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EVALUATION OF PHYTOTOXIC ACTIVITY OF CIPROFLOXACIN TOWARDS COMMON DUCKWEED (*LEMNA MINOR* L.) AND YELLOW LUPIN (*LUPINUS LUTEUS* L.)

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ОЦЕНКА АКТИВНОСТИ ФИТОТОКСИНА ЦИПРОФЛОКСАЦИНА ДЛЯ РЯСКИ (*LEMNA MINOR* L.) И ЖЕЛТОГО ЛЮПИНА (*LUPINUS LUTEUS* L.)

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Medicinal products commonly used may cause harmful effects to the environment. Active substances of pharmaceuticals occur not only in hospital waste or surface waters but also in rivers and seas. Major metabolites or active ingredients can be excreted in urine and faeces. In nature, the excreted substance can operate for some time, depending on the environmental conditions, soil type, physical and chemical properties of the substance. Active substances in excrement can enter arable land when they are used as fertilizers. Antibiotic contamination has repeatedly been demonstrated in habitats in Europe, North America and Asia. One of the frequently detected antibiotics in the environment is ciprofloxacin. Ciprofoxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid) is a fluoroquinolone antibiotic, used for the treatment of gastrointestinal problems, bacterial infections (pneumonia), urinary tract or skin diseases. Global surveillance studies demonstrate that fluoroquinolone resistance rates have increased in the past years in almost all bacterial species. The effect of ciprofloxacin to common duckweed and yellow lupin was investigated. In line with increasing concentration of the medicine, yellow lupin roots elongation and common duckweed fronds growth were inhibited. The content of chlorophyll in tissues decreased with increasing doses of ciprofloxacin. The aim of this study was to determine the phytotoxicity of ciprofloxacin with the use of biological (Lemna Test, Phytotoxkit) and biochemical (biogenic amines) methods.

Lemna minor, Lupinus luteus, ciprofloxacin, morphological and biochemical plant characteristics, Lemna Test, Phytotoxkit

Наиболее часто используемые лекарственные препараты могут оказывать негативное влияние на окружающую среду. Действующие вещества фармацевтических препаратов встречаются не только в медицинских отходах или поверхностных водах, но даже и в реках и морях. Основные метаболиты или активные ингредиенты выделяются с мочой и фекалиями. В течение определенного времени выделенное вещество может оказывать влияние на объекты окружающей среды. Это время зависит от условий, типа почвы, физических и химических свойств вещества. Активные вещества в экскрементах могут войти в пахотный горизонт почв, когда они используются в качестве удобрений. Антибиотическое загрязнение неоднократно наблюдали в среде обитания на территории Европы, Северной Америки и Азии. Один из часто обнаруживаемых антибиотиков в окружающей среде – ципрофлоксацин (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic кислота). Он является производным фторхинолона, используемым для предотвращения заболеваний желудочно-кишечного тракта, лечения бактериальных инфекций (пневмонии), мочевыводящих путей или кожных заболеваний. Глобальные исследования показали, что устойчивость почти всех типов бактериальных инфекций к фторхинолону за прошедшие годы увеличилась. По мере увеличения концентрации препарата удлинение корней люпина и рост ряски ингибировались. В работе изучено влияние ципрофлоксина на ряску и желтый люпин. Содержание хлорофилла в тканях уменьшалось с увеличением дозы ципрофлоксацина. Цель настоящей работы состояла в определении фитотоксичности ципрофлоксацина с использованием биологических (Lemna-тест, Phytotoxkit) и биохимических (определение биогенных аминов) методов.

Lemna minor, Lupinus luteus, ципрофлоксацин, морфологические и биохимические особенности растений, Lemna-Тест, Phytotoxkit

INTRODUCTION

In the recent years, pharmaceuticals have been seen as an emerging environmental protection problem due to their presence in water and soil ecosystems. Active substances of pharmaceuticals occur not only in hospital waste or surface waters but also in rivers and seas [1, 2].

Medicinal products commonly used may cause harmful effects to the environment. Some active ingredients or the major metabolites of medicines can be excreted in urine and faeces by animals or humans and spread in the environment, especially surface water or groundwater and soil. Active substances in excrement can enter arable land when they are used as fertilizers. Antibiotic contamination has repeatedly been demonstrated in habitats in Europe, North America and Asia. In the natural environment the excreted substances may remain active for some time, depending on environmental conditions, kind of soil, chemical and physical properties of substances [3]. The appearance of a drug in the environment, in particular in an aquatic environment attracted the interest of researchers in 1990, and since then it has been increasing [4].

Ciprofoxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid) is a fluoroquinolone antibiotic, prescribed for certain types of bacterial infections such as pneumonia, urinary tract or skin diseases and also used to treat gastrointestinal problems [5]. Between 1995 and 2002 in the United States prescribing fluoroquinolone became three times more frequent, from 7 million visits in 1995 to 22 million visits in 2002 [6]. It is also used in modern agriculture in order to increase productivity of fodder, and support and enhance growth of farm animals. Germs are increasingly more resistant to antibiotics and thus, unlike in the USA, their application as growth stimulators was forbidden in the European Union in 2006 [7]. Global surveillance studies demonstrate that fluoroquinolone resistance rates have increased in the past years in almost all bacterial species [8].

The aim of this study was to determine the phytotoxicity of ciprofloxacin with the use of biological (Lemna Test, Phytotoxkit) and biochemical (biogenic amines) methods. The impact of contaminating fresh water reservoirs and soil on morphological and biochemical characteristics of common duckweed and yellow lupin were determined

MATERIALS AND METHODS

Seed germination and root growth test. Seeds of yellow lupin (*Lupinus luteus* L.) were germinated for seven days in PHYTOTOXKIT™ plates (MicroBio Test Inc., Belgium). Germination and seedling growth occurred at 8/16 hours photoperiod, and temperatures of 20°C at day and 16°C at night. Ninety ml of soil (sand, vermiculite, peat 1:0.3:1, v/v/v) were placed in plastic microbiotest plates. The soil was covered with Whatman No.1 filter-paper and watered with 27 ml distilled water supplemented with ciprofloxacin at final concentrations: 0, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40 mM. The control plants were watered with pure distilled water. The root and shoot length and cotyledon area were estimated using Image Tool for Windows. Dry and fresh mass roots, shoots and biogenic amines were determined too.

Lemna minor (common duckweed) was grown in 10 ml Steinberg’s (OECD 221) [9] solution at 8/16 hours photoperiod, and temperatures of 20°C at day and 16 at night. The response of *Lemna minor* to ciprofloxacin concentrations (identical to lupin) was determined by the growth rate of frond, leaf area, dry and fresh mass of plants and biogenic amines.

Biogenic amines**,** the polyamines were extracted from lyophilized plant material with cold 5% perchloric acid in accordance with Taibi et al., [10]. The lyophilized plant material was shaken with 25 ml 5% $HClO_4$ solution for 30 min and then centrifuged at 16 000g for 30 min at 4°C. The supernatant was evaporated and the residue was re-dissolved in 3 ml 5% $HCl0_4$. The extract was analyzed by using the AA 400 amino acid analyzer (Ingos, Praha, Czech Rep.). The polyamines were separated at 70°C on a 7.0 x 0.37 cm column filled with Ostion Lg ANS and then eluted from the ion-exchange column with two pH 5.65 sodium citrate buffers with addition 1.0 and 2.6 M sodium chloride. The quality and quantity of the polyamines were assayed with a spectrophotometric detector, following their reaction with ninhydrin, and expressed in $\mu M \times g^{-1}$ fresh weight

RESULTS AND DISCUSSION

Physiochemical and biological methods are commonly used to assess contaminants of the environment which lower its quality [11]. It was found that as concentration of ciprofloxacin grew, it impeded the development of morphological characteristics in both common duckweed and yellow lupin. In line with increasing concentration of the medicine, yellow lupin roots elongation and common duckweed fronds growth was inhibited (Fig. 1).

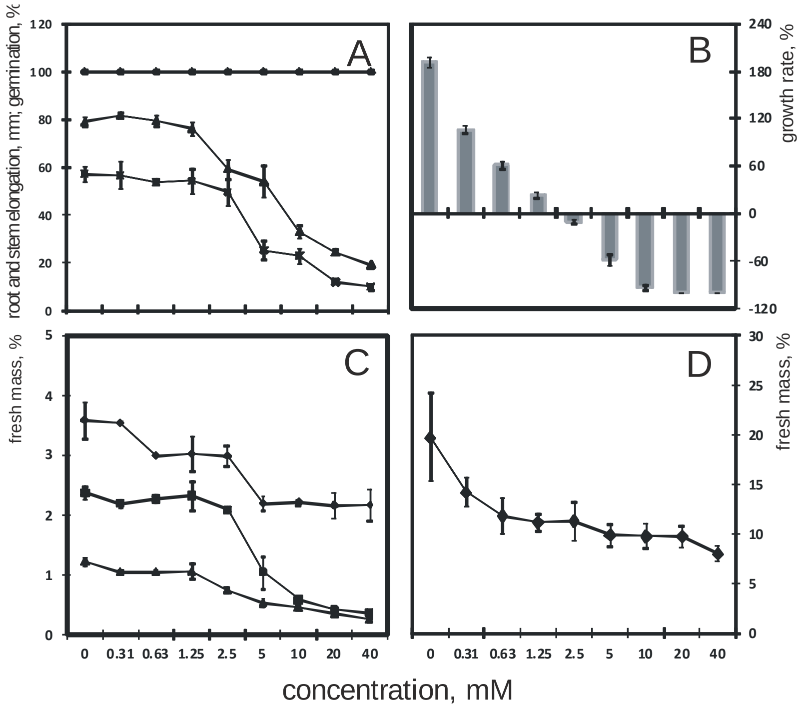

Fig. 1. Root (▲) and stem (■) elongation, and germination (●) (panel A), fresh mass of cotyledon (●), root (▲), stem (■) of yellow lupin (panel C), and the growth rate (panel B) fresh mass (panel D) of common duckweed growing in soil or water supplemented with different ciprofloxacin concentrations. Data points represent the means ± SD for nine replicate samples

Рис. 1. Удлинение корней (▲) и побегов (■), созревание (●) (A), сырая масса семядоли (●), корня (▲), побега (■) желтого люпина (C), скорость роста (B), сырая масса (D) ряски малой, произрастающих в почве или воде, содержащей различные концентрации ципрофлоксацина. Представлены средние арифметические значения и их отклонения для девяти повторностей опыта

The content of chlorophyll in tissues decreased with increasing concentrations of ciprofloxacin. In the control plants, its content was 0.67 mg/g of fresh mass in yellow lupin (Fig. 2).

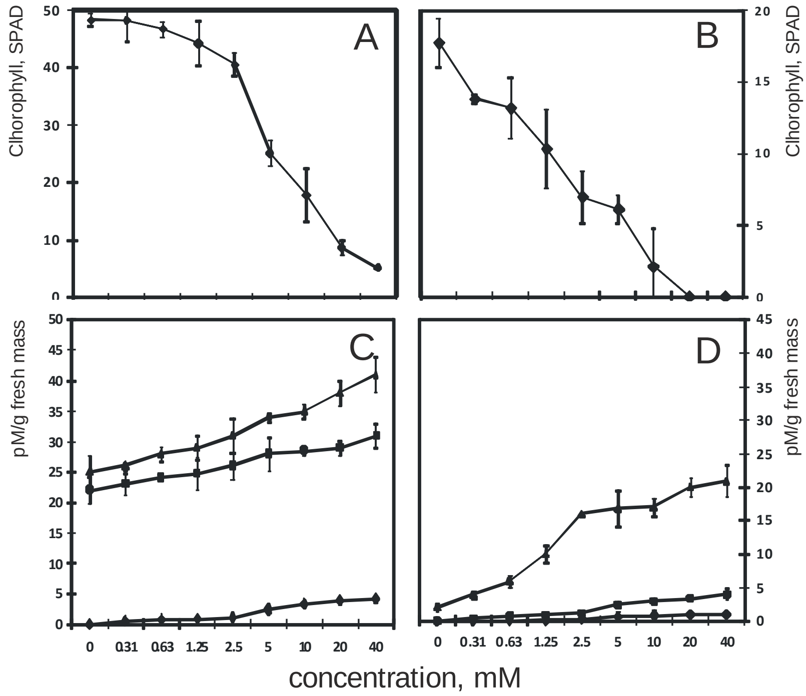

Fig. 2. Chlorophyll (panel A) and biogenic amines (spermine ▲, spermidine ■ and putrescine ●; panel C) of yellow lupin and chlorophyll (panel B) and biogenic amines (spermine ▲, spermidine ■, and putrescine ●; panel D), of common duckweed growing in soil or water supplemented with different ciprofloxacin concentrations. Data points represent the means ± SD for nine replicate samples

Рис. 2. Содержание хлорофилла (A, B) и биогенных аминов (спермин - ▲, спермидин - ■ и путресцин - ●) (C, D) в растениях люпина желтого (A, C) и ряски малой (B, D), произрастающих в почве или воде, содержащей различные концентрации ципрофлоксацина. Представлены средние арифметические значения и их отклонения для девяти повторностей опыта

In the highest concentrations of ciprofloxacin the content of chlorophyll decreased by 75% and 18% in yellow lupin and common duckweed, respectively. Similar trends were observed for the content of biogenic amines, with the highest concentration decreasing its content by 75%. Ebert et al. showed [12] high sensitivity of lesser duckweed to fluoroquinolone. Analysing the tested substance by inspecting the plant features (intensity of leaf and cotyledon colour, and osmotic potential in lupin), it was found that ciprofloxacin was more phytotoxic to common duckweed than to yellow lupin. Reduction of 50 % fresh mass of common duckweed and yellow lupin seedling appeared in 0.23 and 5.2 mM of ciprofloxacin, respectively. Spermine, spermidine and putrescine were detected in common duckweed and lupin seedlings. In common duckweed the level of biogenic amines was two times lower than in lupin seedlings (Fig. 2).

Reduction of 50 % for root elongation, shoot elongation and fresh mass of root and stem, which are a quantitative measure of the phytotoxicity of ciprofloxacin in soil were 2.5 mM, 2.3 mM, 1,26 mM and 0.63 mM for yellow lupin. Reduction of 50 % for growth rate and fresh mass of ciprofloxacin in water were 0.29 mM, 0.22 mM for common duckweed. These values can be used in the future to predict the risk level at which toxic effects will appear.

For the first time, the content of biogenic amines was determined in common duckweed tissues. The research proved that in order to assess contamination of fresh water reservoirs and soil with ciprofloxacin, one can successfully apply common duckweed (Lemna Test) and yellow lupin (Phytotoxkit) as bioindicators. Biological methods should be supplemented with biochemical methods to fully answer the question about contamination of the natural environment.

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