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Ciprofloxacin exposure affects arugula plants growth and the photosynthetic pigments content

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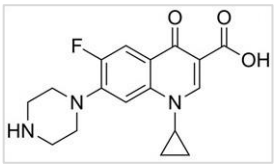
Abstract:
Arugula (*Brassica eruca* L.) seeds have been planted on solidified nutrient solutions that were supplemented with ciprofloxacin at concentrations from 1 to 10^5 μg/L. At seven days, the seedlings were tested for pigments and phenols concentration in their leaves, and their growth parameters were recorded. Over the analysed range of ciprofloxacin concentration, a biphasic, hormesis-type effect was observed. Compared to the control, increased plant height, leaf area and photosynthetic pigments content occurred at low concentrations of ciprofloxacin. At high concentrations, there was a marked dose-dependent inhibition of plant growth and very low chlorophylls and carotenoids content in theirs leaves. The observed phytotoxicity of ciprofloxacin may result from a mechanism similar to its antibiotic action, *i.e*. the inhibition of the function of bacterial topoisomerases. Chloroplasts have their own circular chromosome, whose replication occurs as in prokaryotic cells and may be inhibited by ciprofloxacin.

Keywords: arugula, ciprofloxacin, plant growth, photosynthetic pigments, phenols

1. INTRODUCTION

Environmental pollution with antibiotics has become an issue of real concern due to their widespread use and poor waste management [1].



Ciprofloxacin, a synthetic, broad spectrum antibiotic from the second generation of fluoroquinolones, inhibits bacterial DNA replication and bacterial cell division by interfering with the action of the type II bacterial topoisomerases: DNA gyrase and topoisomerase IV. Topoisomerases are enzymes that solve the topological problems of DNA that are related to its double-stranded structure. These enzymes function by transiently cleaving and then resealing of the sugar-phosphate backbone of DNA, without altering its nucleotide sequence, being of crucial importance in the processes of DNA replication, transcription, recombination and repair [2-4].

2. MATERIALS AND METHODS

2.1. Methods

Arugula (*Eruca sativa* L.) seeds were obtained from a local store. Pharmaceutical grade ciprofloxacin hydrochloride monohydrate, $C_{17}H_{21}ClFN_3O_4$, M=385.82 g/mol, was used to prepare a stock solution of 100 mg/L (10^5 μg/L). From the ciprofloxacin stock solution, a series of 5 dilutions were obtained, with concentrations from 10,000 μg/L to 1 μg/L that were brought to final volumes of 50 mL with a Hoagland solution containing 0.5% agar. The resulting nutrient solutions containing ciprofloxacin were poured into Petri dishes and, when solidified, 20 arugula seeds were planted in each dish. The dishes were kept at room temperature, under natural lighting conditions (photoperiod of 16/8 hours), and temperature of 26/20 °C.

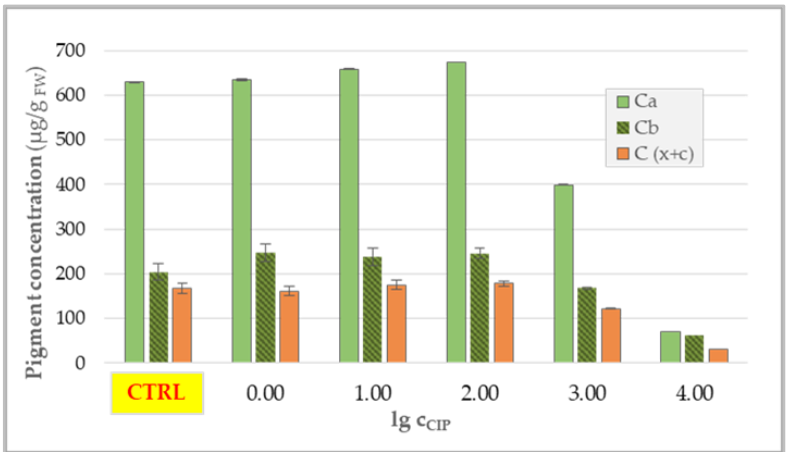
2.2. Analysis methods

Seven days old plants were sampled for pigment extraction and assay. Homogenates were obtained from leaf samples of about 0.03 g and 4 mL of ethanol, that were centrifuged at 8000 rpm for 15 min, at 4 °C, in a Sigma 2-16 centrifuge. Absorption spectra of the supernatants were registered from 350 to 750 nm using a Varian Cary 50 UV-Vis spectrophotometer. Chlorophylls and carotenoids content in the extracts were calculated with the Lichtentaler's equations [5], and the pigment content was reported to the samples fresh weight (μg/g_{FW}). Total soluble phenols (and other reducing substances) in the ethanolic extracts were assayed with the Folin-Ciocalteu reagent [6], and their concentration was expressed as gallic acid equivalents

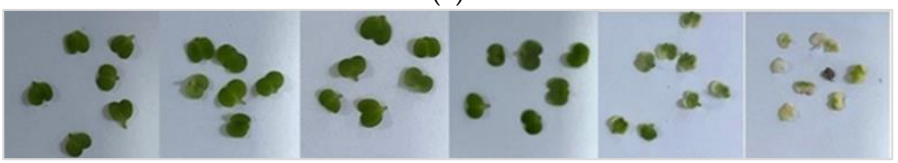
(μmol/g_{FW}). The calculation and graph were done with the Microsoft Excel software, the 2013 version.

3. RESULTS AND DISCUSSION

3.1. Results



(a)



(b)

(c)

Figure 1. (**a**) Leaf pigment concentrations in arugula plants; Ca-chlorophyll a; Cb-chlorophyll b; C(x+c) total carotenoids (xanthophylls and carotenes); (**b**) Leaf samples from the arugula plants (**c**) that were exposed for 7 days to different concentrations of ciprofloxacin in their growth media. Ciprofloxacin concentration was expressed as μg/L.

3.2. Discussion

Leaf pigment concentration in arugula plants that were eposed to ciprofloxacin are presented in Figure 1a; samples of the plants' leaves are presented in Figure 1b, and the plants are presented in Figure 1c. In control plants, average leaf pigment concentrations were 629.48 μg/g_{FW} for chlorophyll a (Ca), 238.70 μg/g_{FW} for chlorophyll b (Cb), and 167.50 μg/g_{FW} for total the carotenoids, C(x+c); total phenols content was 10.40 μmol/g_{FW}.

Chlorophyll a concentration in plant leaves didn't vary significantly compared to the control at concentrations of ciprofloxacin below 10^2 μg/L, but decreased to 63.5% of control value at 10^3 μg/L, while at 10^4 μg/L was only about 10% of the control. Chlorophyll b and carotenoid pigments concentrations varied similarly to chlorophyll a, while the phenols had an opposite trend.

Table 1. Relative values of the photosynthetic pigments and phenols contents in the leaves of arugula plant exposed to ciprofloxacin.

parameter	CTRL	lg c_{CIP}				
		0	1	2	3	4
C(a+b)	100	106	107	110	68	16
Ca/Cb	100	83	90	89	77	37
C(a+b)/C(x+c)	100	110	103	104	94	84
phenols	100	113	115	137	129	139

Decreased chlorophylls' concentrations were related to the marked inhibition of plant growth: smaller leaves, lower height and shorter roots of the plants exposed to high concentrations of ciprofloxacin are suggestive for damaged chloroplast structure and function. Ciprofloxacin and other antibiotics in the class of fluoroquinolones inhibit bacterial growth by interfering with DNA replication, transcription and repair. Chloroplasts in higher plants originate in early autotrophic prokaryotes, to which they resemble, among others characteristics, in chromosome circular structure and multiplication by direct division. In bacteria, ciprofloxacin enter the cell by passive diffusion through the porins channels in cell membrane [7]. A similar mechanism of action could be responsible for the structural and functional impairment of the chloroplasts, leading to the results presented here. At high concentrations, fluoroquinolones can also affect mitochondrial function, generating oxidative stress to which plants respond with antioxidants (*i.e.* phenolic compounds in our experiment).

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