properties suggesting their potential utility as herbicides. The esters *iso*-propyl and *sec*-butyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**6** and **8**, respectively) inhibited root and hypocotyl growth and also reduced dried biomass accumulation in *L. sativa* plantules, behaving similarly to commercial herbicides Glyphosate and Basagran.

The esters *n*-butyl, *n*-pentyl, and *n*-hexyl 2-hydroxy-4-methoxy-6-*n*-pentylbenzoate (**7**, **10**, and **11**, respectively) proved highly phytotoxic to *A. cepa*, with highest activity exhibited by compound **10**, which inhibited root and coleoptile growth in a manner similar to Gesagard and Poast. Esters **6** and **8** may be used as model molecules for future studies of herbicides for dicotyledon control, while ester **10** may serve the same purpose for monocotyledons.

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REFERENCES

1- Pelaez V, Terra FHB, Silva LR. A regulamentação dos agrotóxicos no Brasil: entre o poder de mercado e a defesa da saúde e do meio ambiente. XIV Encontro Nacional de Economia Política / Sociedade Brasileira de Economia Política. 09/06/2009 - 12/06/2009; São Paulo-SP. 22 p. Available from: http://www.sep.org.br/artigo/1521_b91605d431331313c8d7e1098bb1dd34.pdf

2- Londres F. Agrotóxicos no Brasil: Um guia para ação em defesa da vida. Rio de Janeiro: AS-PTA – Assessoria e Serviços a Projetos em Agricultura Alternativa, 2011.

3- Fantke P, Friedrich R, Jolliet O. Health impact and damage cost assessment of pesticides in Europe. *Environ Int.* 2012; 49: 9-17.

3- Rich JD, Gabriel SM, Schultz-Norton JR. *In Vitro* Effects of Herbicides and Insecticides on Human Breast Cells. *ISRN Toxicol*. 2012; Article ID 232461, 9 pages.

5- Aliota G, Cafiero G, De Feo V, Sachi R. Potential Allelochemicals from *Ruta graveolens* L. and their action on Radish seeds. *J Chem Ecol.* 1994; 20: 2761-2775.

6- Ferreira ML, Barbosa LCA, Demuner AJ, Silva AA, Perreira RC. Avaliação da atividade herbicida de algumas quinonas. *Acta Sci*. 2000; 22: 999-1003.

7- Barbosa LCA, Costa AV, Piló-Veloso D, Lopes JLC, Hernandez-Terrones MG, King-Diaze B, Lotina-Hennsene B. Phytogrowth-Inhibitory Lactones Derivatives of Glaucolide B. *Z Naturforsch*. 2004; 59c: 803-810.

8- Kozubek A. Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chem Rev*. 1999; 99: 1-15.

9- Kozubek A, Zarnowski R, Stasiuk M, Gubernator J. Natural amphiphilic phenols as bioactive compounds. *Cell & Mol Biol Lett.* 2001; 6: 351-355.

10- Kozubek A, Tyman JHP. Bioactive phenolic lipids. *Bioact Nat Prod*. (Part K). 2005; 30: 111-190.

11- Stasiuk M, Kozubek A. Biological activity of phenolic lipids. *Cell Mol Life Sci*. 2010; 67: 841-860.

12- Elix JA, Stocker-Wörgötter E. Biochemistry and secondary metabolites. In: Nash TH. Lichen Biology, Cambridge: Cambridge University Press; 2008. p. 104-133.

13- Shrestha G, St. Clair LL. Lichens: a promising source of antibiotic and anticancer drugs. *Phytochem Rev*. 2013; 12: 229-244.

14- Gómez-Serranillos MP, Fernández-Moriano C, González-Burgos E, Divakar PK, Crespo A. Parmeliaceae family: phytochemistry, pharmacological potential and phylogenetic features. *RCS Adv*. 2014; 4: 59017-59047.

15- Nishitoba Y, Nishimura H, Nishiyama T, Mizutani J. Lichen acids, plant growth inhibitors from *Usnea longuissima*. *Phytochemistry.* 1987; 26: 3181-3185.

16- Rojas IS, Lotina-Hennsen B, Mata R. Effect of lichen metabolites on thylakoid electron transport and photophosphorylation in isolated spinach chloroplasts. *J Nat Prod*. 2000; 63: 1396-1399.

17- Peres MTLP, Mapeli AM, Faccenda O, Gomes AT, Honda NK. Allelopathic potential of orsellinic acid derivatives. *Braz Arch Biol Tech.* 2009; 52: 1019-1026.

18- Gianini AS, Marques MR, Carvalho NCP, Honda NK. Activities of 2,4-dihydroxy-6-*n*-pentylbenzoic acid derivatives. *Z Naturforsch*. 2008; 63c: 29-34.
19- Huneck S, Yoshimura I. Identification of Lichen Substances. Springer, Berlin, 1996.
20- Macías FA, Castellano D, Molinillo JMG. Search for a Standard Phytotoxic Bioassay for Allelochemicals. Selection of Standard Target Species. *J Agric Food Chem*. 2000; 48: 2512-2521.
21- Macías FA, Varela RM, Torres A, Molinillo JMG. Potential Allelopathic Guaianolides from Cultivar Sunflower Leaves, var. SH-222. *Phytochemistry*. 1993; 34: 669-674.
22- Brasil. Ministério da Agricultura e Reforma Agrária. Regras para a Análise de Sementes, SNDA/DNDU/CLU, Brasília, 2009.
23- Macías AF, Fernandez A, Varela RM, Molinillo JMG, Torres A, Alves PLCA. Sesquiterpene lactones as allelochemicals. *J Nat Prod*. 2006a; 69: 795- 800.
24- Maguire JD. Speed of germination – aid in selection and evaluation for seedling emergence and vigor. *Crop Sci*. 1962; 1: 176-177.
25- Laboriau LG. A Germinação das Sementes. Washington DC: Secretaria Geral da Organização dos Estados Americanos. 1983.
26- Choi JS, Choi, IS. Inhibitory effect of marine green algal extracts on germination of *Lactuca sativa* seeds. *J Env Biol.* 2016; 37: 207-213.
27- Macías FA, Galindo JCG, Molinillo JMG, Castellano D, Velasco RF, Chinchilla D. Developing new
Herbicide Models from Allelochemicals. *Pest Sci*. 1999; 55: 633-675
28- Macías FA, Marin D, Oliveros-Bastidas A, Chinchilla D, Simonet AM, Molinillo JMG. Isolation and synthesis of allelochemicals from Gramineae: Benzoxazinones and related compounds. *J Agric Food Chem.* 2006b; 5: 991-1000.
29- Serniak LT. Comparison of the allelopathic effecs and uptake of *Fallopia japonica* phytochemicals by *Raphanus sativus*. *Weed Res*. 2016; 56: 97-101.

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