ORIGINAL ARTICLE

THE INFLUENCE OF RESIDUAL ACETAMINOPHEN ON *PHASEOLUS VULGARIS* L. SECONDARY METABOLITES

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Abstract

This study investigated the influence of paracetamol as a pollutant from wastewater on the beans plant (*Phaseolus vulgaris* L.). We used a portable gas exchange system to determine the photosynthetic parameters, gas chromatography-mass spectrometry (GC-MS) technique to determine the volatile organic compounds (VOC) produced by plants, and ultra-high performance liquid chromatography (UHPLC) to analyse the plants pigments (chlorophylls and β-carotene). The plants were grown in pots and treated with diverse concentrations of paracetamol. The residual concentration of paracetamol from the environment can be detrimental to the growth and development of the plants. Paracetamol influences the photosynthetic parameters (net assimilation rate and stomatal conductance), the emission rate of (Z)-3-hexenol, the concentration of 3-carene (one out the 3 monoterpenes identified in plant emission besides α-pinene and camphene), and β-carotene on *Phaseolus vulgaris* L. Chlorophyll was not influenced by paracetamol concentration.

Rezumat

Acest studiu a investigat influența paracetamolului ca poluant din ape reziduale asupra plantei *Phaseolus vulgaris* L. Am utilizat un sistem portabil de schimb de gaze pentru a determina parametrii de fotosinteză, tehnica de cromatografie de gaze-spectrometrie de masă (GC-MS) pentru a determina compușii organici volatili (COV) ai plantelor și cromatografia de lichide de ultra înaltă performanță (UHPLC) pentru analiza pigmenților (clorofile și β-caroten). Plantele au fost crescute în ghivece și tratate cu soluții apoase cu diferite concentrații de paracetamol. Concentrația reziduală a paracetamolului din mediul înconjurător poate fi în detrimentul creșterii și dezvoltării plantelor. Paracetamolul influențează parametrii de fotosinteză (rata netă de asimilare și conductivitatea stomatală), rata de emisie a (Z)-3-hexenolului, concentrația 3-carenului și concentrația β-carotenului la *Phaseolus vulgaris* L. Clorofilele nu au fost influențate de concentrațiile de paracetamol.

Keywords: acetaminophen (paracetamol), *Phaseolus vulgaris* L., photosynthetic parameters, volatile organic compounds, pigments

Introduction

Acetaminophen (paracetamol) is an antipyretic and analgesic medicine, mostly used for mild to moderate pain and to reduce the fever. Paracetamol is the most commonly prescribed drug for patients in case of moderate fever [2, 21] and the first choice analgesic including oral diseases [8, 13]. Due to the fact that paracetamol is one of the most recommended over-the-counter (OTC) drug, its market was valued at around 801.3 million USD in 2014 and is expected to reach 999.4 million USD in 2020 [33]. It has been shown in a report that in the U.S. 36.5% of the inter-viewed population had used paracetamol in two weeks [3], while in Germany 5.2% of a consulted population used it in 7 days [31]. In Sweden, one study reported that more than 70% of the population had used paracetamol during three months [20]. In Romania, studies showed that self-medication administrated by 46.6% from the interviewed population used paracetamol during six months [5, 32].

A great concern about the influence of different pharmaceutical products and also of their metabolites on the environment has been rising due the fact that different antidepressants, antibiotics, antihistamines, analgesics have been found in surface, ground, and drinking waters [6, 7, 12, 14, 15, 24, 27, 29]. The concentrations of paracetamol in River Doubs and the River Lounge have been found to be 273 and 158 ng/L, respectively, being one of the most critical pollutant after sulfamethoxazole and ofloxacine [9]. Previous studies have found paracetamol at concentrations above 65 μg/L in the Tyne River, UK [30]. In a recent study there were observed concentrations of 12.42 μg/L paracetamol in the waste water treatment plants influents of several rivers from Romania [17]. The ecological influence of paracetamol has been demonstrated on bivalve as

Corbicula fluminea, crustaceans (*Daphnia magna*) or fish (*Oncorhynchus mykiss* and *Anguilla anguilla*) [16]. Paracetamol induced oxidative stress in the amphipod species of *Hyalella Azteca* [18]. The published studies related to the influence of paracetamol on plants are scarce. Anyway, the inhibition of *Triticum aestivum* seedlings growth and changes in photosynthetic pigments in the presence of paracetamol has been demonstrated [1]. The researchers have shown that chlorophyll synthesis in wheat leaves has been inhibited by an exposure of 14 days at 2.8 mg/L paracetamol. Soluble protein peroxidase and superoxide dismutase in wheat seedlings have been affected only by exposure to a very high concentration of paracetamol. In another study, the total polyphenols content has been increased for *Lemna minor* plants treated with very low paracetamol concentration (0.1 mg/L) [23], the polyphenols as well as other active substances content of different plants varying obviously depending on a number of other factors [4, 28]. In the present study we examined the influence of paracetamol on bean (*Phaseolus vulgaris*L.) as a model plant.

Materials and Methods

All commercial chemicals and solvents were of reagent grade and were used without further purification. Acetone and paracetamol were purchased from Merck, Germany.

Plant material

Bean plants (*Phaseolus vulgaris* L.) var. Odir, GBBR, Bekescsaba Hungary were seeded in 3 L pots and grown for 3 weeks under artificial light at a rate of 300 μmol/m^2·s. The light/dark period was 12/12 hours. The temperature was 25°C. The plants were watered every two days. Four different paracetamol aqueous solutions were used for plant treatment: 1 g/L, 2 g/L, 3 g/L and 4 g/L.

Photosynthestic parameters determination

Photosynthesis measurements were conducted with a portable gas exchange system GFS-3000 (Waltz, Effeltrich, Germany). The system has an environmental-controlled cuvette with 8 cm^2 window area. Each time, a leaf fully filling the cuvette area was enclosed. The following measurement parameters that have been set were kept constant during the measurements as follows: air flow into the cuvette: 750 μmol/s, CO_2: 385 ppm, PARtop 1000 μmol/m^2·s, relative humidity 65%, cell temperature 25°C. The bean leaves were stabilized under the standard conditions until stomata opened and steady-state CO_2 and water vapour exchange rates were reached.

Volatile organic compounds (VOC) sampling and GC–MS analysis

VOC sampling was performed on adsorbent cartridges *via* the outlets of each cuvette, using steel tube with size ¼ filled with adsorbent [22]. A part of the flow from the cuvettes has been deviated *via* a T tube and sampled in the tube.

The air flow rate was 200 mL/min and the adsorption time was 20 minutes for each sample. The carbotrap tubes were initially conditioned with helium for 60 minutes to remove possible contaminants. After sampling, the tubes were stored at -24°C until analysis.

Tubes were desorbed using a TD 20 thermodesorber (Shimadzu, Japan). A gas chromatograph coupled with mass spectrometer (Shimadzu 2010 plus, GCMSTQ8040, Shimadzu, Japan) was used for determination. The carrier gas was helium. VOCs were separated using a capillary column (1 Accent MS column OPTIMA, Germany) (50 m × 0.2 mm, film thickness 0.33 μm). The program used to separate the volatile compounds was: 40°C for 1 min, 9°C/min at 120°C, 2°C/min at 190°C, 20°C/min 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. For the identification of the compounds in the samples, standard solutions and the mass spectra from NIST 14.0 database were used.

Pigments analysis

Bean leaf samples of 4 cm^2 were frozen in liquid nitrogen. The pigments were extracted in ice-cold acetone (100%) in the presence of calcium carbonate. Then the extract was centrifuged with a Hettich 380R Universal centrifuge (Hettich GmbH, Germany) at 0°C and 9500 g for 3 minutes. The supernatant was removed and the extraction was repeated till the supernatant became colourless. The pooled supernatant was reduced to 1 mL volume and filtered through a 0.45 mm PTFE membrane filter (PALL, USA). The UHPLC (NEXERA8030, Shimadzu, Japan) was calibrated using: chlorophyll a, chlorophyll b, and β-carotene. The extracts were analysed for these pigments by using a NUCLEOSIL 100-3 C18 reversed-phase column (Macherey-Nagel AG, Germany) and the method described in literature by Opriș *et al*. [26].

Statistics

All experiments were performed in triplicates with independent samples of plants. The means were statistically compared with ANOVA and then post hoc Tukey's test was applied using ORIGIN8 (Origin Lab Corp., Northampton, MA, USA). All statistical tests were considered significant at $p < 0.05$.

Results and Discussion

The influence of paracetamol on photosynthetic parameters

Net assimilation rates and stomatal conductance to water vapours were not significant different relative to control for plants treated with 1 g/L paracetamol. Assimilation rate decreased with more than 20% in

case of the plants treated with 2 g/L paracetamol. The same trend has been observed for stomatal conductance to water vapour, but in this case there were no significant differences between plants treated with higher concentration than 2 g/L paracetamol (Figure 1).

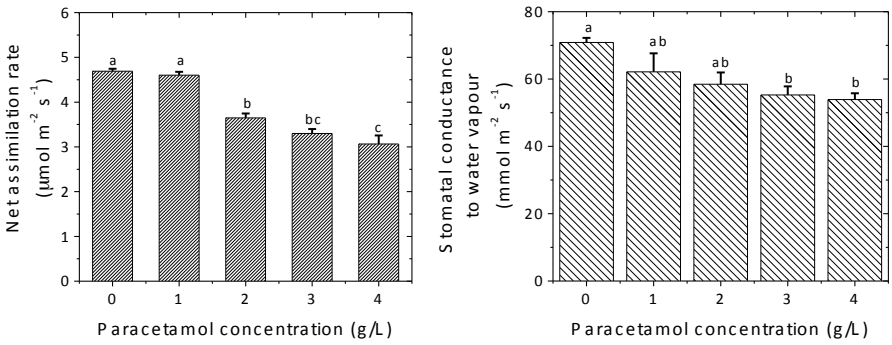

Figure 1.
Changes in net assimilation rate and stomatal conductance to water vapour *per* unit projected leaf area in *Phaseolus vulgaris* L. cv. Odir plants treated with paracetamol

Such behaviour of assimilation rate and stomatal conductance indicates that the potential photosynthetic activity of the plant decreased in all treatments compared to control. Our results are consistent with literature [23] which has shown a decrease in the ratio of chlorophyll fluorescence for plants treated with paracetamol.

Volatile organic compounds emission

To gain further into the effects of paracetamol on plants, emission rates of green leaf volatiles (known as lipoxygenase pathway products) and monoterpenes were analysed. The green leaf volatile compound emitted by *Phaseolus vulgaris* L. leaves identified in the samples was (*Z*)-3-hexenol (Figure 2). As well, three monoterpenes: α-pinene, camphene and 3-carene (Figure 2) were also found in the emission.

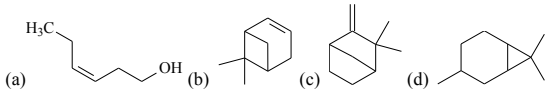

Figure 2.
Chemical structures of the three monoterpenes found: 3-hexenol (a), α-pinene (b), camphene (c), and 3-carene (d)

Anyway, the concentrations of all emitted compounds were very low in the control plants. The stressed plants with low paracetamol concentrations exhibited a very low emission (Figure 3), while the high concentration of paracetamol induced quite high concentration emission of green leaf volatile. However, the emission rate for this lipoxygenase pathway product increased linearly with the concentration of the stress.

Bean is not a constitutive monoterpene emitter under non-stressed conditions. Furthermore, monoterpenes are typically induced in response to abiotic or biotic stresses [10, 11, 12].

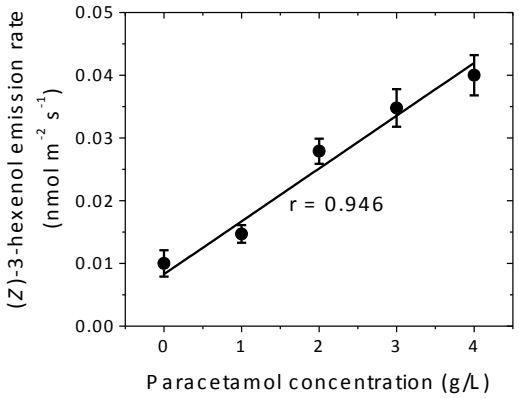

Figure 3.
Emissions of (Z)-3-hexenol from *Phaseolus vulgaris* L. cv. Odir plants in response to paracetamol treatments

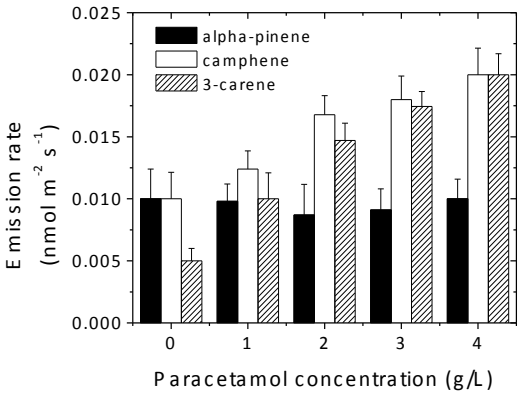

Figure 4.
Emissions of monoterpenes from *Phaseolus vulgaris* L. cv. Odir plants in response to paracetamol treatments

In the present study, very low emissions of three monoterpenes have been found: α-pinene, camphene and 3-carene (Figure 4). From them, only in the case of 3-carene there were different emissions for

increasing stress concentrations. For α-pinene and camphene the pathway flux going to monoterpene synthesis was not disturbed.

Changes in chlorophylls and β-carotene composition

Carotenoids can be rapidly destroyed by oxidative severe stress conditions therefore are no longer available to protect against oxidative damage and from photoinhibition [25]. In our study, paracetamol did not influence significantly the chlorophyll concentrations (Figure 5).

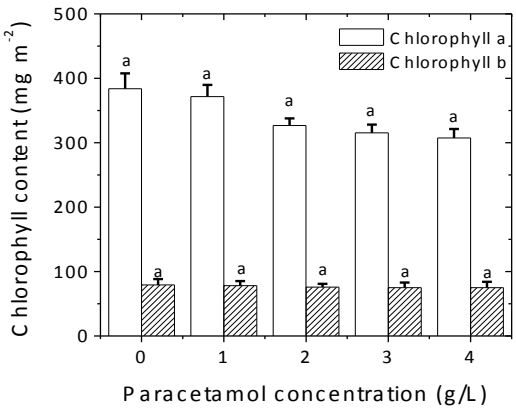

Figure 5.

Effects of treatments with paracetamol on the chlorophyll a and b contents of *Phaseolus vulgaris* L. cv. Odir plants

The β-carotene content in the bean leaves was significantly influenced by the concentration of paracetamol ($p < 0.05$) (Figure 6). We observed a decreased quantity of β-carotene with more than 20% in the case of plants treated with 2 g/L paracetamol and significant decreases at higher concentration of paracetamol ($p < 0.05$).

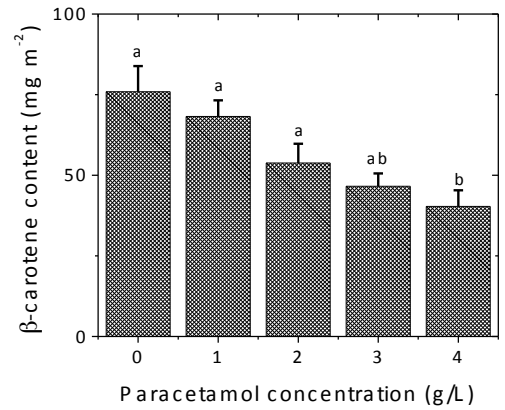

Figure 6.

Changes in β-carotene content in *Phaseolus vulgaris* L. cv. Odir plants in response to treatments with paracetamol

The same effect on photosynthetic pigments of antibiotics and textile dyes has been found in previous studies [10, 26].

The present study revealed the influence of low concentrations of paracetamol on growth and development of bean plant that is an important food product. Nowadays, there are scarce information about the influence of long-term exposure of small concentrations of pharmaceuticals on both plant and human health and development. There is an actual need for studies that approach the long-term effects of low doses of medicines and their degradation products (from the environment and food products exposed to them) on human health. A more challenging study will include the behaviour of mixtures of medicines and also the interactions of mixtures of drugs with other pollutants from the environment on human health.

Conclusions

Paracetamol is influencing the growth and development of bean plant (*Phaseolus vulgaris* L.). Our data have shown that photosynthetic parameters, such as net assimilation rate and stomatal conductance, decreased when higher concentrations than 2 g/L of paracetamol were used. We have also determined that the emission of green leaf volatile scale strengthens with the stress (paracetamol concentration), while monoterpenes emission is not affected by paracetamol. Even more, β-carotene concentrations decrease with the drug concentrations.

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References

1. An J., Zhou Q.X., Sun F.H., Zhang L., Eco-toxicological effects of paracetamol on seed germination and seedling development of wheat (*Triticum aestivum* L.). *J. Hazard Mater.*, 2009; 169: 751-757.
2. Bailey B., Trottier E.D., Managing Pediatric Pain in the Emergency Department. *Pediatr. Drugs*, 2016; 18: 287-301.
3. Boudreau D.M., Wirtz H., Von Korff M., Catz S.L., St John J., Stang P.E., A survey of adult awareness and use of medicine containing acetaminophen. *Pharmacoepidemiol. Drug Saf.*, 2013; 22: 229-240.
4. Bungău S., Bâldea I., Copolovici L., Determination of ascorbic acid in fruit using a Landolt type method. *Rev. Chim. Bucharest*, 2003; 54(3): 213-216.
5. Bungău S., Bungău C., Ţiţ D.M., Pallag A., The influence of specialized university studies on self-medication. *Rev. Rom. Bioet.*, 2015; 13(1): 153-158.
6. Bungău S., Bungău C., Ţiţ D.M., Studies about last stage of product lifecycle management for a pharmaceutical product. *J. Environ. Prot. Ecol.*, 2015; 16(1): 56-62.

7. Bungău S., Suciu R., Bumbu A., Cioca G., Ţiţ D.M., Study on hospital waste management in medical rehabilitation clinical hospital, Băile Felix. *J. Environ. Prot. Ecol.*, 2015; 16(3): 980-987.
8. Cazacu I., Leucuta D.C., Baciut M., Baciut G., Mogosan C., Haramburu F., Fourrierréglat A., Loghin F., Acute postoperative pain and analgesics consumption in oral and maxillofacial surgery. *Farmacia*, 2016; 64(4): 539-543.
9. Chiffre A., Degiorgi F., Bulete A., Spinner, L., Badot P.M., Occurrence of pharmaceuticals in WWTP effluents and their impact in a karstic rural catchment of Eastern France. *Environ. Sci. Pollut. Res.*, 2016; 23: 25427-25441.
10. Copaciu F., Opriş O., Coman V., Ristoiu D., Niinemets U., Copolovici L., Diffuse water pollution by anthraquinone and azo dyes in environment importantly alters foliage volatiles, carotenoids and physiology in wheat (*Triticum aestivum*). *Water Air Soil Pollut.*, 2013; 224: 1478.
11. Copolovici L., Kaennaste A., Remmel T., Niinemets U., Volatile organic compound emissions from *Alnus glutinosa* under interacting drought and herbivory stresses. *Environ. Exp. Bot.*, 2014; 100: 55-63.
12. Copolovici L., Niinemets U., Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. *Plant Cell Environ.*, 2010; 33: 1582-1594.
13. Dumitrache M.A., Ionescu E., Sfeatcu R., Ginghina O., Burcea Dragomiroiu G.T.A., Petre A., The pharmacist's role in preventive and pharmaceutical treatment for oral diseases. *Farmacia*, 2016; 64(6): 966-969.
14. Fodor A., Petrehele A., Bungău S., Ţiţ D.M., Tetracycline and metabolites in agricultural farmland soil and *Petroselinum Crispum* var. *Neapolitanum* roots and leafs. A*nnals Oradea Univ., Fasc. Environ. Prot.*, 2013; XX(A): 1-6.
15. Fodor A., Petruş-Vancea A., Petrehele A.I.G., Bungău S.G., Accumulation Properties and Foliar Limb Effects of Tetracycline and Metabolites in *Lactuca sativa* L., *Eruca Sativa* L. and *Spinacia oleracea* L.. *Nat. Res. Sust. Develop.*, 2015; 2015: 57-66.
16. Freitas R., Coelho D., Pires A., Soares A., Figueira E., Nunes B., Preliminary evaluation of *Diopatra neapolitana* regenerative capacity as a biomarker for paracetamol exposure. *Environ. Sci. Pollut. Res.*, 2015; 22: 13382-13392.
17. Gheorghe S., Petre J., Lucaciu I., Stoica C., Nita-Lazar M., Risk screening of pharmaceutical compounds in Romanian aquatic environment. *Environ. Monit. Assess.*, 2016; 188(6): 379.
18. Gomez-Olivan L.M., Neri-Cruz N., Galar-Martinez M., Vieyra-Reyes P., Garcia-Medina S., Razo-Estrada C., Dublan-Garcia O., Corral-Avitia A.Y., Assessing the oxidative stress induced by paracetamol spiked in artificial sediment on *Hyalella azteca*. *Water Air Soil Pollut.*, 2012; 223: 5097-5104.
19. Grigore R.A., Mihai A.M., Ciolan D.F., Tarmure V., Ionescu E., Pain therapy associated with orthodontic treatment. *Farmacia*, 2014; 62(6): 1062-1071.
20. Hedenrud T., Hakonse H., Purchase habits, use of paracetamol, and information sources on a reregulated Swedish pharmacy market: A population-based study. *Health Policy*, 2017; 121: 35-41.
21. Kanabar D.J., A clinical and safety review of paracetamol and ibuprofen in children. *Inflammo-pharmacology*, 2017; 25: 1-9.
22. Kannaste A., Copolovici L., Niinemets U., Gas Chromatography-Mass Spectrometry method for determination of biogenic volatile organic compounds emitted by plants. *Pl. Isoprenoids: Meth. Prot.*, 2014; 1153: 161-169.
23. Kummerova M., Zezulka S., Babula P., Triska J., Possible ecological risk of two pharmaceuticals diclofenac and paracetamol demonstrated on a model plant *Lemna minor*. *J. Hazard Mater.*, 2016; 302: 351-361.
24. Mompelat S., Thomas O., Le Bot B., Contamination levels of human pharmaceutical compounds in French surface and drinking water. *J. Environ. Monit.*, 2011; 13: 2929-2939.
25. Munné-Bosch S., Peñuelas J., Photo- and anti-oxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta*, 2003; 217: 758-766.
26. Opriş O., Copaciu F., Soran M.L., Ristoiu D., Niinemets U., Copolovici L., Influence of nine antibiotics on key secondary metabolites and physiological characteristics in *Triticum aestivum*: Leaf volatiles as a promising new tool to assess toxicity. *Ecotox. Environ. Saf.*, 2013; 87: 70-79.
27. Orbeci C., Nechifor G., Stănescu R., Removing toxic compounds from wastewater. *Environ. Eng. Manag. J.*, 2014; 13(9): 2153-2158.
28. Pallag A., Bungău S.G., Ţiţ D.M., Jurca T., Sîrbu V., Honiges A., Horhogea C., Comparative study of polyphenols, flavonoids, and chlorophylls in *Equisetum Arvense* T. populations. *Rev. Chim. Bucharest*, 2016; 67(3): 530-533.
29. Popescu D.E., Bungău C., Prada M., Domuţa C., Bungău S., Ţiţ D.M., Waste management strategy at a public university in Smart City context. *J. Environ. Prot. Ecol.*, 2016; 17(3): 1011-1020.
30. Roberts P.H., Thomas K.V., The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Sci. Total Environ.*, 2006; 356: 143-153.
31. Sarganas G., Buttery A.K., Zhuang W., Wolf I.K., Grams D., Rosario A.S., Scheidt-Nave C., Knopf H., Prevalence, trends, patterns and associations of analgesic use in Germany. *BMC Pharmacol. Toxicol.*, 2015; 16: 1-13.
32. Ţiţ D.M., Bungău S., Nistor Cseppento C., Copolovici D.M., Buhaş C., Disposal of unused medicines resulting from home treatment in Romania. *J. Environ. Prot. Ecol.*, 2016, 17(4): 1425-1433.
33. ***www.atozresearch.com