Diclofenac Influence on Photosynthetic Parameters and Volatile Organic Compounds Emission from *Phaseolus vulgaris* L. Plants

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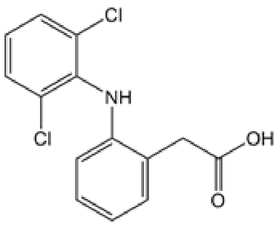
Diclofenac, both from human and veterinary consumption, may arrive in landfills or in the wastewater treatment plants, becoming an environmental pollutant. Therefore, we aimed to study the influence of diclofenac on plants growth and development. We chose as model plant the bean (Phaseolus vulgaris L.) that was watered with different concentrations of aqueous diclofenac solutions (0-0.4 g/L). The plants exhibited linear decreased values of net assimilation rates and stomatal conductance to water vapors with increased diclofenac's concentrations. Emission of 3-hexenol was determined to scale up with diclofenac concentration, therefore this compound may be proposed as stress marker. Also in the emission of bean plants were detected 3 different monoterpenes (α-pinene, camphene and 3-carene), their concentration increasing with elevated concentration of diclofenac. We can conclude that diclofenac may affect the plants photosynthetic parameters and also might disturb the methylerythritol phosphate pathway (MEP) in plastids.

Keywords: diclofenac, photosynthetic parameters, volatile organic compounds emission, Phaseolus Vulgaris l. plants

2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid (Diclofenac) (scheme 1) is one of the most popular non-steroidal anti-inflammatory drugs along with ibuprofen, aspirin, acetaminophen, ketoprofen, naproxen [1], and subject of a lot of studies [2-5].



Scheme 1. Structure of Diclofenac

It is used to reduce pain, fever and inflammation, being sold without prescription in many countries worldwide [6].

In general, annual pharmaceuticals consumption could arrive at an average of 150 g per capita in many industrialized countries [7]. Diclofenac is prescribed as oral tablets, topical gel, or suppositories, being sold under different trade names. The total consumption of diclofenac is almost impossible to be determined, but Intercontinental Marketing Services (IMS) health data have been estimated that around 940 tons of diclofenac is consumed in one year. Even more, the total sale of diclofenac in 2011 has been around 1.5 billion dollars [8]. According to IMS health data around 1500 tons of diclofenac is consumed globally only for human medicine [9]. In Europe, the most usage of diclofenac has been reported in Germany with around 86 tons in 2001 [10], while in England the total consumption has been 26.13 tons per year [11].

Medicines are polluting the environment [12-15], mainly water sources [16-22] and soil [23-28], and affect the plants growth and development. Studies regarding the medicines concentrations that reach into the soil and their impact on plants is scary. The main sources of diclofenac pollution are the human and veterinary routes from which diclofenac ends up in landfills or in the wastewater treatment plants [7]. The medium concentration in German rivers for examples has been determined at 0.15 μg/L but concentration of 1.2μg/L has been determined [29]. It has been demonstrated that living organisms could be affected by pharmaceutical contamination [30] but they could be used as phyto-indicators [31]. Diclofenac has also been shown to induce cytological changes in rainbow and brown trout tissues after 21 days of exposure at 50 μg/L diclofenac [32]. Furthermore, mussels could accumulate diclofenac from the water if they are exposed even at small concentrations [33]. The number of the studies regarding the diclofenac effects on the plants is very low. It has been demonstrated that diclofenac reduces the seedlings growth and determined the increasing of enzymatic activity in three leguminous plants (lupin, pea, lentil) [34] but this compound has less phytotoxic effect than different antibiotics. Biochemical processes of duckweed plants have been affected even at a very low concentration (10 μg/L) of diclofenac [35].

In the present study we observed the influence of diclofenac on bean (*Phaseolus vulgaris* L.) as a model plant. Consequently, we determined the photosynthetic parameters, the emission of volatile organic compounds and concentration of pigments of bean in the presence of diverse concentrations of diclofenac.

Experimental part

Materials and methods

All commercial chemicals and solvents are reagent grade and were used without further purification. Acetone was purchased from Merck-Schuchardt, Germany.

Bean plants (*Phaseolus vulgaris* L.) var. Ecaterina, Agrosel, Campia Turzii, Romania, were seeded in 3 L pots and grown for 3 weeks under artificial light at a rate of 300 μmol m^{-2} s^{-1}. The light / dark period was 12-12 h and temperature was 25°C. The plants were watered every

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second days. Four different diclofenac solution were used for plant treatment: 0.1 g/L, 0.2 g/L, 0.3 g/L and 0.4 g/L.

A portable gas exchange system GFS-3000 (Waltz, Effeltrich, Germany) has been used for photosynthesis measurement at following parameters: .air flow into the cuvette - 750μmol/s, CO_2 - 385 ppm, PARtop 1000 mol m^{-2} s^{-1}, relative humidity 65%, leafs temperature 25°C.

A part of the flow from the gas exchange system cuvette has been deviated via a T tube and sampled in a tube filled with adsorbent as described in [36].

Tubes were desorbed using a TD 20 thermodesorber (Shimadzu, Japan) and injected in a gas chromatograph coupled with mass spectrometer (Shimatzu 2010 plus, GCMSTQ8040, Japan). The carrier gas was helium and a capillary column (1 Accent MS column OPTIMA Germany) (50 m × 0.2 mm, film thickness 0.33 μm) has been used for separation of the compounds. The program used to separate the volatile compounds is: 40 °C for 1 min^{-1}, 9°C min^{-1} at 120°C, 2 °C min^{-1} at 190°C, 20°C min^{-1} at 240°C, 240°C for 5 min.

The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range m/z 48–400, the transfer line temperature was set at 250 °C and ion-source temperature at 200 °C. The compounds have been identified using their mass spectra by comparison with the mass spectra from database (NIST 14.0).

For the pigments analysis we used the same method as described in [37].

Results and discussions

The influence of diclofenac on photosynthetic parameters

Net assimilation rates and stomatal conductance to water vapors are significant different relative to control for plants treated even with 0.1 g/L diclofenac. For both parameters a linear decrease has been observed (fig. 1).

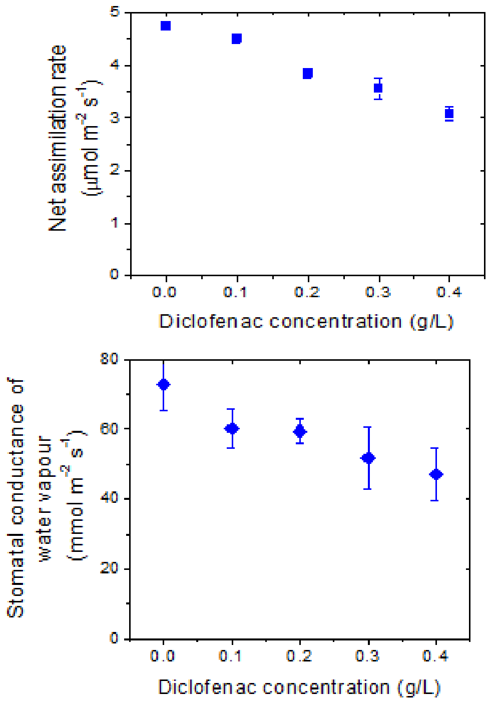


Fig. 1. Changes in net assimilation rate and, stomatal conductance to water vapor per unit projected leaf area in *Phaseolus vulgaris* L. cv. *Ecaterina* plants treated with diclofenac

Such behavior of assimilation rate and stomatal conductance indicated that the potential of photosynthetic activity of the plants decreased in all treatments compared to control. Kummerova et al. have been shown the same behavior in *Lemna minor* plants treated with 0.1 mg diclofenac [35]. They have been demonstrated that values of non-photochemical quenching (NPQ) are increasing until more than 60 % relative to control for plants treated with diclofenac. Such behavior indicates an excess-radiant energy dissipation to heat in photosynthetic system II (PSII) antenna complexes during light-adapted state.

Volatile organic compounds emission

Emission rates of lipoxygenase pathway volatiles (named green leaf volatiles) and monoterpenes were analyzed in order to show the effects of diclofenac on bean plants. The green leaf volatiles are emitted by plants in case of different abiotic or biotic stresses (see for review [38]). *Phaseolus vulgaris* L. leaves emitted (Z)-3-hexenol (scheme 2) as a response of diclofenac stress. Even more, the emission rates are scaling up with diclofenac concentrations as this compound could be used as stress marker. Our results are in good correlation with other published papers which have been shown that different C6 aldehydes emission from tomato leaves scale increase with stress temperatures [39].

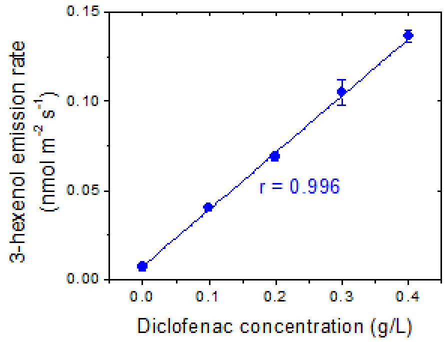


Fig. 2. Emissions of (Z)-3-hexenol from *Phaseolus vulgaris* L. cv. *Ecaterina* plants in response to diclofenac treatments

The emission of 3 different monoterpenes (α-pinene, camphene and 3-carene) was found in case of diclofenac stress. *Phaseolus vulgaris* L. is not a constitutive monoterpene emitter under non-stressed conditions, monoterpenes emission being typically induced in response to abiotic or biotic stresses [40, 41].

The α-pinene and camphene (scheme 2) emissions are statistically significant higher than control but there is

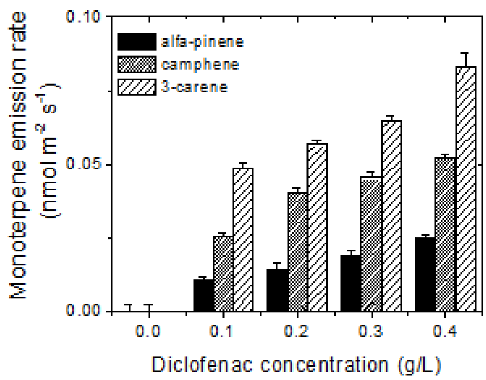
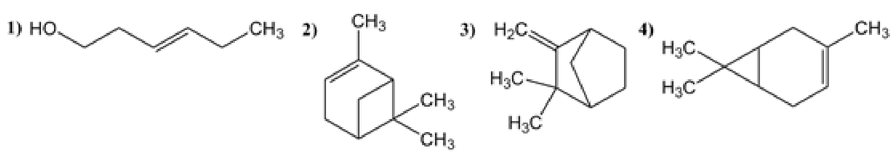


Fig. 3. Emissions of monoterpenes from *Phaseolus vulgaris* L. cv. *Ecaterina* plants in response to diclofenac treatments



Scheme 2. Structures of volatile organic compounds emitted in case of diclofenac stress on *Phaseolus vulgaris* L.: 1) 3-hexenol, 2) α-pinene, 3) camphene, and 4) 3-carene

no statistical difference in the emission in case of all 4 treatments with diclofenac. In contrast, 3-carene emission is increasing with diclofenac soil concentrations. The fact could be explain by the disturbance in methylerythritol phosphate pathway (MEP) in plastids due to diclofenac.

Changes in chlorophylls and β-carotene composition

The roles of the carotenoids are to protect against oxidative damage and from photo inhibition but due to oxidative severe stress conditions they could be rapidly destroyed [42]. The chlorophyll a and b concentrations decrease for plants leaves treated with diclofenac as can be seen in figure 4.

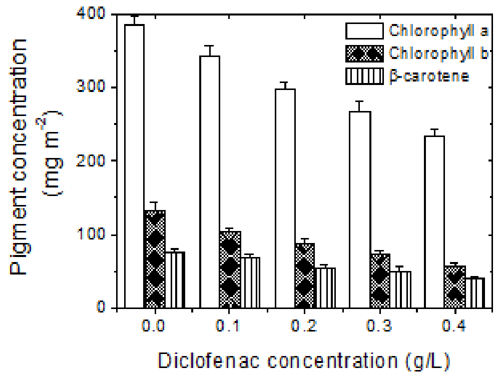


Fig. 4. Effects of treatments with diclofenac on the chlorophyll a and b and β-carotene contents of *Phaseolus vulgaris* L. cv. *Ecaterina* plants

It has been demonstrated that chlorophyll fluorescence is not influenced by the very low concentration of diclofenac (less than 0.1 mg/L) but for concentrations higher than 0.1 g/L both pigments concentration decreased, which means that there are exhibited important effects on PSII.

The presence of diclofenac influences the β-carotene content in the bean leaves. Even those carotenoids play an important role in photo protection of photosynthetic apparatus, in the presence of diclofenac their function is not exhibited very clear.

In some of our previous studies we have shown the same moderate effect on photosynthetic pigments of antibiotics and textile days [37, 41, 43]

Conclusions

It has been shown that diclofenac could affect the plants photosynthetic parameters and could influence the metabolic pathways. Green leaf volatiles are scaling up with the diclofenac concentration and could be used as a stress signals.

The results attained in the study help us to better estimate the influence of medicines on plants.

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