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The effect of the number of alkyl substituents on imidazolium ionic liquids phytotoxicity and oxidative stress in spring barley and common radish seedlings

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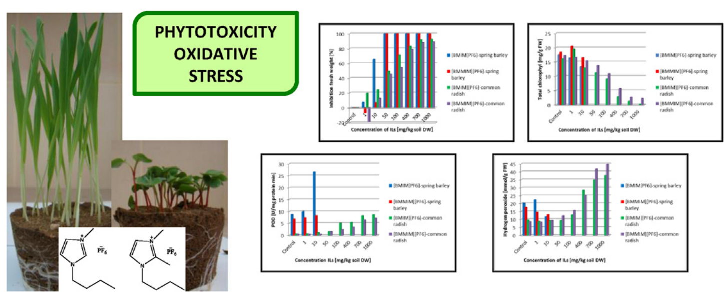
HIGHLIGHTS

- [BMIM][PF_6] and [BMMIM][PF_6] were characterized by similar phytotoxicity.
- ILs at high concentration were cause oxidative damage in plants.
- The increased level of ILs in soil causes a decrease in content of plant pigments.
- The increased concentration of ILs in the soil increases the activity of POD.
- The common radish revealed the higher tolerance to the ILs as compared to barley.

GRAPHICAL ABSTRACT



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ABSTRACT

Increasing amounts of two ILs: 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM][PF_6] and 1-butyl-2,3-dimethylimidazolium hexafluorophosphate [BMMIM][PF_6], were introduced to soil in which spring barley (*Hordeum vulgare*) and common radish (*Raphanus sativus* L. subvar. *radicula* Pers.) seedlings were cultivated, in order to evaluate the phytotoxicity of ionic liquids with imidazolium cation with two or three alkyl substituents attached. The results of the study i.e. the inhibition of the length of plants and their roots, as well as the yield of fresh weight of plants, clearly showed that differences in the number of substituents did not affect the toxicity of these ILs. Although, radish was more resistant to the applied ionic liquids than barley. Ionic liquids led to a decrease in the content of all assimilation pigments and induced oxidative stress in the plants, as showed by an increase in malondialdehyde (MDA) content, and changes in the level of H_2O_2 and antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). The best biomarkers of oxidative stress in both plants were the changes in chlorophyll content and the increase in POD activity. Both spring barley and radish exposed to [BMIM][PF_6] and [BMMIM][PF_6] accumulated a large amount of fluoride ions, which further increased the toxicity of these compounds for both plants.

1. Introduction

In the search for environmentally friendly chemicals, mostly

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solvents, the world of science has focused its attention on ionic liquids (ILs). Ionic liquids are characterized by ionic structure, and can be both liquids and solids across a wide range of temperatures. Interest in these salts is due to their properties, such as low melting points, negligible vapor pressure, polarity, high thermal and electrochemical stability, high ionic conductivity, non-flammability and good catalytic properties. Due to these features, ILs are called “green solvents”, they are considered to be perfect substitutes for volatile organic solvents, and are used in biocatalysis processes, analytical chemistry, separation and extraction, electrochemistry, as well as in the pharmaceutical industry, biotechnology and nanotechnology (Bruzzone et al., 2011; Ferlin et al., 2013; Messali et al., 2013; Peric et al., 2013).

However, studies conducted in recent years have placed a big question mark over the “green” nature of ionic liquids. The toxicity of ILs for microorganisms, fungi, algae, higher plants, invertebrates and vertebrates (Pham et al., 2010; Petkovic et al., 2012; Peric et al., 2013; Cvjetko Bubalo et al., 2014a) has already been showed beyond all doubt. Ionic liquids, as with all chemical compounds, can reach the soil environment, where they are taken up by plants, thus affecting their growth and development. Therefore, there are an increasing number of reports in the literature describing the effect of ILs on terrestrial plants (Biczak et al., 2010, 2013a, 2013b, 2015; Matzke et al., 2009; Studzińska and Buszewski, 2009). The degree of ILs impact on terrestrial plants was determined in these studies primarily based on plant growth inhibition; however, according to Cvjetko Bubalo et al. (2014b), the mechanism of ILs toxicity has not yet been fully understood. Therefore, in the last few years, the scientific literature more often presents the opinion that ionic liquids evoke oxidative stress in the examined organisms (Liu et al., 2013, 2014; 2015a, 2015b; 2016a; Biczak, 2016; Biczak et al., 2016; Pawłowska and Biczak, 2016).

There is a predominant belief in the literature that the phytotoxicity of ionic liquids depends largely on the kind of cation and the length of substituent (Matzke et al., 2008; Studzińska and Buszewski, 2009; Biczak et al., 2014a; Cvjetko Bubalo et al., 2014a), and to a small degree depends on the kind of anion (Studzińska and Buszewski, 2009; Biczak et al., 2014b; Cvjetko Bubalo et al., 2014a; Liu et al., 2016a, 2016b). At present, there are no reports linking ILs phytotoxicity with the total amount of alkyl groups at ILs cation. Existing studies of a similar nature relate only to the antimicrobial activity of these compounds. Therefore, the aim of this study was to determine and compare the toxic effect of ionic liquids with an imidazole cation containing 2 and 3 alkyl substituents for spring barley and common radish. ILs which are popular and often used in chemical synthesis, electrochemical or biotechnological processes, i.e. 1-butyl-3-methylimidazolium hexafluorophosphate, and 1-butyl-2,3-dimethylimidazolium hexafluorophosphate, were used in the study (De Diego et al., 2009; Roy et al., 2014; Zhao et al., 2014; Xu et al., 2015). In order to compare the toxicity of imidazolium ionic liquids, and concurrently show which of the cations to a higher degree determines the toxic activity of these substance on terrestrial plants, the level of oxidative stress in monocotyledonous and dicotyledonous plants was also determined in the present study in addition to traditional phytotoxicity biomarkers, i.e. shoot length, root length, and yield of fresh and dry weight. It was possible to realize the aim via the determination of the content of MDA, H_2O_2, assimilation pigments and activity of SOD, POD and CAT. As PF_6^- anions were used in the study, the level of fluoride ions was determined both in spring barley and radish. This can be collected by the plants from the soil under these conditions and accumulated, and then converted into fluoroacetate which is highly toxic to animal organisms (Baunthiyal and Pandey, 2012). The choice of spring barley for the study was dictated by the evidence that it is the fourth most common cereal species in production and acreage, and radish is a popular vegetable, enriching the human diet in a number of micro- and macroelements and vitamins (Schubert and Jahren, 2011; Dragišić Maksimović et al., 2013; Arias-Baldrich et al., 2015).

2. Materials and methods

2.1. Chemicals

The imidazolium ionic liquids: 1-butyl-3-methylimidazolium hexafluorophosphate (≥98% purity) and: 1-butyl-2,3-dimethylimidazolium hexafluorophosphate (≥98% purity) used in the study was purchased from Sigma-Aldrich Chemical Co. The structure of the tested ILs is illustrated in Suppl. Fig. 1.

2.2. Growth condition and treatment

A pot experiment for the determination of phytotoxicity of the ILs was carried out in the vegetation hall of the Department of Biochemistry and Ecotoxicology at Jan Długosz University in Częstochowa based on the OECD/OCDE 208/2006 Guide.

A monocotyledonous plant – the spring barley (*Hordeum vulgare*) and a dicotyledonous plant – the common radish (*Raphanus sativus* L. subvar. *radicula* Pers.) were used in the experiment. Identical seeds of the plants, originating from the same source, were sown into 90 mm-diameter plastic plant pot, which was filled with the reference soil and a soil thoroughly mixed with the examined ILs. The treatment concentration of ILs were set to 0, 1, 10, 50, 100, 400, 700 and 1000 mg per 1 kg soil dry weight (DW). The soil used in the experiment was light loam with a dissolved matter of approximately 10%, an organic carbon of 9.5 g kg^{-1} and pH equal to 6.0. Throughout the testing period (14 days), constant substrate moisture content at the level required for the plants (of about 70% field water capacity), a constant temperature 20 ± 2 °C and a constant illumination of 160 μmol m^{-2} s^{-1} were maintained in the system of 16 h/day and 8 h/night.

The evaluation of the phytotoxicity of the ILs was determined and based on the comparison of fresh weight and dry weight content, shoot length and root length and germination potential and germination rate. The shoot length and root length were measured as described by Wang et al. (2009). Results were expressed as yields of fresh weight inhibition, shoot length and root length inhibition in comparison to the control. The effective concentrations (EC_{50}) were estimated by the non-linear regression using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Germination potential (GP) and germination rate (GR) were designated as described by Liu et al. (2014). Seeds were considered germinated when both the plumule and radicle extended to more 2 mm from their junction.

Furthermore, in plant leaves, MDA and H_2O_2 content, photosynthetic pigments content and antioxidant enzymes activities were measured and compared. Higher ILs concentrations were not included in these measurements because for some ILs, inhibition was too strong to harvest plant material.

2.3. Determination of total fluoride content

Total fluoride content in plant material was assayed with the method described by Eyde (1982). Dried and ground samples are fused in nickel crucibles with sodium hydroxide. To the melt is added water, diluted hydrochloric acid and citrate buffer solution. The filtered solution is measured with an electrode. Fluoride concentration was determined in the presence of TISAB III buffer using the potentiometric method with an Orion Research ion-selective electrode. The total fluoride content was expressed as mg kg^{-1} of DW.