POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 32(3): 427–438, Y. 2017

EFFECT OF 3-PYRIDYLKETOXIME AND ITS QUATERNARY PYRIDINIUM SALTS ON MIZE GERMINATION*

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Key words: phytotoxicity, quaternary pyridinium ketoximes, 3-pyridyl ketoxime, maize.

Abstract

In the present work the impact of the 3-pyridyl ketoxime and its quaternary pyridinium bromides with various alkyl chain lengths on the monocot plant – maize (*Zea mays* L.) was tested. The study was carried out on two systems – hydroponic and soil. After a seven-day period of these experiments, the number of germinated seeds was counted and the length of maize radicle and shoot was also measured. 3-pyridyl ketoxime and pyridinium ketoxime bromides had varying effects on the germination of maize seeds and the development of radicle and shoot. Toxicity of these compounds depended largely on the structure of the compound, and the systems on which the plant was grown. The toxic effects of tested compounds on maize development were higher in hydroponic systems compared with the soil system.

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* This work was supported within the project "Engineer of the Future. Improving the didactic potential of the Poznan University of Technology" – POKL.04.03.00-00-259/12, implemented within the Human Capital Operational Programme, co-financed by the European Union within the European Social Fund and the Polish Ministry of Science and Higher Education as project No 03/32/DSMK/0516 and No 03/32/DSMK/0520.

WPŁYW 3-PIRYDYNOKETOKSYMU ORAZ JEGO CZWARTORZĘDOWYCH SOLI PIRYDYNIOWYCH NA KIEŁKOWANIE KUKURYDZY

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Słowa kluczowe: fitotoksyczność, czwartorzędowe bromki pirydynioketoksymów, 3-pirydyloketoksym, kukurydza.

Abstrakt

W pracy przedstawiono wpływ oksymu ketonu 3-pirydylowego oraz jego czwartorzędowych bromków zawierających różną długość łańcucha alkilowego na kiełkowanie i początkowy rozwój rośliny jednoliściennej – kukurydzy (*Zea mays* L.). Badania przeprowadzono w dwóch układach – hydroponicznym i glebowym. Po upływie siedmiu dni od założenia doświadczenia zliczono liczbę wykiełkowanych nasion oraz zmierzono długość korzeni i pędów kukurydzy. 3-pirydyloketoksym oraz jego czwartorzędowe bromki pirydyniowe wywierały zróżnicowany wpływ na kiełkowanie nasion kukurydzy, jak i rozwój korzeni i pędów. Toksyczność tych związków zależała w dużym stopniu od struktury związku oraz układu, w którym roślina była uprawiana. Silny efekt zahamowania kiełkowania oraz rozwoju nasion kukurydzy obserwowano w układach hydroponicznych, natomiast mniejszą inhibicję rozwoju nasion podczas upraw kukurydzy w glebie. Prawdopodobnie jest to związane z sorpcją analizowanych związków w matrycy glebowej i mniejszą biodostępnością.

Introduction

Interest in quaternary ammonium salts and ionic liquids is currently very high. The quaternary pyridinium salts can be used in a wide range of applications including pesticides, fungicides and preparations bactericidal, as well as extractants of metals from aqueous solutions (ABELE et al. 2003, VÖRÖS et al. 2014, KONIDARIS et al. 2011, KLIACHYNA et al. 2014, WIESZCZYCKA et al. 2013). Pyridyl ketoximes quaternary derivatives have the ability to reactivate the enzyme acetylcholinesterase, when it is fully inhibited by organophosphorus compounds, including pesticides and chemical warfare agents, and they are of particular pharmacological interest (EYER 2003). With such a broad spectrum of possibilities their usefulness is also certainly associated with the fact that they may enter the environment, both at the production and due to improper management of waste products. Ecotoxicological analysis is one of the essential elements of the assessment of the suitability and risks of chemicals. Many authors tested the dependence of compound structures on the toxicity for different organisms. The results showed that the toxic properties of this group of compounds are mainly dependent on the type of cation.

The modification of the anion in molecules of the ionic liquid usually affected changes in the physico-chemical properties of the compound. It was also noted that a long chain extension in the substituent alkyl pyridinium or imidazolium increases the toxicity of these compounds (CHENG et al. 2009, LATAŁA et al. 2009, PERNAK et al. 2011 and 2012, GRABIŃSKA-SOTA 2011, PETKOVIĆ et al. 2011, MATZKE et al. 2007, CVEJTKO BUBALO et al. 2014, KULACKI and LAMBERTI 2008). Among other things, therefore, a very important part of the research of the newly synthesized compounds is an analysis of their interaction with the environment and living organisms, including analysis of phytotoxicity. Their impact on soil and water is certainly of considerable concern at the time of their disposal.

From the above, the most interesting to study was analyses of the effect of 3-pyridylketoxime and quaternary pyridinium ketoxime salts on germination model monocotyledonous plant – maize (*Zea mays*). In addition, the obtained results will enable an assessment of risk of environmental contamination with such compounds.

Materials and Methods

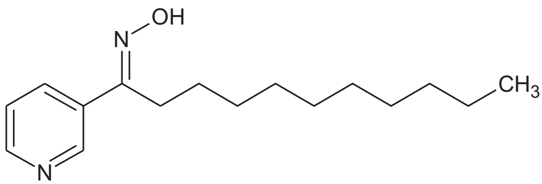
Chemicals

The tested compounds were synthesized on the based methods described by WIESZCZYCKA et al. (2013). Structures of tested compounds were shown at Figure 1. At first 1-(3-pyridyl)-undecane-1-one was synthesized by treating 3-pyridylcaronitrile with decylmagnesium bromide. And next, to the obtained ketone was added hydroxylamine hydrochloride in the presence of sodium carbonate (at pH 7). The synthesized 1-(3-pyridyl)-undecane-1-one oxime was stirred and heated (60^{o}C) for 12h with appropriate alkyl halides (bromide propane or bromide pentane) in ethanol as diluent to give quaternary pyridinium ketoximes (WIESZCZYCKA et al. 2013). The yields of the pyridinium ketoxime bromides were 87 to 89%. The structure and purity of the compounds obtained was confirmed by spectroscopic methods (nuclear magnetic resonance (^{1}H and ^{13}C NMR)).

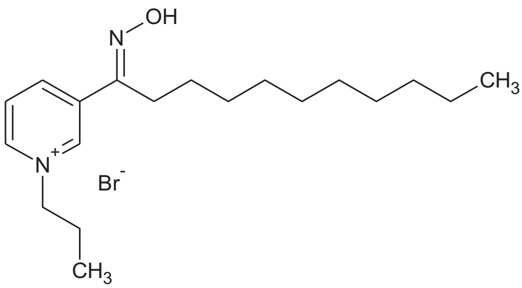
Treatments and experimental design

The analysis of impact the 3-pyridyl ketoxime and its quaternary pyridinium ketoximes on germination of maize (*Zea mays* L.) was exanimated in hydroponic and soil systems. The tested compounds were dissolved in methanol and then prepared in the aqueous solutions containing 10, 25, 50, 100, 500 and 1000 mg l^{-1} respectively. The addition of methanol to the samples was not more than 5%. The control samples were made with distilled water. Aqueous solutions of the tested compounds or water were added to the soil and mixed.

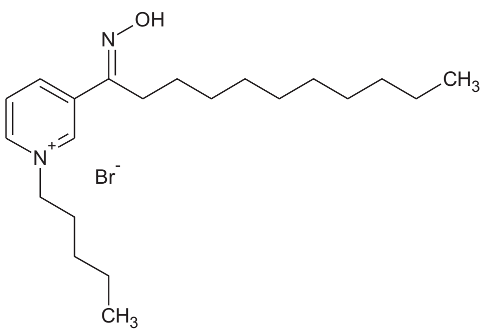
First, investigation of the effect of the pyridylketoxime and its quaternary bromides was carried out in the hydroponic. The maize seeds were placed in Petri plate on a piece of filter paper covered with a thin layer of cotton wool and moistened with 10 ml of water or compounds solution of the appropriate concentration.



1-(3-pirydyl)-undecan-1-one oxime (**3PC10**)



3-[1-(hydroximino)-undecyl]-1-propyl-pyridinium bromide (**3PC10-PrBr**)



3-[1-(hydroximino)-undecyl]-1-penyl-pyridinium bromide (**3PC10-PnBr**)

Fig. 1. The structures of tested compounds

The examination of germination inhibition in the soil system was carried out on plates Phytotoxkit^{TM}. On each plate 100g soil was added containing the appropriate concentration of the test substance (0, 10, 25, 50, 100, 500 and 1000 mg kg^{-1} dry weight of soil (d.w.s.)). Ten seeds were placed on each plate. All tests were carried out in 5 replications (5 plates for each concentration of test compounds – analysis for 50 seeds). The prepared samples were left for 7 days at 21°C.

Soil composition

Composition of the soil used in the experiment was as follows (mg kg^{-1} of soil): 81 P, 88 K, 69 Mg, pH 5.92, C organic content of 1.01% (10.1 g kg^{-1} of soil). According to the international classification WRB (*World reference...* 2015), the examined soils should be classified as Phaeozems (Haplic Phaeozems), while according to the FAO Soil Taxonomy (*Soil taxonomy* 1999), as Mollisols (Typic Endoaquolls). The influence of 3-pyridyl ketoxime and its quaternary pyridinium ketoximes was investigated using the phytotoxicity test based on the ISO-11269-2:2012 International Standard (*Soil quality...* ISO-11269-2 2012).

Calculation

After end of the experiments, the number of germinated seeds was counted and the radicle and shoot length were measured. The germination index (GI) was calculation based on the formula (MOSSE et al. 2010):

$$\mathrm{GI} = \left(\frac{G_x}{G_c}\right) \cdot \left(\frac{L_x}{L_c}\right) \cdot 100\ [\%]$$

where:
G_x and G_c represent the number of germinated seeds, respectively: x – sample c – control
L_x and L_c is the length of radicle respectively: x – sample, c – control.

The pyridine ketoximes and its quaternary derivatives were obtained at Poznan University of Technology in Department of Organic Chemistry. The experiments about impact of these compounds on plant germination were also carried out at Poznan University of Technology in 2015.

Statistical analysis

All the experiments were carried out in 5 replications and each collected sample was tested in duplicate. The mean values and standard deviations (SD) of the experimental data were calculated using software Microsoft Excel.

Results

The studies have showed that all tested compounds stimulated radicle growth of maize at the concentration of 10 mg l^{-1} (Figure 2). The increasing of compounds concentration caused the inhibition of radicle growth. It could be observed that the greatest inhibitory effect on radicle growth caused oxime 3PC10. In contrast, all tested substances with concentrations of 500 mg l^{-1} and 1000 mg l^{-1} showed a complete inhibition of the seeds germination processes. The presence of compounds of concentration 10 mg l^{-1} did not inhibit the shoot

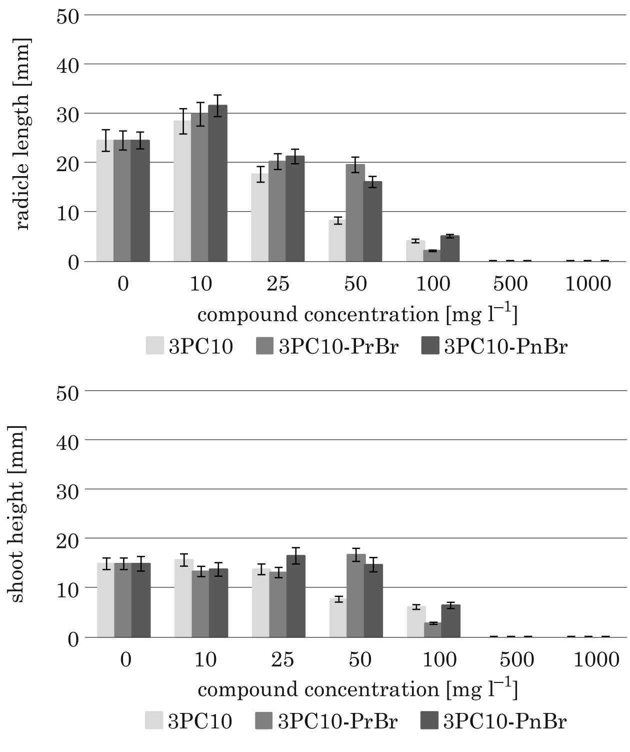


Fig. 2. Effect of 3-pyridylketoxime and its quaternary pyridinium ketoxime salts on the radicle and shoot growth of maize in hydroponic system

growth (Figure 2). Moreover, in the case of oxime 3PC10 growth stimulating properties relative to the control sample without oxime and its derivatives were observed.

This demonstrates the beneficial effects of oxime 3PC10 at the lowest concentration on the seeds germination, and also the further development of the plant. Increasing 3PC10 concentration in the medium to above 50 mg l^{-1} resulted in a significant (50%) decrease in the average shoot length. Slightly less effect on the shoot growth after exposure to 10 mg l^{-1} of 3PC10-PnBr was recorded. However, 3PC10-PnBr stimulated growth of shoot at concentration 25 mg l^{-1}. But when tested, results for 3PC10-PrBr, in the concentration range 10–25 mg l^{-1} had no effect on the shoot growth, but when the concentration increased to 50 mg l^{-1}, the shoot length increased above 15% in comparison to the control. In general, germination and growth inhibition was more pronounced when higher concentrations of oxime and its quaternary bromide were used. The shoot length was decreased for about 50% (3PC10 and 3PC10-PnBr) and 80% (3PC10-PrBr) after exposure to 100 mg l^{-1}. However all compounds used at a concentration 500 mg l^{-1} and higher caused a complete stop of the germination processes of maize seeds (Figure 2).

On the base of the number of seeds germinated and the radicle length compared to controls germination index (formula 1) was calculated and then plotted the dependence of the germination index to the concentration of tested compounds (Figure 3). It could be observed that with a concentration 10 mg l^{-1}, all compounds have a stimulating effect on the maize seeds germination. That confirms the GI value more than 100%. With the increase of the compounds concentration in the solution, germination index decreased. Only in the case of 3PC10-PrBr at a concentration of 50 mg l^{-1} could be observed a slight increase

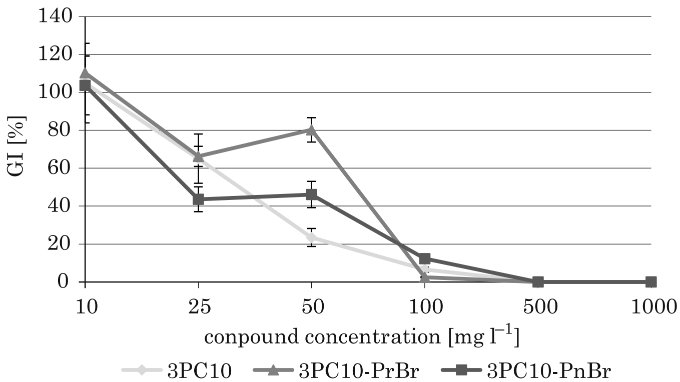


Fig. 3. Dependence of germination index on 3-pyridylketoxime and quaternary pyridinium ketoxime salts concentration in hydroponic systems

in GI values, relative to a concentration of 25 mg l^{-1}. The lowest GI values observed at a concentration of 100 mg l^{-1}. At 500 mg l^{-1} and above the germination process did not occur.

Studies on the effect of 3-pyridyl ketoxime and its quaternary pyridinium ketoxime salts on the maize germination were also performed for soil system. Figure 4 shows the dependence of the radicle and shoot growth on the compounds concentration. As could be seen, these compounds resulted in the varied effect on radicle growth (Figure 4). The application of 3PC10-PrBr at a concentration of 50 mg kg^{-1} d.w.s. stimulated radicle growth about 15% compared to the control, which was not exposed to compounds. However, all tested substances inhibited seeds germination and radicle growth above

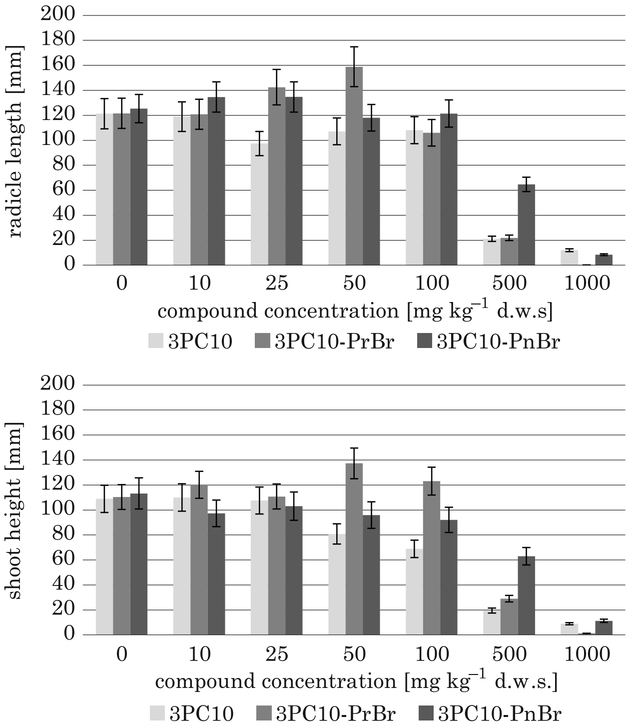


Fig. 4. Effect of 3-pyridylketoxime and quaternary pyridinium ketoxime salts on the radicle and shoot growth in soil system

100 mg kg^{-1} d.w.s, and 500 mg kg^{-1} d.w.s. inhibited radicle growth by about 50% (3PC10-PnBr) and 80% (3PC10 and 3PC10-PrBr). The highest inhibition of radicle growth of seed maize was observed when exposed to 1000 mg kg^{-1} d.w.s. Then the radicle length did not exceed 10% of the radicle length of the control.

During the impact study of the 3-pyridylketoxime and its quaternary salts on the growth and development maize, the shoot growth was also tested (Figure 4). In this case, the increase of oxime 3PC10 and 3PC10-PnBr concentration in the soil resulted in inhibition of the shoot length in all tested compound concentrations. However, the cultivation in a soil containing 50 and 100 mg kg^{-1} d.w.s. of 3PC10-PrBr induced stimulation of shoot growth relative to the control samples watered only with deionized water. Application of 3PC10-PrBr at a concentration of 500 and 1000 mg kg^{-1} d.w.s., as well as for other tested substances, resulted in significant inhibition of the shoot length. The highest inhibition of growth in the aboveground parts was observed for the concentration of 1000 mg kg^{-1} d.w.s.

In the final stage of the study the germination index was determined and then the graphs were plotted in correlation to the germination index to the oxime and quaternary pyridinium ketoxime salts concentration (Figure 5). When analysing Figure 5 it was observed that there was a marked decrease in germination index (GI) above 100 mg kg^{-1} d.w.s. Only in the case of the 3PC10-PrBr concentration of 50 mg kg^{-1} d.w.s. there was a 30% increase of germination index, whether evidenced by GI value exceeding 100%.

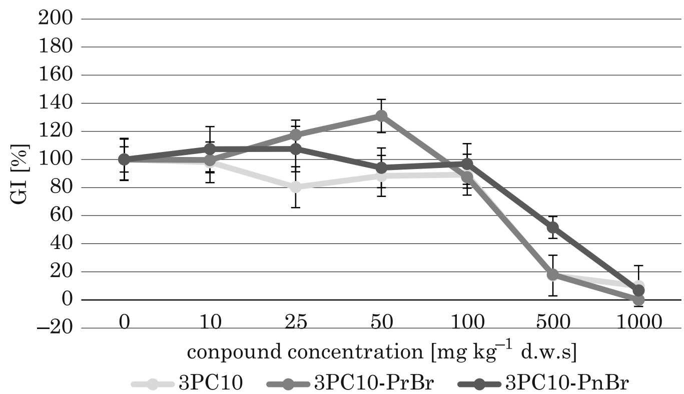


Fig. 5. Dependence of germination index on 3-pyridylketoxime and quaternary pyridinium ketoxime salts concentration

Discussion

In this study the influence of structure pyridine derivatives and alkyl chain length linked to the nitrogen atom of pyridine ring on its toxicity was tested. In general, germination and growth inhibition was more pronounced when a higher concentration of oxime and quaternary pyridinium ketoximes salts were used. Also, once the seeds germinated the inhibition of radicle growth was more pronounced than growth of shoot, which could be explained by direct contact between compounds and radicle. The study was carried out in two systems, hydroponic and soil. Comparing growth in both systems, it could be seen that 3-pyridylketoxime and quaternary pyridinium ketoxime salts in the soil matrix exhibit less toxic than the effect hydroponic systems. This phenomenon could be caused by the sorption process of chemicals compounds in the soil matrix which may reduce the availability and thus smaller seeds in contact with the stressor agent. However, using the hydroponic system, seeds were exposed to direct contact with the chemical, which may adversely affect the germination of seeds and the subsequent development of the plant. In the literature, toxicity of different ionic liquids and quaternary salts are described, but most of them noted that the ionic liquids toxicity increased with the increase in length of the alkyl chain, which is in accordance with studies on various model organisms (CVEJTKO BUBALO et al. 2014, THUY et al. 2010, MATZKE et al. 2007, STUDZINSKA and BUSZEWSKI 2008). The observed effect of the length chain could be explained by mode of toxic action which takes place through membrane distribution due to the ionic liquids structural similarities to detergent, pesticides and antibiotics (ZHANG et al. 2012, LI et al. 2012). The long alkyl chains in the cation caused the increase the possibility of their interaction with cell membrane phospholipid bilayers and hydrophobic domains of membrane proteins, leading to disruption of membrane physiological functions and in consequence to the greater toxicity (RADOŠEVIČ et al. 2013, CVEJTKO BUBALO et al. 2014). However, in the study about influence of 3-pyridylketoxime and quaternary pyridinium ketoxime salts it was observed that pyridinium ketoxime bromides were less toxic for maize than precursor 3-pyridylketoxime, especially in the hydroponic system. But the analysis of the influence of the alkyl chain length was observed with different results than those described in the literature. Quaternary pyridinium salt with chain contain 3 carbon atoms at concentration 50–100 mg kg^{-1} d.w.s. stimulated shoot and radicle growth. However the compound with pentyl chain at concentration 500 i 1000 mg kg^{-1} d.w.s. was less toxic than compound with propyl chain.

Conclusions

3-pyridylketoxime and quaternary pyridinium ketoxime salts had varying effects on the germination of maize seeds. Phytotoxicity of these compounds depended largely on the structure of the compound, and the substrate on which the plants were grown. Higher toxicity of tested chemicals was observed in hydroponic system compared to the maize grown on the soil. The results indicate almost ten-fold lower toxicity of tested chemicals in the soil than in the hydroponic system. This was probably due to sorption processes that occur in the soil matrix, while in an aqueous system, seeds were in direct contact with solutions of chemical substances. Generally, in the hydroponic cultivation all the tested compounds inhibited germination of seeds at the concentration of above 100 mg l^{-1} and the concentration which reached 500 mg l^{-1} resulted in complete inhibition of seeds germination. However, at low concentrations, in the range of 10 mg l^{-1} to 25 mg l^{-1}, they stimulated the germination of maize seeds. The toxic concentration of analysed compounds become evident above 100 mg l^{-1}. Less toxicity was indicated at soil cultivation than in hydroponic. The application of these substances at a concentration of 500 mg kg^{-1} d.w.s. resulted in a significant inhibition of radicle and shoot of maize, and the presence of substances with a concentration of 1000 mg kg^{-1} d.w.s. resulted in both the shoot length and radicle length not exceeding 10 mm, which represents about 10% of the shoot length or radicle length of control sample not exposed to chemical agent.

Translated by Authors

Accepted for print 4.09.2017

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