Aleksandra WOJCIECHOWSKA [1], Anna PARUS [1], Irmina WOJCIECHOWSKA [1], Hubert IWIŃSKI [1], Karolina WIESZCZYCKA [1], Piotr SZULC [2], Zbyszek ZBYTEK [3]
[1] Politechnika Poznańska, Instytut Technologii I Inżynierii Chemicznej
ul. Berdychowo 4, 60-965 Poznań, Poland
e-mail: anna.parus@put.poznan.pl
[2] Uniwersytet Przyrodniczy w Poznaniu, Wydział Rolnictwa i Bioinżynierii, Katedra Agronomii
ul. Dojazd 11, 60-632 Poznań, Poland
[3] Przemysłowy Instytut Maszyn Rolniczych, ul. Starołęcka 31, 60-963 Poznań, Poland

ANALYSIS OF PHYTOTOXICITY OF PYRIDINE-2-, -3-, -4-AMIDOXIMES

Summary

Pyridine amidoximes are compounds used in production of substances with definite chemical and biological properties. They are used among other things as drugs, fungicides and bactericides. An important aspect of research consists in determination of influence of a chemical compound on the natural environment, including assessment of influence on land plants. The aim of the presented study included synthesis of pyridine-2-,-3- and -4-amidoximes and assessment of their influence on growth and development of a monocot model plant – maize (Zea mays L.). Analysis of the obtained results showed that all the compounds inhibited germination at concentrations above 500 mg·l⁻¹. Therefore it can be concluded that phytotoxic effect of the compounds can be observed at concentrations above 500 mg·l⁻¹.
***Key words**: phytotoxicity, amidoximes, pyridine derivatives, germination index*

ANALIZA FITOTOKSYCZNOŚCI 2-, 3- i 4-PIRODYNOAMIDOKSYMÓW

Streszczenie

Pirydynoamidoksymy są związkami stosowanymi w produkcji substancji o określonych właściwościach chemicznych i biologicznych. Znalazły one zastosowanie m.in. jako leki, środki grzybo- i bakteriobójcze. Ważnym aspektem badań jest określenie wpływu związku chemicznego na środowisko naturalne, w tym również ocena wpływu na rośliny lądowe. Celem prezentowanych badań była synteza 2-, 3- i 4-pirydynoamidoksymów oraz ocena ich wpływu na wzrost i rozwój modelowej rośliny jednoliściennej – kukurydzy zwyczajnej (Zea mays L.). Analiza otrzymanych wyników wskazuje, że wszystkie związki hamowały kiełkowanie w stężaniu powyżej 500 mg·l⁻¹. Na tej podstawie stwierdzić można, że charakter fitotoksyczny związki te wykazują w stężeniach powyżej 500 mg·l⁻¹.
***Słowa kluczowe**: fitotoksyczność, amidoksymy, pochodne pirydyny, indeks kiełkowania*

1. Introduction

Pyridine amidoximes are key compounds used in production of numerous heterocyclic compounds. The compounds are precursors for synthesis of substances with definite chemical and biological properties. Amidoximes are often used as drugs, fungicides and bactericides. Due to their properties, they can also be successfully used as components of cytostatic drugs, antidepressants and anti-allergic agents. Production of crop protection agents is an important branch of industry using pyridine amidoximes. Pyridine amidoximes are also known as metal ligands, especially of Zn, Ni and Cu ions [1, 5, 7, 8, 9, 10, 11, 15]. The latest literature reports also note the possibility of using pyridine-O-alkyl-amidoximes as extractants of metal ions, e.g. uranium and nickel from aqueous systems [2].

Introduction of each chemical compound to the industry as well as trade includes also the necessity of conducting a range of analyses establishing its influence on the natural environment. It results from the possibility of chemicals' entering the natural environment both in the stage of production and during improper management of waste products. Toxic influence on plants can take place in different plant developmental stages, and it is also subject to their growth conditions and systematic affiliation. Some substances exhibit an effect only in the place of their absorption, while others can move in the plant after being absorbed, and accumulate in its above-ground parts, which results in inclusion of the substances in the trophic chain.

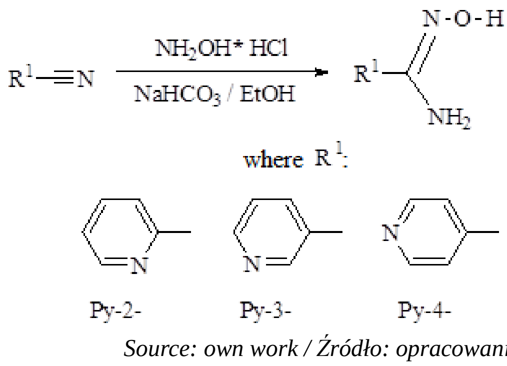
The aim of the study was to synthesize pyridine-2-, -3- and -4-amidoximes and assessment of their influence on growth and development of a monocot model plant – maize (*Zea mays* L.).

2. Materials and methods

The conducted research included two stages. The first one consisted in synthesis of model compounds, which then were analyzed in terms of phytotoxicity.

2.1. Synthesis of model compounds

Pyridine-2-, -3- and -4-amidoximes were obtained in one-stage reaction of an appropriate cyanopyridine and hydroxylamine hydrochloride and sodium hydrogen carbonate with efficiency over 90% (Scheme 1). Structures of all the obtained compounds were confirmed with spectroscopic methods: ^{1}H NMR, ^{13}C NMR and FT-IR.



Source: own work / Źródło: opracowanie własne

Scheme 1. Synthesis of pyridine amidoximes
Schemat 1. Schemat syntezy pirydynoamidoksymów

2.2. Phytotoxicity analysis

In the next stage of the research, influence of pyridine-2-, -3- and -4-amidoximes on germination capability of higher plants was analyzed. The experiment employed a representative of monocot plants – maize (*Zea mays* L.). Pyridine amidoximes were solved in methanol, and then their aqueous solutions were prepared, containing respectively: 10, 25, 50, 100, 500 and 1000 $mg \cdot l^{-1}$ of the analyzed compound. Aqueous solutions of 10 ml with appropriate concentrations were administered to Petri dishes with previously prepared filter paper discs. 10 maize seeds were laid down on such prepared medium of each Petri dish (5 dishes for each concentration of the examined compounds – analysis of 50 seeds) and kept for 7 days at a temperature of 25°C. 10 ml of distilled water was added to the control sample. After the definite period of time, the amount of germinated seeds was analyzed, and also the weight and length of radicle and epicotyl was determined. The values enabled calculation of germination index (GI) according to the following formula:

$$GI = \Sigma(\frac{Gt}{Tt}), \quad (1)$$

where:
Gt – number of seeds germinated on *t* day,
Tt – number of days of the experiment when the measurement was made,
and also germination capability (G) according to the following formula [15]:

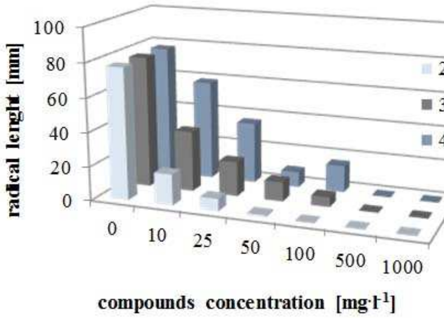
$$G(\%)=(\frac{Gx}{Gc})\cdot 100\% \quad (2)$$

where:
Gx – number of germinated seeds,
Gc – total number of examined seeds.

On the basis of the obtained results, concentrations of the compounds were determined when 50% of organisms were affected at a definite time (EC50).

3. Results of research

In the present study, analysis of phytotoxicity of pyridine-2-, -3- and -4-amidoximes was conducted by assessment of their influence on germination of maize seeds. Influence of concentration of the examined compounds on mean radicle length of germs was presented in Figure 1. All the examined compounds already at a concentration of 10 $mg \cdot l^{-1}$ inhibited growth of radicles. Irrespective of the type of a compound, together with an increase in its concentration in the medium, they caused greater inhibition of radicle growth when compared to the control sample. Only in the case of pyridine-4-amidoxime, a slight growth of radicles at a concentration of 100 $mg \cdot l^{-1}$ was observed when compared to the other compounds. Seeds germinating on that medium had nearly three-times greater mean length of radicles (15.75 mm) than it was in the case of the other two isomers (5.67 mm and 0 mm). The greatest inhibiting influence on radicle development was observed for pyridine-2-amidoxime, while in case of concentrations of 500 $mg \cdot l^{-1}$ and 1000 $mg \cdot l^{-1}$, total inhibition of germination processes was observed. Similarly as in the case of mean length of radicles, their greatest weight was noted in germs growing on a medium with 10 and 25 $mg \cdot l^{-1}$ of pyridine-4-amidoxime. Pyridine-2-amidoxime had the greatest negative influence on weight of radicles. A considerable reduction in radicle weight was noted when doses of the examined compounds were increased to 100 $mg \cdot l^{-1}$.



Source: own work / Źródło: opracowanie własne

Fig. 1. Dependence of radical length on pyridine-amidoximes concentration
Rys. 1. Zależność długości korzenia od zastosowanej dawki związku

A value higher than 4.55 g was not noted in any of the examined samples. The obtained radicle weight values of all the samples are presented in Table 1. Measurements of epicotyl length showed that at a dose of 10 $mg \cdot l^{-1}$, both pyridine-2-amidoxime and pyridine-4-amidoxime have a slightly inhibiting effect on epicotyl growth. However, in case of pyridine-3-amidoxime, its stimulating effect was noted. It proved that the effect of the used concentration of the compound on germination and development of seeds was beneficial. A slightly lesser influence on epicotyl growth was noted for pyridine-2-amidoxime at a concentration of 25 $mg \cdot l^{-1}$. Analysis of the other compounds at a concentration of 50 $mg \cdot l^{-1}$ showed a decrease in epicotyl growth. Increase in pyridine-4-amidoxime concentration up to 100 $mg \cdot l^{-1}$ in a medium resulted in an increase in mean epicotyl length when compared to the other compounds. All the compounds at a concentration of 500 $mg \cdot l^{-1}$ caused total inhibition of the germination process of maize seeds (Figure 2). Analysis of epicotyl weight showed a similar tendency to that of epicotyl mean length (Table 2).

Table 1. Changes in radicle weight after using different concentrations of the analyzed compounds
Tab. 1. Zmiany masy korzeni zarodkowych po zastosowaniu różnych stężeń analizowanych związków

Compound	Concentration [$mg \cdot l^{-1}$]					
	10	25	50	100	500	1000
	Radicle weight [g]					
C*	4.55					
2PAO	2.05	0.9	0.2	0	0	0
3PAO	2.15	0.9	0.55	0.5	0	0
4PAO	2.95	1.35	0,9	0.1	0	0

*control sample, 2PAO – pyridine-2-amidoxime, 3PAO – pyridine-3-amidoxime, 4PAO – pyridine-4-amidoxime

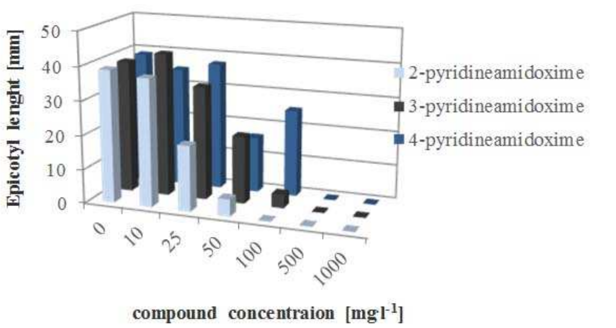
Source: own work / Źródło: opracowanie własne

Table 2. Changes in epicotyl weight after using different concentrations of the analyzed compounds
Tab. 2. Zmiany masy epikotyli po zastosowaniu różnych stężeń analizowanych związków

Compound	Concentration [mg·l^{-1}]					
	10	25	50	100	500	1000
	Epicotyl weight [g]					
C*	6.85					
2PAO	7.3	1.55	0.50	0	0	0
3PAO	6.55	5.05	1.3	0.30	0	0
4PAO	6.15	5.15	1.75	2.0	0	0

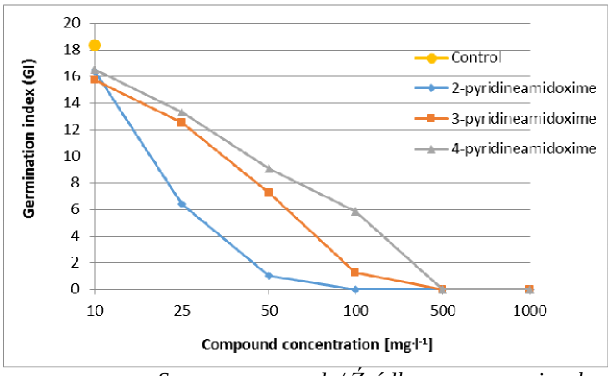
*control sample, 2PAO – pyridine-2-amidoxime, 3PAO – pyridine-3-amidoxime, 4PAO – pyridine-4-amidoxime

Source: own work / Źródło: opracowanie własne



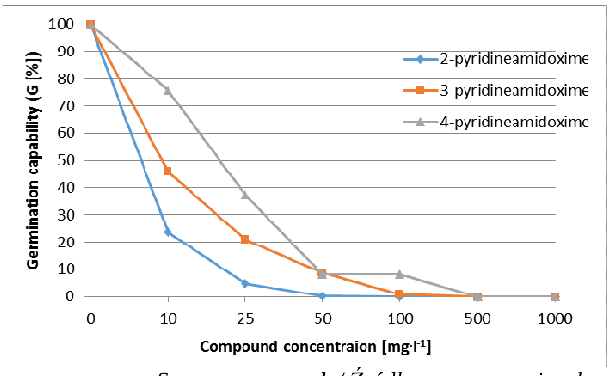
Source: own work / Źródło: opracowanie własne

Fig. 2. Dependence of epicotyl length on pyridine-amidoximes concentration
Rys. 2. Zależność długości epikotylu od zastosowanej dawki związku



Source: own work / Źródło: opracowanie własne

Fig. 3. Dependence of germination index (GI) on pyridine-amidoximes concentration
Rys. 3. Wartość indeksu kiełkowania (GI) w zależności od stężenia zastosowanego związku



Source: own work / Źródło: opracowanie własne

Fig. 4. Dependence of germination capability (G%) on pyridine-amidoxime concentration
Rys. 4. Zdolność kiełkowania (G%) dla różnych stężeń analizowanych związków

The highest weight was noted for seeds growing on a medium with 10 mg/l of pyridine-2-amidoxime. Higher values were observed for the isomer than for the control. Only epicotyl weight of kernels growing on a medium with 50 mg·l^{-1} of pyridine-2-amidoxime was relatively low in comparison with epicotyl mean length. Figure 3 shows values of germination index of the analyzed compounds depending on the used concentrations. It could be observed at a concentration of 10 mg/l that all the compounds exhibited the highest germination capability of maize seeds. However, it was only 16% and it was lower than the value for the control sample. Together with an increase in compound concentration in the medium, germination index was decreasing. The lowest values of GI were observed for a compound concentration of 100 mg·l^{-1}, while at concentrations of 500 mg/l and higher, the germination process did not take place. The graph of germination capability (G%) (Figure 4) is similar to that of Figure 3. At a concentration of 10 mg·l^{-1}, it could be observed that all the compounds showed the highest germination capability of maize seeds. Together with an increase compounds concentration in the medium, germination capability was decreasing. The lowest values (G%) could be observed at a compound concentration of 100 mg·l^{-1}, while at concentrations of 500 mg·l^{-1} and higher, the germination process did not take place. On the basis of the obtained results, EC50 values were determined for the examined compounds. The results are presented in Table 3. The highest value of EC50 was observed for pyridine-4-amidoxime, and the lowest for pyridine-2-amidoxime, which was over twice lower than the highest value.

Table 3. EC50 values for the analyzed compounds
Tab. 3. Wartość EC50 dla analizowanych związków

Compound	EC50 [mg·l^{-1}]
2PAO	28
3PAO	53
4PAO	69

2PAO – pyridine-2-amidoxime, 3PAO – pyridine-3-amidoxime, 4PAO – pyridine-4-amidoxime

Source: own work / Źródło: opracowanie własne

4. Discussion

The effect of chemical compounds is very popular, especially quaternary ammonium salts and herbicides. The authors analyzed effect of organic compounds on plant, which include to the other units, monocots and dicots. The most of these works showed that increasing the concentration of compounds resulted in increased toxicity. For example, Bałczewski et al. [3] described that barley was a more resistant plant which fairly well tolerates test ionic liquid concentration up to 200 mg/kg of soil, but these chemicals inhibited growth of radish at concentration 100 mg/kg of soil. Pernak et al. [12] reported the 1,3-dialkoxmethylimidazolium tetrafluoroborate salts introduced to the soil at concentration to exert a phytotoxic effect on monocotyledonous plants. Studzińska and Buszewski [13] have proved that hazardous effects of imidazolium ionic liquids were closely connected with organic matter content in soil.

In the literature were described the effect of pyridine carboxylic acids and their amides on plant growth. They used lower concentrations (up to 10 mg/L) those employed by us. Among other things, they are shown the positive effects of plant development in the presence of 3-pyridine carboxylic acid amide, but negative for dicarboxylic acid amides and the other two isomers (i.e., compounds having a functional group at position 2 or 4 of the pyridine ring). The authors also observed inhibition of plant growth in the presence of pyridine acids and amides concentration of 0.001M above [4, 15].

The presented results about effect of pyridineamidoximes confirm that the phytotoxicity was depended on the concentration compound, but significantly effects of toxicity phenomenon depended on structures of analyzed compounds and exactly on the position of functional group in the pyridine ring.

5. Conclusions

Synthesized pyridine amidoximes are of considerable beneficial industrial use, but they do not always have a beneficial effect on plants. Therefore their analysis in terms of potential harmful properties and safe doses is so important prior to their introduction into the environment. In the present study, pyridine-2-, -3- and -4-amidoximes were synthesized, which can exhibit also biologically active properties, and therefore affect plants. Hence the main aim was to initially analyze their phytotoxicity.. All the compounds inhibited germination at a concentration above 500 $mg^{.}l^{-1}$. On this basis it can be concluded that the phytotoxic effect of the compounds occurs at concentrations above 500 $mg^{.}l^{-1}$.

6. References

[1] Abele E., Abele R., Lukevics E.: Pyridine oxime: synthesis, reactions and biological activity. Review. Chemistry of Heterocyclic Compounds, 2003, 39(7), 825-865.

[2] Astheimer L., Schenk H., Witte E., Schwochau K.: Development of sorbers for the recovery of uranium from seawater. Part 2. The accumulation of uranium from seawater by resins containing amidoxime and imidoxime functional groups. Separation Science and Technolgy, 1983, 18, 307.

[3] Bałczewski P., Bachowska B., Białas T., Biczak R., Wieczorek W.M., Balińska A.: Synthesis and phytotoxicity of new ionic liquids incorporating chiral cations and/or chiral anions. Journal of Agricultural and Food Chemistry, 2007, 55, 1881-1892.

[4] Berglund T., Ohlsson A.B., Rydström J., Jordan B.R., Strid A.: Effect of nicotinamide on gene expression and glutathione levels in tissue culture of *Pisum sativum*. Journal of Plant Physiology, 1993, 142, 676-684.

[5] Eloy F., Lenaers R.: The Chemistry of amidoximes and related compounds. Chemical Reviews, 1962, 62, 155-183.

[6] G-Yu An., Yuan B., Tao J., Ai-Li Cui, H-Zh. Kou.: Synthesis, structure and magnetic properties of trinuclear transition metal complexes based on pyridine-2-amidoxime. Inorganica Chimica Acta, 2012, 387, 401-406.

[7] Karaguzel O., Cakmakci S., Ortacesme V., Aydinoglu B.: Influence of seed coat treatments on germination and early seedling growth of Lupinus varius (L.). Pakistan Journal of Botany, 2004, 36(1), 65-74.

[8] Kliachyna M., Santoni G., Nussbaum V., Renou J., Sanson B., Colletier J.P., Arboleas M., Loiodice M., Weik M., Jean L., Renard P.Y., Nachon F., Baati R.: Design, synthesis and biological evaluation of novel tetrahydroacridine pyridine- aldoxime and -amidoxime hybrids as efficient uncharged reactivators of nerve agent-inhibited human acetylcholinesterase. European Journal of Medicinal Chemistry, 2014, 78, 455-467.

[9] Konidaris K.F., Giouli M., Raptopoulou C.P., Psycharis V., Verginadis I.I, Vasiliadis A., Afendra A.S., Karkabounas S., Manessi-Zoupa E., Stamatatos T.C.: Initial employment of pyridyl oximes and dioximes in zinc(II) chemistry: Synthesis, structural and spectroscopic characterization, and biological evaluation. Inorganica Chimica Acta, 2011, 376, 470-478.

[10] Mlícková K., Sebelaa M., Cibulka R., Fréborta I., Peca P., Liska F., Tanizawa K.: Inhibition of copper amine oxidases by pyridine-derived aldoximes and ketoximes. Biochimie, 2001, 83, 995-1002.

[11] Nicolaides D.N., Varella E.A.: Acid derivatives volume 2. John Wiley & Sons, New York, 1992.

[12] Pernak J., Sobaszkiewicz K., Foksowicz-Flaczyk J.: Ionic liquids with symmetrical dialkoxymethyl-substituted imidazolium cations. Chemistry - A European Journal, 2004, 10, 3479-3485.

[13] Studzińska S., Buszewski B.: Study of toxicity of imidazolium ionic liquids to watercress (*Lepidium sativium L.*). Analytical and Bioanalytical Chemistry, 2008, 393, 983-990.

[14] Taguchi H., Maede M., Nishitari H., Shimabayashi Y., Iwai K.: Effect of cinchomeronic acid and related compounds on the growth of Radish seedlings. Bioscience, Biotechnology and Biochemistry, 1992, 56(12), 1921-1923.

[15] Vörös A., Mucsi Z., Baán Z., Timári G., Hermecz I., Mizsey P., Finta Z.: An experimental and theoretical study of reaction mechanisms between nitriles and hydroxylamine. Organic & Biomolecular Chemistry, 2014, 12, 8036-8047.

The project was funded from the Polish Ministry of Science and Higher Education as project No 03/32/DSPB/0500 and 03/32/DSMK/0516.