INFLUENCE OF GAMMA RADIATION ON SECONDARY METABOLISM IN LICHENS Cladonia substellata AND Cladonia verticillaris

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ABSTRACT

Lichens are organisms formed from a symbiotic relationship between a fungus and an alga. These when submitted to different doses and types of radiation are encouraged to produce their substances in quantities different from those that would produce without the intervention of radiation. The objective of this research was to determine the influence of gamma rays on the production of usnic acid from Cladonia substellata and on the production of fumarprotocetraric acid from Cladonia verticillaris. Lichens samples were submitted to gamma irradiation Co-60 source, receiving different doses (0, 5, 10, 50 and 100 Gy) of gamma irradiator. After six months samples were collected and submitted to the extraction of its phenols. The extracts were subjected to thin-layer chromatography, and read from Biochrom Libra S22 spectrophotometer. The qualitative assessment of the chemical composition of lichens stalks irradiated or not, revealed by thin layer chromatography production of usnic acid and fumarprotocetraric throughout the experiment. Regarding the quantification of fumarprotocetraric acid was observed a production significantly higher in extracts obtained from irradiated lichens, the largest production obtained by the dose of 50 Gy. As for the production of usnic acid, the highlight was the dose of 10 Gy. Thus we can conclude that the lichens Cladonia verticillaris and Cladonia substellata when submitted to gamma radiation in the laboratory, produce fumarprotocetraric acid and usnic acid, respectively, but the radiation dose influences the metabolism and its subsequent biosynthesis.

1. INTRODUCTION

Lichens are defined as symbiotic organisms composed of fungi, the micobionte, and one or more species of photosynthesizers, the photobiont, which can be green alga or cyanobacteria [1, 2, 3]. This symbiosis can range from parasitism to mutualism strict, depending on the taxonomic groups of fungi and algae (or cyanobacteria) that are involved [4]. Of this "union" are produced lichens substances [1, 5]. These substances, in most phenols are responsible for Most of the benefits from lichens, including:
applications in the pharmaceutical industry, textiles, perfums and cosmetics, and is also referred to as agricultural use [6, 7].

After the Chernobyl accident, where a large amount of radioactive elements were released to the environment, the use of lichens as bioindicators and biomonitors of radioactive pollution has been extensively used [8, 9, 10], since lichens accumulate high levels of heavy metals and radionuclides compared to vascular plants [11, 12]. On the other hand, much little has been studied on the effect of ionizing radiation on lichen metabolism [13].

Lichens when submitted to different doses and types of radiation are encouraged to produce their substances in different amounts when compared with the production without the intervention of radiation [14, 15, 16] mainly due to their sensitivity to external agents [17].

In this context, the objective of this research was to determine the influence of different doses of gamma radiation on the bioproduction of the majority compounds of Cladonia verticillaris (Raddi) Fr and Cladonia subtellata Vainio, the fumarprocetraric acid (FUM), usnic acid (USN) respectively.

2. MATERIAL AND METHODS

2.1. Collecting the lichen material

For the experiments were collected clumps of Cladonia subtellata Vainio and Cladonia verticillaris (Raddi) Fr in city of Alhandra – PB (Fig. 1). The collection area is characterized by open wooded savanna, with many grasses, shrubs and trees of small and medium enterprises, present characterized by soils with high content of quartz sand dystrophic currently receiving the name of Entisols. Samples were deposited at the UFP herbarium of the Botany Department (UFP), registration No. 46687 and 63705, respectively.

Figure 1. Local collection of lichen samples.
2.2. Gamma Irradiation in Co-60 source of the material lichen
Lichens samples were placed in paper envelopes (12g) and submitted to gamma irradiation in gamma irradiator (Co-60 - Radiator, Excel Gammacell 220), receiving doses of 0, 5, 10, 50, 100 Gy, with dose rate of 7.795 kGy / h.

2.3. Assembly of the experiments
After submission to gamma radiation, the lichens irradiated and their control groups were packed separately in a Petri dish with lid, being five repetitions for each experiment.

2.4. Collection of lichen material and obtaining the extracts
After 90 days of the assembly of the experiments, lichens samples were collected from each Petri dish, and its phenols extracted by sewage system with organic solvents.

2.5 Analysis of samples
2.5.1. Thin Layer Chromatography (TLC)
Organic extracts of C. substellata and C. verticillaris, irradiated or not, were subjected to ascending thin-layer chromatography [18].

2.5.2. Assay spectroscopic
The extracts (0.01 mg/mL) were read by spectrophotometer BIOCHROM model Libra® 22S at wavelengths of 254, 310 and 366 nm [19].

2.6. Statistical analysis
The data obtained were submitted to analysis of variance, involving radiation doses. Then, was performed regression analysis to quantify the lichens substances by using the statistical program SISVAR ®.

3. RESULTS AND DISCUSSION
Qualitative analysis of the chemical composition of irradiated lichen stems and their control groups, revealed by thin layer chromatography (TLC), with the aid of standard substances and the staining reaction bands, production of fumarprocetraric acid (FUM), for C. verticillaris (Fig. 2) and usnic acid (USN) by C. substellata (Fig. 3), the main compounds produced by species, respectively [20] in all samples evaluated. However, in CCDs additional bands could be visualized. These bands correspond to auxiliary substances produced by species: atranorin (ATR) in C. verticillaris and stictic acids, and constit, criptostictic in C. substellata [20].
Lichens when submitted to gamma radiation, or ultraviolet, tend to produce more of its phenols, because for functioning as photoreceptors and/or photoinductor, protect the interior of the lichen thallus, preserving their biological functions [21, 1]. Stalks of *Cladonia verticillaris* promoted greater fumarprocetraric acid synthesis when directly exposed to the sun in a natural environment [22]. The same lichen, when protected by the shadow of the canopy of *Anacardium occidentalis*, prioritized the accumulation of chlorophyll and other photosynthesizers pigments.

From the graphics produced by spectrophotometry, was determined the amount of lichens substances produced after 180 days of the experiment in *C. verticillaris* (Fig. 4) and *C. substellata* (Fig. 5).
Figure 4. Fumarprotocetraric acid (FUM) by *C. verticillaris* depending on the radiation dose.

Figure 5. Usnic acid (USN) by *C. substellata* depending on the radiation dose applied.

It is observed that after 180 days of experiments for the production of FUM *C. verticillaris* follows an increasing trend up to a dose of 50 Gy, where this production is 122.84% higher than the control sample, however at a dose of 100 Gy this production was 55.86% lower than the control sample, not irradiated. For the production of USN by *C. substellata*, there is a pattern of increase until the dose of 10 Gy when production is 117.3% higher than non-irradiated sample, but after this dose the production tends to decrease, especially in the dose of 100 Gy which presents a production of USN only 0.05% over in relation of the control sample.

Increasing the dose absorbed in the tissues of the lichen may have prevented the synthesis of the USN, as adaptation factor to this situation. It is possible that high doses of gamma radiation are greater than the carrying capacity of the cells. In this respect, it
can be observed damage to the plasma membrane of *Cladonia arbuscula*, *Cetraria islandica* and *Hypogymnia physodes* by the effect of gamma radiation at doses of 500, 5,000 and 50,000 Gy. With emphasis on *H. physodes* irradiated with 50,000 Gy, which showed a 400% increase in leakage of electrolytes and 542.85% in the escape of potassium, when compared to control sample [13].

Studies with gamma radiation doses on the acute or chronic lichen *Cladonia sylvatica* show a delay in evidence of the effects caused. No damage was reported to a prolonged period for growth of the stem [23; 24]. The same type of behavior was recorded for *C. verticillata* irradiated with acute doses of gamma radiation [25]. The species showed no damage or activation of growth, even when exposed for prolonged period, a regime of high brightness and low light intensity.

More detailed studies relating lichens and radiation were conducted with ultraviolet radiation considered non-ionizing radiation. The USN showed high ability as sunblock, when compared to commercial standards [26].

It is known that lichen substances are shaped crystal when deposited on the surface of the hyphae micobionte, and the shape, color, transparency or opacity, enable the crystal to select the type and dose of radiation absorbed by lichens [1; 21]. Thus, there may be increasing the temperature of the stem to the dissolution of snow and ice that is deposited on tufts lichens in temperate climates, or cold, as a strategy to increase the photosynthetic capacity or by preventing the penetration of radiation that unbalances the metabolism of the species. In this case, the emphasis in the production of secondary metabolites is fully justified.

The results can also be ratified due to the damage to the lichens *Cladonia sylvatica*, *Cladonia mitis* Sandst and *C. verticillata* after being irradiated with acute or chronic doses of gamma radiation [25; 24; 27; 28].

Therefore, based on the present results and the theoretical framework mentioned you can check interference in the metabolism of lichen species, when they are subjected to gamma irradiation. At the same time, you can assign the lichen the activation capacity of the biosynthesis of its phenols, or even translocation of them inside the stem, as a defense to aggressive agents of the medium (or radiation and pollutants).

4. CONCLUSIONS

Gamma radiation induces the lichens *C. verticillaris* and *C. substellata*, an increase in production of acid fumarprocetraric and usnic, respectively, as likely a defense mechanism against increased activity of gamma radiation, but there is a tolerance threshold for such, which determines the decrease in production of these substances at higher doses.

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REFERENCES


