STUDIA UBB CHEMIA, LVI, 4, 2011 (p. 17 - 25)

UV-VIS STUDY REGARDING THE INFLUENCE OF TWO POTENTIAL ENVIRONMENTAL POLLUTANTS ON THE TOTAL FLAVONOID CONTENT IN *TRITICUM AESTIVUM* L. AND *SECALE CEREALE* L.*

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ABSTRACT. Waste waters produced by the textile industry and the animal wastes from the intensive farming which are generally used for field irrigation are considered pollutant sources for the environment. These pollutants can be taken by soil and then by plants. The flavonoids are secondary metabolites of plants; therefore their determination in plants is necessary because they have different important roles in body of plants. The spectrophotometric determination of the total flavonoid content from *Triticum aestivum* L. (wheat) and *Secale cereale* L. (rye) treated with two potential environmental pollutants (Nylosan Red N-2RBL - textile azo dye and ampicillin - antibiotic) is presented in this paper. The studied plants were periodically watered with solutions of Nylosan Red N-2RBL and ampicillin respectively, prepared at different ($1.5\cdot10^{-5}$, $1.5\cdot10^{-4}$, $1.5\cdot10^{-3}$, 1 mg/L) concentrations. The total flavonoids from plants were extracted by sonication in a solvent mixture of ethanol:water in a ratio of 80:20 (v/v). The quantitative determination of total flavonoids was performed with an UV-Vis 1800 Shimadzu spectrophotometer using aluminium chloride as reagent and rutin as standard. In both treatments, a decrease in total flavonoid content compared to the control (wheat and rye untreated samples) has been observed.

Keywords: *UV-Vis spectroscopy, flavonoids, textile azo dye, antibiotic, Triticum aestivum* L.*, Secale cereale* L.

INTRODUCTION

The environment is contaminated with numerous organic compounds, used extensively in various fields. Two major classes of environmental pollutants, often mentioned in literature are the textile dyes which are considered toxic [1] and the antibiotics which are pseudo-persistent and may play an important role in the development/maintenance/ transfer/spread of their resistance of bacteria [2].

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* This paper was presented at the "17th International Symposium on Separation Sciences. News and Beauty in Separation Sciences, Cluj-Napoca, Romania, September 5-9, 2011"

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Textile manufacturing is one of the largest industrial sources generating of waste waters because a high quantity of water is used to obtain the textile products (125-150 L water for 1 kg textile product). Thus, the resulted waste waters contain a large number of water-soluble chemical pollutants that is a serious problem for environment [3, 4]. In the textile industry, more than 100.000 different dyes are commercially available and it is estimated that about 15% of the world dye production is lost during the dyeing process [5]. The most common dyes used in the textile industry are the azo dyes [6]. Intensive irrigation of agricultural lands with water polluted with various industrial effluents severally effects soil fertility and plant growth. Carbohydrate, protein and chlorophyll contents are related to plant growth, a decrease of their content is a clear indication of the toxic nature of the dye industry effluents [7]. In a recent study the effects of the toxic dyes on narrow-leaved cattails were expressed in terms of relative plant growth rate and the appearance of symptoms such as necrosis, and chronic or acute wilting [8].

The other class of pollutants included in this study is the antibiotics that have been used for several decades in human medicine for their antibacterial properties [9]. Antibiotics are also extensively used in veterinary medicine as growth promoters or to prevent diseases. The major concern with agricultural antibiotic usage have been their presence in animal-based food products, the development and spread of antibiotic resistant bacteria, and the transport to aquatic environments from lands amended with antibiotic-laden manure [10]. Several studies have been investigated the potential risks for a range of antibiotics to be taken up from soil by plants, and also have been assessed the potential risks of this exposure route in terms of human health [11]. Other major concern surrounding antibiotics uptake by plants is the contamination of food supply that is associated with health risks [10]. Many studies report the impact of antibiotic exposure to plants. For example sulfadimethoxine was found to reduced significantly root, stalk, and leaf growth in millet (*Panicum miliaceum* L.), pea (*Pisum sativum* L.), corn (*Zea mays* L.), and barley (*Hordeum vulgare* L.) [12, 13]. Also, streptomicyn was found to inhibit chlorophylls synthesis in barley [14] and fluoroquinolone antibiotics to inhibit the photosynthesis of plants [15].

Flavonoids are secondary metabolites categorized as phenolic compounds, widely found throughout plants and prokaryotes. More than 6500 flavonoids have been identified. Flavonoids protect plants against various biotic and abiotic stresses, exhibit a diverse spectrum of biological functions and play an important role in the interaction between plants and environment. Flavonoids have roles against frost, drought resistance and may play a functional role in plant, heat acclimation and freezing tolerance. Flavonoids absorb the harmful UV radiation which can induce cellular damage. Another important role of flavonoids is that they are responsible for protecting the plants from microbes and insects [16]. The determination of flavonoids in plants is necessary because they have an important role in plant development.

This study is focused on the spectrophotometric determination of total flavonoid content in *Triticum aestivum* L. (wheat) and *Secale cereale* L. (rye), treated with the textile azo dye Nylosan Red N-2RBL (NR) and the ampicillin (AMP) antibiotic in four ($1.5 \cdot 10^{-5}$, $1.5 \cdot 10^{-4}$, $1.5 \cdot 10^{-3}$, 1 mg/L) concentrations.

RESULTS AND DISCUSSION

The quantitative analysis of total flavonoid content in plants was performed using UV-Vis spectrophotometer. The method is based on the reaction of total flavonoids with aluminum chloride ($AlCl_3$) resulting a yellow compound. Its maximum of absorbance was identified at 420 nm, being characteristic for flavonoids, expressed as rutin. The calibration plot was drawn through seven points, using concentrations ranging between 0.02 - 0.4 mg/mL. A good correlation coefficient of 0.99942 was obtained. In Figure 1 are presented the calibration plot of rutin and its corresponding UV-Vis spectra.

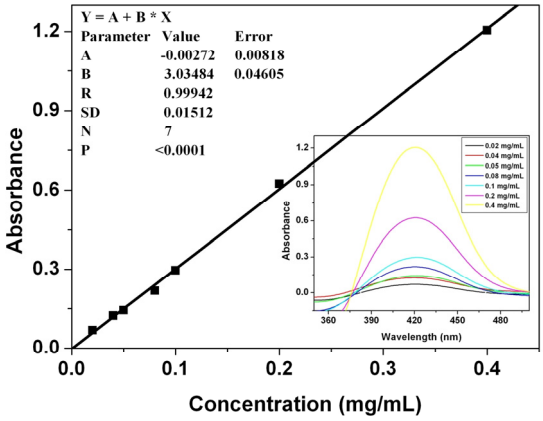


Figure 1. Calibration plot of rutin and its corresponding UV-Vis spectra.

As it was specified in a previous paper [17] for the extraction of total flavonoids from plants, several extraction methods were tested: maceration, microwave assisted solvent and sonication, the last one being the most effective. Some solvent mixtures of ethanol:water in different ratios 100:0, 80:20, 70:30, 60:40, 50:50 and 40:60 (v/v) were tested. The best results were obtained with the ratio 80:20 (v/v) that was used further for the extraction of total flavonoids from wheat and rye treated previously with NR and AMP pollutants.

Further, the extractions of total flavonoids from plants treated with NR and AMP was performed as is described in the experimental section. In both treatments, a decrease of the total flavonoid content compared to control plants (untreated wheat and untreated rye) was observed.

In the case of wheat treated with NR the decrease of total flavonoid content is proportional with the applied concentrations of textile azo dye. The tests on rye do not show so proportional decrease between the concentrations of NR used for watering the plants, compared to wheat tests. In this case it was observed a significant decrease in total flavonoid content for 1 mg/L NR concentration, approximately 65%, and for all other concentrations used the decrease is approximately 50% compared to control (Figure 2).

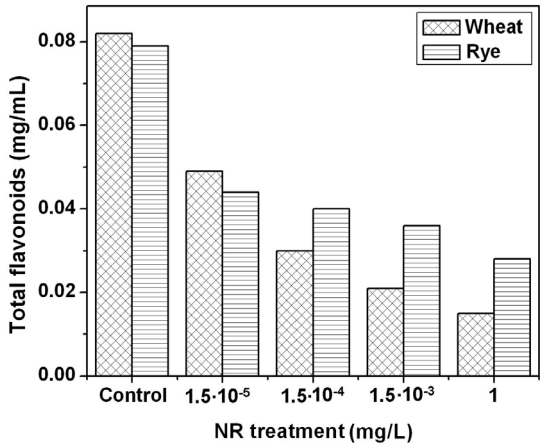


Figure 2. The total flavonoid content in wheat and rye treated with NR in different concentrations.

For AMP treatments on wheat, a decrease of total flavonoid content was observed, approximately 60% compared to control, even at the lowest concentration used for watering the plants ($1.5 \cdot 10^{-5}$ mg/L). The amounts of total flavonoids decrease with increasing the concentration of AMP. The differences between the decreases of total flavonoids are not very significantly influenced by the concentrations of AMP used for plant treatment, especially in the case of rye. This aspect it might be caused by the nature of plant according to that the plants resist differently to these abiotic stress conditions after a certain period of time. In the case of the rye treated with AMP, for all concentrations used in this test, the total flavonoid content decreased with approximately 30% compared to control (Figure 3).

For the identification and quantification of total flavonoids in treated plants, a simple, cheap and easy to use UV-Vis method was used.

The method used was according to Romanian Pharmacopoeia and the quantitative determination was expressed as rutin. For the calibration plot, a correlation coefficient of 0.99942 was obtained.

In both treatments with NR and AMP, a continuous and uniform decrease of total flavonoid content compared to control samples of (untreated wheat and rye) can be observed. The lowest amount of total flavonoids is observed in the case of NR textile azo dye, for both treated plants (wheat and rye). This fact explains that the class of azo dyes is considered more toxic for environment.

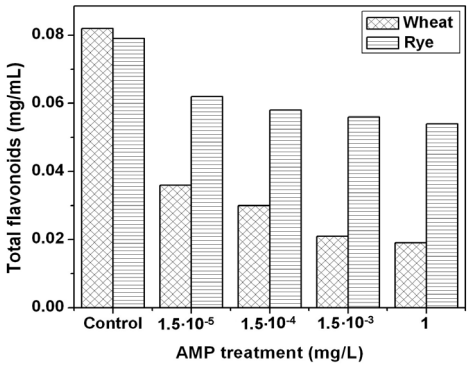
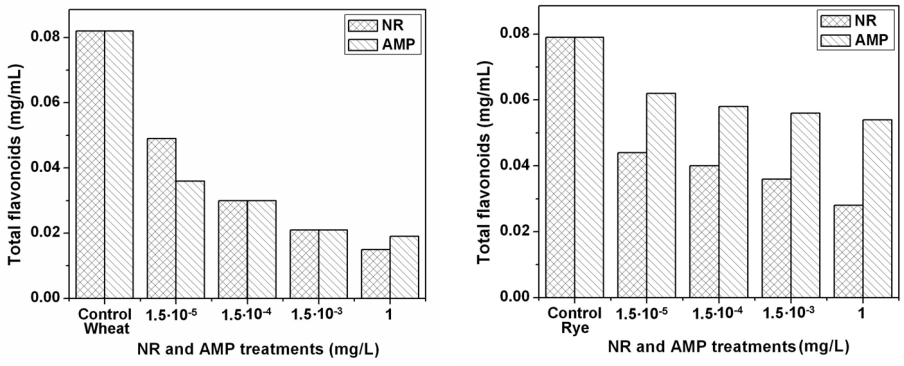


Figure 3. The total flavonoid content in wheat and rye treated with AMP in different concentrations.

The both treatments with NR and AMP reduce more drastically the total flavonoid content in the case of wheat (Figure 4a) in comparison with that of rye (Figure 4b). The two pollutants act practically similar over the wheat (Figure 4a), except for $1.5 \cdot 10^{-5}$ mg/L concentration when ampicillin reduces more total flavonoids than Nylosan Red. In the case of rye (Figure 4b), the azo dye reduces drastically the total flavonoids than the antibiotic. The concentration of total flavonoids decreases slowly according to the increase of NR and AMP concentration respectively.



(a) (b)

Figure 4. The total flavonoid content in wheat (a) and rye (b) treated with NL and AMP in different concentrations.

CONCLUSIONS

Due to the importance of flavonoids for living organisms, their identification and determination in plant tissue play an important role for plant growth.

The determination of total flavonoid content in wheat and rye, treated with the NR azo-textile dye and the AMP antibiotic at four different concentrations ($1.5 \cdot 10^{-5}$, $1.5 \cdot 10^{-4}$, $1.5 \cdot 10^{-3}$, 1 mg/L) was performed using UV-Vis technique.

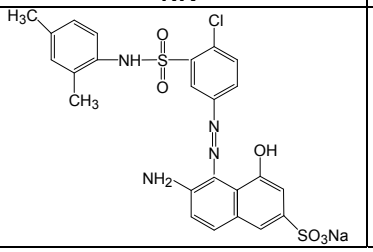
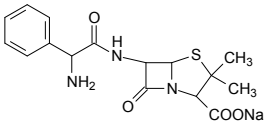
UV-Vis spectrophotometric method is a simple, accessible and inexpensive technique, easy to use in order to obtain information concerning the action of studied pollutants over the total flavonoids from plants. The UV-Vis measurements of wheat and rye samples untreated and treated with Nylosan Red N-2RBL (textile azo dye) and ampicillin (beta-lactam antibiotic) give us useful data regarding the possible toxic effects of these two classes of compounds over the plants and by these over the human and animal health. According to our studies, the toxic effects of these compounds depend on the matrix over they have contact and act.

EXPERIMENTAL SECTION

Chemicals: The Nylosan Red N-2RBL textile azo dye [18, 19] and ampicillin sodium salt antibiotic [20] were purchased from Clariant Produkte (Switzerland) AG, respectively from Antibiotice (Romania) (Table 1). Ethanol of 96% was acquired from Nordic Invest (Romania). $AlCl_3$ 99%, sodium acetate (AcNa) used for sample preparation and rutin trihydrate 97% used for quantitative analysis were purchased from Alfa Aesar (Germany). Water purified by a Milli-Q Ultrapure water purification system (Millipore, USA) was used for experiments.

Table 1. Data of selected compounds used for plant treatments.

Common name/ Abbreviation	**Nylosan Red N-2RBL/ NR**	**Ampicillin/ AMP**
Class	Azo dye	Antibiotic
Chemical name	Sodium 6-amino-5-[[4-chloro-3-[[(2,4 dimethylphenyl) amino]sulfonyl]phenyl]azo]-4-hydroxynaphthalene-2-sulfonate	Sodium [2S-[2α,5α,6β (S*)]]-6-(aminophenyl-acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
CAS number	71873-39-7	69-52-3
Chemical formula	$C_{24}H_{20}ClN_4O_6S_2Na$	$C_{16}H_{18}N_3O_4SNa$
Molecular weight ($g \cdot mol^{-1}$)	583.01	371.39

Common name/ Abbreviation	Nylosan Red N-2RBL/ NR	Ampicillin/ AMP
Structural formula		

Apparatus: UV-Vis 1800 Shimadzu spectrophotometer with software UV Probe Ver. 2.31.

Plant material: The wheat and rye were the studied plants and they were grown by periodically watering with different solutions of NR and AMP respectively, and monitored during 39 days. Each studied plant was submitted to five different treatments consisting in one for control (untreated) and four treated with pollutant (NR and AMP respectively) at four different concentrations ($1.5 \cdot 10^{-5}$, $1.5 \cdot 10^{-4}$, $1.5 \cdot 10^{-3}$, 1 mg/L). All treatments including controls were carried out in duplicate. The detailed procedure consisted as follows: each test was conducted in aluminum recipients (22×10×6 cm); into each recipient, separately 70 seeds of wheat and rye respectively were sown at a depth of 1 cm; the plants were regularly watered at intervals of 3 days, with 70 mL of NR and AMP solutions of different concentrations. The average temperature of the room where the experiments were done was 22°C. Until the extraction of total flavonoids, the plants were watered 13 times with NR and AMP solutions respectively.

Stock solution: For the quantitative analysis of total flavonoid content in wheat and rye, the calibration plot of rutin was performed. The stock solution of rutin was prepared at 1 mg/mL concentration.

Working solutions: The working solutions of rutin in concentration of 0.02, 0.04, 0.05, 0.08, 0.10, 0.20, 0.40 mg/mL were prepared by diluting the stock solution.

Measuring solutions: The measuring solutions were prepared as follows: 1 mL working solution with 1.5 mL of NaAc 100 g/L, and 2.5 mL $AlCl_3$ 25 g/L, were brought with ethanol to a final volume of 10 mL.

Blank solutions: The blank solutions were consisted in 1 mL working solution, 8 mL water and brought with ethanol to the same final volume of 10 mL.

Sample preparation: The extraction procedure of flavonoids from wheat and rye is presented below. An amount of 0.5 g plant material was powdered with liquid nitrogen and then was added 20 mL of ethanol:water

mixture in ratio of 80:20 (v/v). For maceration, the extracts were kept 10 min in oven at temperature of 35°C, and then were sonicated for 30 minutes at the same temperature. Each extract was filtered through Macherey-Nagel filters (MN 640 d·ø 125 mm) and it was brought to a final volume of 25 mL with the same solvent mixture. The quantitative determination of total flavonoids was done by UV-Vis method using $AlCl_3$ as reagent and rutin as standard [21].

Data handling: All data shown in figures were performed using ORIGIN 8 (OriginLab Corporation, MA, USA) software.

ACKNOWLEDGMENTS

This paper was realized with the support of EURODOC "Doctoral Scholarships for research performance at European level" project, financed by the European Social Fund and Romanian Government.

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