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Potential phenolic bioherbicides from *Cladonia verticillaris* produce ultrastructural changes in *Lactuca sativa* seedlings

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ABSTRACT

The possibilities for using phenolics, extracted from the lichen *Cladonia verticillaris* with different organic solvents, as bioherbicides have herein been studied through observation of the ultrastructural changes produced in *Lactuca sativa* seedlings. The different extracts mainly contain protocetraric and fumarprotocetraric acids and very small amounts of atranorin. It has been observed that the roots of lettuce seedlings grow more rapidly in the presence of the phenols than in their absence. This fact is supported by a minor number of lobes and less indentation of the parenchymatous cells as well as a major appearance of active dictyosomes in their cytoplasm. Nevertheless, seedling leaves developed in the presence of these extracts show drastic degenerative changes. Intergranal lamellae of chloroplasts disappear whereas thylakoids are melted in amorphous masses. In some cases, the number of dictyosomes increases in parenchymatous cells and mitochondria disorganize their internal membranes, though in a minor degree of that observed for chloroplasts.

1. Introduction

The use of traditional herbicides, which diminishes the costs of agricultural production, many times results in a negative environmental impact. Probably, this is the main reason why no new herbicides with a new target site have been commercialized in nearly 20 years (Dayan et al., 2012). Thus, the study of plant allelochemicals is currently developed in the search of new natural herbicides in order to avoid the ecological impact that the chemically-synthesized compounds produce (Duke et al., 2002). For example, secondary metabolite extracts from the leaves of *Ailanthus altissima* are powerful herbicidal and insecticidal substances. They produce a strong inhibitory effect on seed germination and plant growth of *Medicago sativa* (Tsao et al., 2002). A phytotoxin, xanthinosin, has been isolated from *Xanthium italicum*. This sesquiterpene lactone significantly affects the growth of both *Lactuca sativa* and *Amaranthus mangistanum* as well as impedes seed germination (Shao et al., 2012). The phenolic compound 3,4-dihidroxy-acetophenone, isolated from leachates of *Picea schenkiana* needles also inhibits germination and plant growth of lettuce, *Cucumis sativus* and *Phaseolus radiatus* (Ruan et al., 2011).

Under this point of view, lichens produce allelopathic phenolics that could be used as natural herbicides. This idea is sustained on three experimentally verified facts: 1. their allelopathic action against higher plants; 2. their solubility in water, which facilitates their use as phytosanitary compounds and 3. their biodegradability by soil microorganisms, that impedes their accumulation in cultured soils.

Concerning the first point, Nieves et al. (2011) found that methanolic extracts of *Everniastrum sorocheilum* (Parmeliaceae), *Usnea roccellina* (Parmeliaceae) and *Cladonia confusa* (Cladoniaceae) inhibit germination and root growth of *Trifolium pratense*. Lecanoric, barbatic and gyrophoric acids behave as uncouplers of the photosynthetic electron transport in isolated chloroplasts of tobacco and spinach (Endo et al., 1998; Takahagi et al., 2006). (−)-Usnic acid inhibits the biosynthesis of both chlorophylls and carotenoids by acting on the enzyme 4-hydroxyphenyl pyruvate dioxigenase, inducing death of *L. sativa* seedlings (Romagni et al., 2000) and raises the susceptibility of chlorophylls to photodegradation (Latkowska et al., 2006). The same compound as well as its (+) enantiomer inhibit transpiration and water photolysis of corn and sunflower seedlings (Lascève and Gaugain, 1990; Vavasseur et al., 1991; Legaz et al., 2004; Latkowska et al., 2006; Lechowski et al., 2006).

Responses to germination and initial growth of *L. sativa* (lettuce) subjected to organic extracts and purified compounds of *C. verticillaris* were analyzed by Tigre et al. (2012). *C. verticillaris* extracts induce modifications of the size of leaf area and the length of seedling hypocotyl of lettuce seedlings whereas root development occurred. During growth experiments, seedlings exposed to ether or acetone extracts showed

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diminished hypocotyl and stimulated root growth, compared to the controls. Increases of extract concentrations led to the formation of abnormal seedlings. The main components of these extracts, fumarprotocetraric and protocetraric acids (Fig. 1), induced at all the assayed concentrations an increase of leaf area of lettuce seedlings, indicating a possible bioherbicide potential of these acids. In contrast, hypocotyl and root hyper-elongation was observed only in the presence of protocetraric acid.

Toledo et al. (2003) reported that the substances composing the phenolic fraction of *Lethariella canariensis*, which were lixiviated by rainwater and deposited in the soil, disable the germination of cabbage, lettuce, pepper and tomato seeds. On the other hand, it has been described that lichen phenols retained by the soil can be used as a substrate for growth of soil microorganisms, which use them as a carbon source, such as usnic and perlatolic acids from *Cladina stellaris* (Stark and Hyvärinen, 2003). This biodegradability is an additional inducement to advance and to insist on the study of the use of allelochemicals as bioherbicides, since they would not accumulate irreversibly in the soils.

Concerning the second point (water solubility), Zagoskina et al. (2013) found that water-soluble phenolics in the lichens *Peltigera aphthosa*, *Solorina crocea*, *Cetraria islandica*, *Flavocetraria nivalis*, *Cladonia uncialis*, and *Cladonia arbuscula* were represented by 7–12 phenolic compounds with similar qualitative composition in the species of the same order. In addition, water solubility of lichen phenolics can be enhanced after conjugation to sugars and amino acids (Nikolaev et al., 2014), and polyamines (Fontaniella et al., 2001).

On the other hand, small organic molecules originating from aboveground vegetation generally constitute an important C source for the soil microbial community. Results obtained by Stark and Hyvärinen (2003) for soil microorganisms living under *C. stellaris* mats suggest that the usnic and perlatolic acids that leach from the lichens form a source for energy for the microbial community in the soil under the lichen carpet. Both *Mortierella isabellina* (Kutney et al., 1978) and *Mucor globosus* (Kutney et al., 1984) fungi isolated from soils produce, respectively, hydrolysis or deacylation of usnic acid.

Since many of these lichen compounds inhibit growth, respiration and photosynthesis of sensitive plants, these changes must be accompanied by changes in the cellular ultrastructure that supports the above mentioned physiological functions, which constitutes the aim of this research.

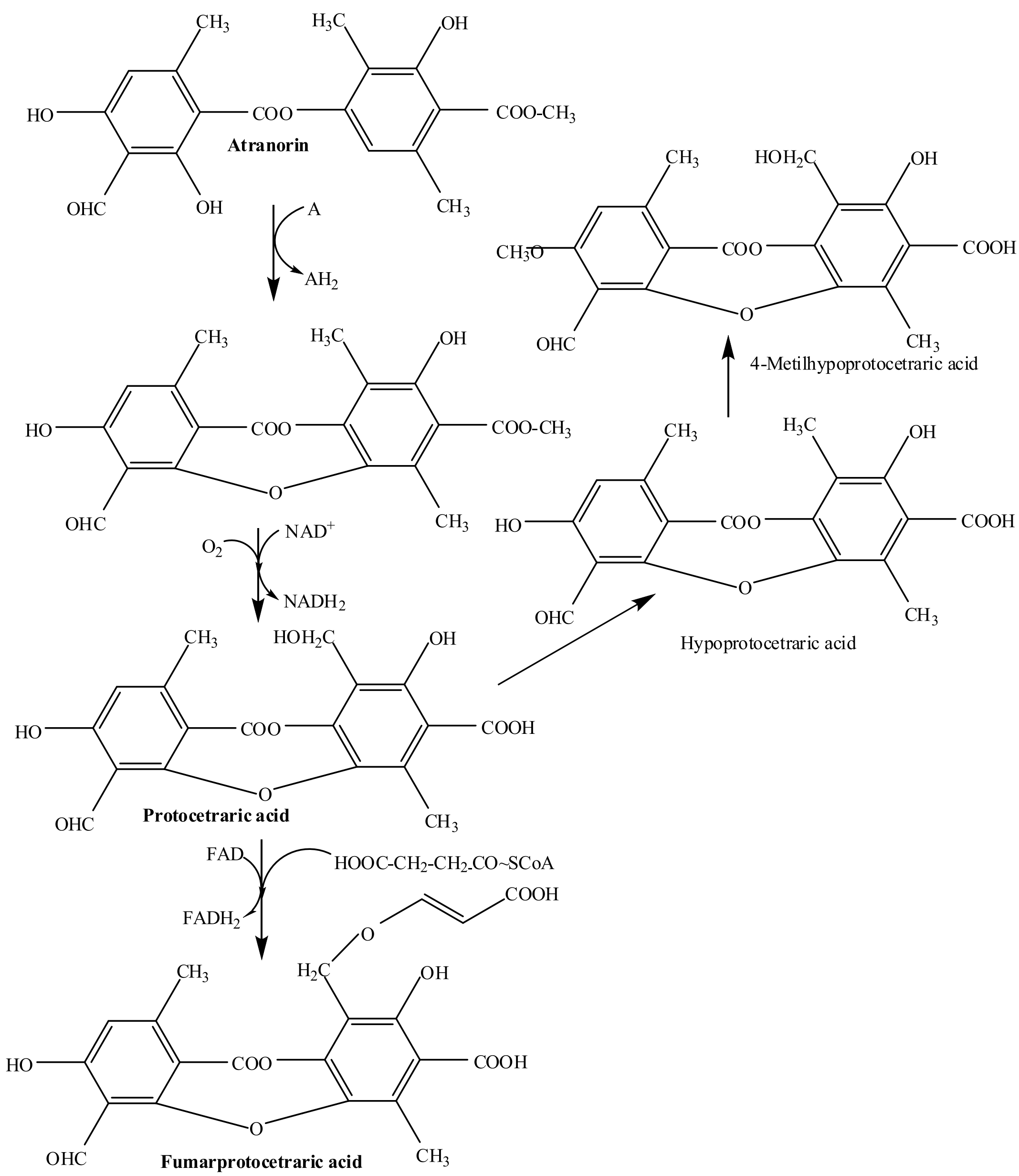

Fig. 1. Scheme showing chemical structure and proposed biosynthetic pathway of atranorin-derived depsidones in the lichen *Cladonia verticillaris*.