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Full Paper

Herbicidal and Plant-growth Stimulating Effects of Phenolic Compounds Isolated from Lichens

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Abstract: The depsides atranorin (7) and diffractaic acid (1), the depsidones hypostictic (2) protocetraric (3), salazinic (4) acids, the xanthone secalonic acid (5), and usnic acid (6) were evaluated for their phytotoxic potentials against the target species *Allium cepa* cv. *Baia periforme* (onion, Monocotyledoneae). The bioassays, carried out under laboratory conditions, revealed that diffractaic (1) and hypostictic (2) acids stimulated plant growth; secalonic acid (5) stimulated seed germination and radicle growth, while reducing coleoptile length. Usnic acid (6) promoted seed germination and stronger inhibition of radicle and coleoptile growth. Protocetraric (3) and salazinic (4) acids and atranorin (7) exhibited a herbicidal effect, inhibiting seed germination and reducing radicle and coleoptile growth—features that suggest their utility as natural herbicides. These results invite further investigation to elucidate the mode of action of these compounds and to synthesize them for field experiments.

Keywords: lichen substances; allelopathic activity; *Allium cepa*; phenolic compounds

1. INTRODUCTION

The current demand for large-scale food production has greatly increased the agricultural use of synthetic pesticides, but considerable efforts have been made to reduce their use, both to diminish their levels in foods and to decrease the environmental impact of agriculture [1].

The search for natural pesticides has gained impetus following the observation of undesirable effects of synthetic herbicides on ecosystems. Plants have chemical defense mechanisms that involve phytotoxins, or allelochemicals—secondary-metabolism compounds that provide protection against other plants, phytophagous insects, and herbivorous predators. This phenomenon is known as allelopathy [2].

Allelopathic agents reported from higher plants include coumarins, flavonoids, alkaloids, tannins, certain quinones, hydroxamic acids, sesquiterpene lactones, and various mono-, sesqui-, di-, and triterpenes, among other compounds, synthesized from either the acetate or the shikimate pathways [3]. Many secondary compounds from plants, microorganisms, and lichens are toxic to insects, microbes, and other organisms. Through the acetate-polymalonate pathway, lichens produce phenolic substances that appear to play anti-herbivory roles [4]. Secondary metabolites of lichens are most likely involved in defense mechanisms against insects and herbivores, parasitism by other fungi, and competition from bryophytes, vascular seedlings, and other lichens [5].

Barbatic, evernic, diffractaic, and 4-*O*-demethylbarbatic acids are known to inhibit growth in *Lactuca sativa* seedlings. Acting as PSII-inhibiting herbicides, these compounds interrupt photosynthetic electron transport in isolated chloroplasts. Of these

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depsides, 4-*O*-demethylbarbatic acid exhibits the highest activity [6]. Emodin and its analogues are highly active against grasses, causing malformations and bleaching in early seedlings. Analogues of rhodocladonic acid cause root malformations in both dicotyledon and monocotyledon seedlings. (–)Usnic acid indirectly inhibits carotenoid biosynthesis by inhibiting 4-hydroxyphenylpyruvate dioxygenase (HPPD), and the *in vitro* activity of this acid is superior to that of other synthetic inhibitors of this enzyme currently in use as herbicides [5, 7]. Phenolic compounds deriving from depsides have also been tested as potential herbicides. Orsellinic and β-methyl orsellinic acids and 4-*O*-methyl derivatives have been shown to inhibit growth in *L. sativa* seedlings [8]. Other phenolic compounds include those present in the herbicide Letharal®—a mixture of 12 phenolic substances, most of them phenol-substituted [9]. The homologous series from methyl to pentyl orsellinate and compounds with ramified chains—namely, *iso*-propyl, *sec*-butyl, and *tert*-butyl orsellinates—have been assayed in the germination of *L. sativa* and *A. cepa*. None of the orsellinates tested significantly affected the germination of *L. sativa*, whereas ethyl, *n*-propyl, *n*-butyl, *iso*-propyl, and *n*-butyl orsellinates significantly influenced *A. cepa* germination. Also, *A. cepa* proved more sensitive to these compounds at concentrations of 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9} M [8]. Protocetraric and fumaprotocetraric acids showed herbicide and allelopathic effects on *L. sativa* seeds [10].

In continuation of our research, this article reports the isolation of diffractaic (**1**), hypostictic (**2**), protocetraric (**3**), salazinic (**4**), secalonic (**5**), and usnic (**6**) acids and atranorin (**7**) (Figure 1), along with the results of their allelopathic bioassays. Each compound was tested at concentrations of 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9} M on *A. cepa* cv. Baia periforme.

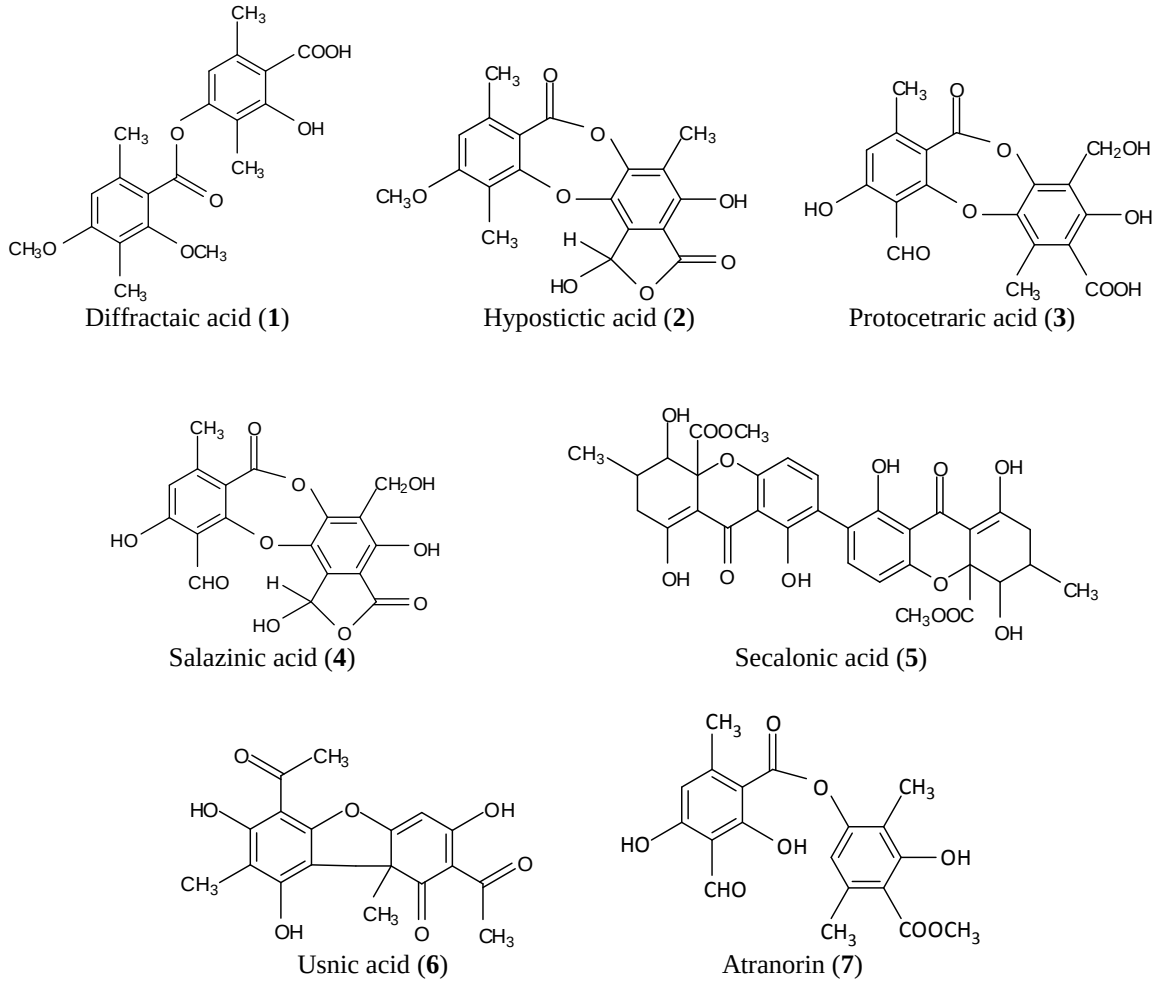

Figure 1. Structures of the lichen compounds tested on *Allium cepa* cv. Baia periforme (onion, Monocotyledoneae).

2. MATERIAL AND METHODS

General procedures

Si-gel (Merck 230-400 mesh) was used for the chromatography column. Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker DPX-300 spectrometer using the solvent as an internal reference. Melting points were determined on a Uniscience Melting Point apparatus without corrections. Optical rotation measurements were recorded on a Perkin Elmer 341 polarimeter at 589 nm. Thin-layer chromatography (TLC) was performed on plates pre-coated with silica gel 60 F254 (Merck), and the spots visualized by spraying the plates with a 10% H_2SO_4 methanol solution, followed by heating. The solvents were purified by distillation.

Lichens

The lichens *Parmotrema dilatatum* (Vain.) Hale (CGMS 49840), *Usnea subcavata* Motika (CGMS 49843), *Parmotrema cetratum* (Ach.) Hale, and *Pseudoparmelia sphaerospora* (Nyl.) Hale (CGMS 49837) were collected near Piraputanga village, Aquidauana county, Mato Grosso do Sul state, Brazil (20°27′21.2″S, 55°29′00.9″W, approx. 200 m in altitude; *P. cetratum* on rock, *P. dilatatum*, *U. subcavata* and *P. sphaerospora* on corticicolous substrate in open woods). The species were identified by Dr. Mariana Fleig, of the Universidade Federal do Rio Grande do Sul, and Dr. Marcelo P. Marcelli, of the Instituto de Botânica de São Paulo and the vouchers were deposited at the Campo Grande Herbarium of the Universidade Federal de Mato Grosso do Sul (CGMS 49840, for *P. dilatatum*; CGMS 49843, for *U. subcavata*; CGMS 37950, for *P. cetratum*; and CGMS 49837, for *P. sphaerospora*).

Extraction and isolation of compounds

Extraction of atranorin and protocetraric acid (from *P. dilatatum*), usnic and diffractaic acids (from *U. subcavata*), secalonic and hypostictic acids (from *P. sphaerospora*), and salazinic acid (from *P. cetratum*) were conducted according to Honda *et al*. [11]. The talli (20.0-40.0 g) were powdered and exhaustively extracted with hexane, followed by acetone, at room temperature. The extracts were concentrated *in vacuo*. Each hexane extract was fractionated in a silica gel column and eluted with hexane and hexane–ethyl acetate mixtures of increasing polarity to yield atranorin (*P. dilatatum and P. cetratum*) and usnic and diffractaic acids (*U. subcavata*) and secalonic acid (*P. sphaerospora*). From the acetone extracts of *P. dilatatum, P. sphaerospora*, and *P. cetratum*, protocetraric, hypostictic, and salazinic acids were isolated, respectively. These compounds were purified with a small volume of acetone in an ice bath, followed by centrifugation. The procedure was repeated until obtaining compounds of >95% purity, as determined by TLC and NMR. The resultant structures were confirmed by analyzing their NMR spectra (^{1}H, ^{13}C, and DEPT 135°) and comparing these with published data [12-19]. **Supplementary Material** available: NMR data (^{1}H and ^{13}C) of compounds **1-7**.

Bioassays

Commercially available seeds of *A. cepa* cv. Baia periforme were employed. The germination and growth bioassays were conducted in Petri dishes (6 cm), each containing 50 seeds distributed in a randomized design on a sheet of Whatman paper moistened with 1.0 mL of test solution and Tween 80 (100 μg/mL, 1.5 mL). The plates were placed in a growth chamber and kept at 15 °C. Four replicates were performed for each concentration [20]. The test solutions at 10^{-3} M were prepared using acetone, while those at 10^{-5}, 10^{-7}, and 10^{-9} M were obtained by dilution. The control tests were conducted under the same conditions, in the absence of compounds. To evaluate seed emergence, readings were taken daily, in accordance with Leather and Einhellig [21]. The experiment was concluded when the germination pattern repeated for three consecutive days. Radicle and coleoptile lengths were measured (10 seeds per dish) three days after germination.

Statistical treatment

Germination and growth assay values were subjected to ANOVA to detect significant differences. Dunnett's test was applied for multiple comparisons of mean length values at the various concentrations in relation to controls. A significance level of 5% was adopted for all statistical tests.

3. RESULTS AND DISCUSSION

Allium cepa cv. Baia periforme (onion, Monocotyledoneae) was selected as the target species

for its rapid growth and visible response in bioassays. The results obtained are presented in Table 1.

The compounds exhibited either stimulant or inhibitory effects on germination and on radicle and coleoptile growth, depending on the formulation tested. Seed germination was promoted by diffractaic (**1**) (10^{-3} and 10^{-9} M), hypostictic (**2**) (10^{-3} M), secalonic (**5**) (10^{-3} and 10^{-5} M), and usnic (**6**) (10^{-3} and 10^{-5} M) acids, but inhibited by protocetraric (**3**) and salazinic (**4**) acids and atranorin (**7**) (Figure 2). Diffractaic (**1**), hypostictic (**2**), and secalonic (**5**) acids significantly promoted radicle growth, depending on concentration.

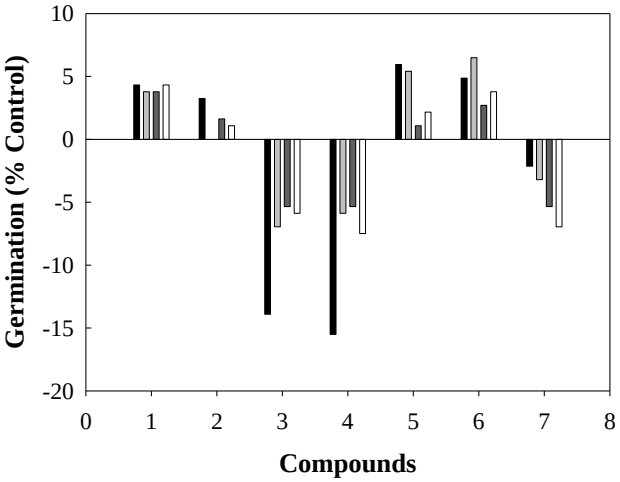

Figure 2. Effects of the lichen compounds investigated (**1-7)** on germination of *Allium cepa*. (■) 1×10^{-3} M; (■) 1×10^{-5} M; (■) 1×10^{-7} M; (□) 1×10^{-9} M.

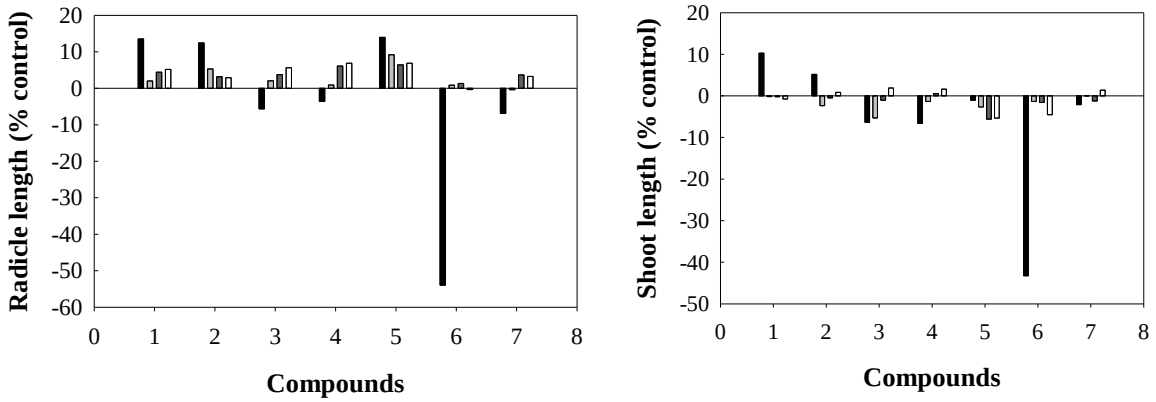

Figure 3. Effects of the lichen compounds investigated (**1-7)** on growth of *Allium cepa* seedlings. (■) 1×10^{-3} M; (■) 1×10^{-5} M; (■) 1×10^{-7} M; (□) 1×10^{-9} M.

Coleoptile growth was stimulated by diffractaic (**1**) and hypostictic (**2**) acids, but inhibited by protocetraric (**3**), salazinic (**4**), secalonic (**5**), and usnic (**6**) acids and atranorin (**7**). Usnic acid (**6**) at 10^{-3}M concentration promoted stronger inhibition of two processes investigated, which inhibited radicle and coleoptile growth by 54% and 43%, respectively. This effect may be consequent to the spatial arrangement of its molecule, which possibly influenced its biological activity, and to the high concentration employed, which may have affected secondary molecular sites [22]. According to Lascève & Gaugain [23], usnic acid induces morphological changes in radicles.

These changes may have resulted from an ability of usnic acid to interfere with a range of physiological processes, among them the permeability of algal membranes to organic substances, without significant modification of ionic permeability [24]. At low concentration (5×10^{-5} M), usnic acid has been reported to induce stomatal closure, reducing water loss. In field experiments performed in arid environments to test its antitranspiration activity in sunflowers, usnic acid decreased water loss without

Table 1. Germination and growth of *Allium cepa* seeds treated with lichen compounds (**1-7**), compared with untreated controls.

	Germination (%; mean ± SD)					**Root length (mm; mean ± SD)**					**Coleoptile length (mm; mean ± SD)**				
	Control	**10^{-3} M**	**10^{-5} M**	**10^{-7} M**	**10^{-9} M**	**Control**	**10^{-3} M**	**10^{-5} M**	**10^{-7} M**	**10^{-9} M**	**Control**	**10^{-3} M**	**10^{-5} M**	**10^{-7} M**	**10^{-9} M**
1 Diffractaic acid	46.3 ±0.203	48.3* ±0.259	48.0 ^{ns} ±0.172	48.0 ^{ns} ±0.295	48.3* ±0.149	6.30 ±0.216	7.15* ±0.208	6.43 ^{ns} ±0.206	6.58 ^{ns} ±0.236	6.63 ^{ns} ±0.126	9.30 ±0.231	10.25* ±0.129	9.28 ^{ns} ±0.222	9.28 ^{ns} ±0.150	9.23 ^{ns} ±0.126
2 Hypostictic acid	46.3 ±0.203	47.8 ^{ns} ±0.187	46.3 ^{ns} ±0.381	47.0 ^{ns} ±0.323	46.8 ^{ns} ±0.209	6.30 ±0.216	7.08* ±0.171	6.63 ^{ns} ±0.222	6.50 ^{ns} ±0.183	6.48 ^{ns} ±0.126	9.30 ±0.231	9.78* ±0.171	9.08 ^{ns} ±0.150	9.25 ^{ns} ±0.173	9.38 ^{ns} ±0.096
3 Protocetraric acid	46.8 ±0.259	40.3* ±0.387	43.5 ^{ns} ±0.120	44.3 ^{ns} ±0.502	44.0 ^{ns} ±0.207	6.20 ±0.141	5.85 ^{ns} ±0.129	6.33 ^{ns} ±0.171	6.43 ^{ns} ±0.287	6.55 ^{ns} ±0.208	9.38 ±0.189	8.78* ±0.096	8.88* ±0.222	9.28 ^{ns} ±0.096	9.55 ^{ns} ±0.129
4 Salazinic acid	46.8 (0.259)	39.3* ±0.316	44.0 ^{ns} ±0.283	44.3 ^{ns} ±0.366	43.3 ^{ns} ±0.199	6.20 ±0.141	5.98 ^{ns} ±0.096	6.25 ^{ns} ±0.265	6.58* ±0.222	6.63* ±0.126	9.38 ±0.189	8.75* ±0.173	9.25 ^{ns} ±0.300	9.43 ^{ns} ±0.206	9.53 ^{ns} ±0.150
5 Secalonic acid	46.3 ±0.203	49.0* ±0.354	48.8* ±0.127	46.8 ^{ns} ±0.131	47.3 ^{ns} ±0.226	6.30 ±0.216	7.18* ±0.171	6.88* ±0.150	6.70* ±0.183	6.73* ±0.96	9.30 ±0.231	9.20 ^{ns} ±0.183	9.05 ^{ns} ±0.191	8.78* ±0.189	8.80* ±0.141
6 Usnic acid	46.3 ±0.203	48.5* ±0.338	49.3* ±0.197	47.5 ^{ns} ±0.170	48.0 ^{ns} ±0.124	6.30 ±0.216	2.90* ±0.141	6.35 ^{ns} ±0.238	6.38 ^{ns} ±0.189	6.28 ^{ns} ±0.150	9.30 ±0.231	5.28* ±0.126	9.18 ^{ns} ±0.206	9.15 ^{ns} ±0.129	8.88* ±0.126
7 Atranorin	46.8 ±0.259	45.8 ^{ns} ±0.287	45.3 ^{ns} ±0.326	44.3 ^{ns} ±0.195	43.5 ^{ns} ±0.175	6.20 ±0.141	5.78* ±0.206	6.18 ^{ns} ±0.096	6.43 ^{ns} ±0.189	6.40 ^{ns} ±0.082	9.38 ±0.189	9.18 ^{ns} ±0.189	9.38 ^{ns} ±0.189	9.25 ^{ns} ±0.379	9.50 ^{ns} ±0.183

^{ns} Mean value not significantly different from controls; *mean values significantly different from controls.

affecting CO_2 uptake—a noteworthy effect that suggests the possibility of using this acid on trees to prevent excessive drying [25].

In the present study, allelopathic effects were more evident on growth than on germination, particularly for protocetraric (**3**) and salazinic (**4**) acids and atranorin (**7**). Ferreira & Áquila [26] pointed out that although germination is less sensitive than plantlet growth to the action of allelochemicals, changes in germination and growth patterns may result from effects on membrane permeability, DNA transcription and transduction, action of secondary messengers, respiration, and conformation of enzymes and receptors.

No patterns have been detected for the effects of compounds pertaining to any given chemical class, particularly in the case of protocetraric (**3**) and salazinic (**4**) acids. In fact, isolated functional groups in a molecule are not always sufficient to impart it with significant biological activity. Rather, the entire molecular structure may have to be taken into account to explain its higher potential for biological activity [27].

Exposed to protocetraric acid (**3**), *L. sativa* seedlings were reported to develop greater leaf areas at all concentrations tested, in addition to demonstrating hypocotyl and root stimulus [10]. Lichen compounds have exhibited different effects, depending on the target species, with usnic acid (**6**) having more marked effects on onion, while not inhibiting growth in lettuce plantlets, even at high concentrations. Diffractaic acid (**1**), however, stimulated radicle growth and inhibited hypocotyl length in lettuce, while also promoting development of onion plantlets [6].

The lichen substances assayed can manifest their allelopathic potential on diverse communities, given the evidence that they can be disseminated by lixiviation into the environment, where they accumulate by resisting microbiological activity, penetrating the cell walls of seeds and acting as allelochemicals [8].

The results obtained reveal that these compounds exert allelopathic activities when applied to monocotyledon species, making them good candidates for use as herbicides for weed control**.**

4. CONCLUSION

Diffractaic (1) and hypostictic (2) acids were found to stimulate plant growth, protocetraric (3) and salazinic (4) acids and atranorin (7) exhibited a herbicidal effect, inhibiting germination of onion seeds and slowing radicle and coleoptile growth—features that suggest their utility as natural herbicides. Usnic acid (6) promoted seed germination and slowed both radicle growth (by 54%) and coleoptile growth (by 43%) when tested at 10^{-3} M. These findings invite further studies to elucidate the mode of action of the compounds investigated, including their synthesis in amounts sufficient for field experiments.

5. ACKNOWLEDMENTS

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6. REFERENCES AND NOTES

[1] Fomsgaard, I. S. *J. Agric. Food Chem.* **2006**, *54*, 987. [CrossRef]

[2] Pinto, A. C.; Silva, D. H. S.; Bolzani, V. S.; Lopes, N. P.; Epifânio, R. A. *Quim. Nova* **2002**, *25*, 45. [CrossRef]

[3] Einhellig, F. A. In: *Allelopathy* – Organisms, *Processes and Applications*. Iderjit, K.M.M.; Dakshini, F.A. E., eds. ACS Symposium Series, 1993, 582, 1–22.

[4] Nash III, T. H. In: *Lichen Biology*, 1st. ed. Nash III, T. H., ed. Cambridge: Cambridge University Press, 1996, chapter 1.

[5] Dayan, F. E.; Romagni, J. G. *Pesticide Outlook* **2001**, *12*, 229. [CrossRef]

[6] Nishitoba, Y.; Nishimura, H.; Nishiyama, T.; Mizutani, J. *Phytochemistry* **1987**, *26*, 318. [CrossRef]

[7] Romagni, J. G.; Meazza, G.; Dhammika, N. N. P.; Dayan, F. E. *FEBS Letters* **2000**, *480*, 301. [CrossRef]

[8] Marante, F. J. T.; Castellano, A. G.; Rosas, F. E.; Aguiar, J. Q.; Barrera, J. B. *J. Chem. Ecol*. **2003**, *29*, 2049. [CrossRef]

[9] Peres, M. T. L. P.; Mapeli, A. M.; Faccenda, O; Gomes, A. T.; Honda, N. K. *Braz. Arch. Biol. Technol.* **2009**, *52*, 1019. [CrossRef]

[10] Tigre, R. C.; Silva, N. H.; Santos, M. G.; Honda, N. K.; Falcão, E. P. S.; Pereira, E. C. *Ecotox. Environ. Safe.* **2012**, *84*, 125. [CrossRef]

[11] Honda, N. K.; Pavan, F.R.; Coelho, R. G.; Leite, A. S. R.; Micheletti, A. C.; Lopes, T. I. B.; Misutsu, M. Y.; Beatriz, A.; Brum, R. L.; Leite, C. O. F. *Phytomedicine* **2010**, *17*, 328. [CrossRef]

[12] Bayir, Y.; Odabasoglu, F.; Cakir, A.; Aslan, A.; Suleyman, H.; Halici, M.; Kazaz, C. *Phytomedicine* **2006**, *13*, 584. [CrossRef]

[13] Carvalho, A. E.; Barison, A.; Honda, N. K.; Ferreira, A. G.; Maia, G. *J. Electroanal. Chem.* **2004**, *572*, 1. [CrossRef]

[14] Sundholm, E. G.; Huneck, S. *Chem. Scripta* **1981**, *18*, 233.

[15] Huneck, S.; Yoshimura, I. *Identification of Lichen Substances*, Berlin, Springer, 1996.

[16] Eifler-Lima, V. L.; Sperry, A.; Simbandhit, S.; Boustie, J.; Tomasi, S.; Shenkel, E. *Magn. Res. Chem.* **2000**, *38*, 472. [CrossRef]

[17] Yosioka, I.; Yamauchi, H.; Murata, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1972**, *20*, 1082. [CrossRef]

[18] Honda, N. K. Liquens de Mato Grosso do Sul – Estudo Químico e Avaliação da Atividade Biológica. [Doctoral dissertation]. Araraquara, Brazil: Departamento de Química Orgânica, Instituto de Química, Universidade Estadual Paulista – UNESP, 1997. [link]

[19] König, G. M.; Wright, A. D. *Phytochem. Anal.* **1999**, *10*, 279. [CrossRef]

[20] Brasil. Ministério da agricultura. Regras para a análise de sementes. 1992, 365 p.

[21] Leather, G. R.; Einhellig, F. A. In: The Science of Allelopathy. Putnam, A.R.; Tang, C.S., eds. New York, John Wiley and Sons, 1986, p 133-146.

[22] Duke, S. O.; Romagni, J. G.; Dayan, F. E. *Crop Protection* **2000**, *19*, 583. [CrossRef]

[23] Lascève, G.; Gaugain, F. *J. Plant Physiol*. **1990,** *136*, 723. [CrossRef]

[24] Vainshtein, E. A. *Fiziol. Rast.* (Moscow) **1985**, 3*2*, 1153.

[25] Carbonnier, J. In: Recherches et applications dans le domaine des antitranspirants stomatiques. Carbonnier, J.; Laffray, D., eds. Paris: ,Agence de Cooperátion Culturelle et Technique, 1986, p 147–171.

[26] Ferreira, A. G.; Aquila, M. E. A.. *Rev. Bras. Fisiol. Veg.* **2000**, *12*, 175.

[27] Barbosa, L. C. A.; Maltha, C. R. A.; Borges, E. E. L. *Quim. Nova* **2002**, *25*, 203. [CrossRef]