Polish Journal of Agronomy
2016, 24, 16–22

doi: 10.26114/pja.iung.267.2016.24.03

Phytotoxicity of pyridineamidoximes against two crop plants: preliminary evaluation

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Abstract. The study involved the analysis of the impact of pyridineamidoximes on the germination and early development of plants belonging to different taxonomic units (monocots and dicots). Maize (*Zea mays*) and sunflower (*Helianthus annuus*) were selected as the objects. Culture was carried out in vertical plastic Phytotoxkit™ containers (Phytotoxkit, Tigret company, Belgium). Number of germinated seeds, root length and shoot height were recorded. Based on the results, germination index and percentage inhibition of roots and shoots growth were calculated. 2-, 3- and 4-pyridineamidoximes showed varied influence on the plants under study. Generally the toxic effect of pyridineamidoximes on the plants depends on both the position of the functional group in the pyridine ring, as well as on the concentration of the compound used. The results showed a higher susceptibility of sunflower vs. maize to the compounds. Phytotoxic response in maize occurred typically at the highest concentration (above 500 mg kg^{-1} d.w.s.), while the sunflower symptoms inhibition due to the presence of the test compounds occurred at the lowest (25 and 50 mg kg^{-1} d.w.s.) and the highest (1000 mg kg^{-1} d.w.s.) concentration.

key words: pyridineamidoximes, phytotoxicity, maize, sunflower

INTRODUCTION

Chemical manufacturing, transport and utilization contribute to the high environmental contamination. The essence of the construction of amidoxime is the presence, on the same carbon atom, of the amine and hydroxyimine moiety, which makes these compounds versatile reagents used in the synthesis of many organic compounds, especially those containing a heterocyclic ring. Pyridineamidoximes also have important biochemical properties and as such have been used as antibacterial agents, bacteriostatic, antivirals, and as agents with herbicidal and fungicidal properties. In recent years, there have been also references to the synthesis and use of pyridineamidoximes and their *O*-alkyl derivatives as potential extractants of metals from aqueous leaching ores or aqueous waste solutions (EP 0007679 A1,1980; US 3290320 A, 1966; US 5776944 A, 1998; Astheimer et al., 1983; Nishimura et al., 1967; Mlícková et al., 2001). Therefore, prior to their use on industrial scale it is crucial to proactively characterize their potential environmental hazards by determining their persistence in water and soil, (bio)degradation, migration in groundwater; bioaccumulation in aquatic or terrestrial organisms and the toxicity (Thuy Pham et al., 2010). Even the evidence of increasing environmental pollution, which suggest need for the development of simplier and more dependable tests to the determine the harmful effects of chemicals that accumulate in the abiotic and biotic environment, there are still a lack of reports about the influence of different kinds of compounds in soil, especially a new chemical compounds, on plant development.

In view of the above, we deemed it interesting to study the effects of pyridineamidoximes on the germination and early development of maize (*Zea mays*) and sunflower (*Helianthus annuus*).

MATERIALS AND METHODS

2-, 3- and 4-pyridineamidoximes were obtained in reaction, which was devised based on the work of Bernasek (1957) (Fig. 1). An appropriate 2-, 3- or 4-cyanopyridine was added to hydroxylamine hydrochloride and sodium hydrogen carbonate in ethanol as diluent. The mixture was heated for a few hours. The reaction was terminated when the white crystals were precipitated. The crude product was recrystallized three times from ethanol. As a result, 2-pyridineamidoxime (**2PAO**) was obtained at a yield of over 90% (m.p. 119–120 °C), 3-pyridineamidoxime (**3PAO**) at a yield of 83% (m.p. 131–132 °C), and 4-pyridineamidox-

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Received: 15 January 2016; revised 18 February 2016

ime (**4PAO**) at a yield of 86% (198–200 °C). Structures of all the obtained compounds were confirmed with spectroscopic methods: ^{1}H NMR and ^{13}C NMR.

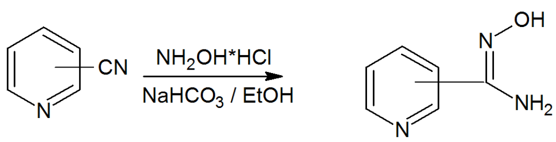

Figure 1. Scheme of compounds synthesis

The second part of the study involved the analysis of the impact of pyridineamidoximes on the germination and early development of plants belonging to different taxonomic units. Maize (*Zea mays*) and sunflower (*Helianthus annuus*) were selected as the objects. Culture was carried out in vertical plastic Phytotoxkit™ containers (Phytotoxkit, Tigret company, Belgium). 90 ml of soil were applied to the plates, and then a quantity of the analyzed substance (25 ml) was added so that their effective concentrations were 25, 50, 100, 500 and 1000 mg kg^{-1} of soil dry weight. Soil without analyzed compound and watered with deionized water was used as the control samples. Phytotoxkit plastic containers were placed in the dark, constant parameters such as temperature (25 ± 1 °C) and humidity (80%) were maintained. On thus prepared soil, 10 seeds of maize or sunflower were put (3 plates for each concentration of the test compounds). After the end of the experiment the number of germinated seeds was counted and the root length and shoot height were measured. Based on the results, the percentage inhibition of roots and shoots growth, was calculated:

$$\text{Inhibition} = \left(\frac{L_c - L_x}{L_c}\right) \cdot 100\%$$

or

$$\text{Inhibition} = \left(\frac{P_c - P_x}{P_c}\right) \cdot 100\%$$

where: L – length of root
P – height of shoot
x – mark of the sample
c – mark of the control

On the basis of the obtained results the (GI) for each treatment was calculated using equation:

$$GI = N \cdot L$$

where: N – number of germinated seeds
L – mean root length.

In the next step the concentrations of the compounds which inhibited root growth at 50% (IC_{50}) were determined.

The soil used in the experiment contains (in 1 kg): 81 mg P, 88 mg K, 69 mg Mg, pH (in KCl) was 5.92, C organic content of 1.01% (10.1 g kg^{-1} soil), 2.4 mg N-NH_4 kg^{-1} d.w.s. and 9.2 mg N-NO_3 kg^{-1} d.w.s., particle size distribution: 1–0.1 mm = 95%, 0.1–0.02 mm = 5%, < 0.02 mm = 0%. Typologically, the field soil was of black earth type, black earth subtype with cambic horizon. The influence of pyridineamidoximes was investigated using the phytotoxicity test based on the ISO-11269-2:2003 International Standard (ISO-11269-2, 2003). Toxic effect was observed when inhibition of germination or growth of root and shoot was more than 30%.

Maize and sunflower provided by Department of Agronomy in Poznan, Poland, were used for that study. The germination tests were carried out in Petri dishes, where 50 maize seeds were sown per dish on a wet filter paper. That experiment was made in three independent runs and each sample was prepared in four replicates. The tests were performed according to the ISTA 2013 methodology. Germination index and sprouting energy (after 4 days exposure) were recorded. Results for sunflower seed: sprouting energy 99 ± 1.2 and germination index: 96 ± 0. Results for maize seed: sprouting energy 99 ± 1.4 and germination index 97 ± 2.3.

Average values were calculated for each analysed group. All phytotoxicity tests were carried out in three parallels. Average values were evaluated with their standard deviations (SD). Results were plotted with Microsoft Excel software. The data was compared by the Student's *t* test and statistical significance was set as $p < 0.05$.

RESULTS

In order to evaluate the impact of pyridineamidoximes on maize and sunflower, plants were treated with different concentrations of three pyridineamidoximes and growth parameters (germination inhibition, shoot height and root length inhibition in comparison to control) were analysed. OECD (2006) and US EPA (1996) norms recommend that seed germination in control plants reaches at least 70% and 90%, respectively. In our experiments 90 to 100% seeds were germinated in control samples, for both analysed plants. The reduction of germinated maize seeds was observed at 250 and 500 mg kg^{-1} d.w.s for 3-pyridineamidoxime (70 and 80%, respectively), and for 2-pyridineamidoxime at concentration 1000 mg kg^{-1} d.w.s., which was above 70%. For sunflower seeds, the reduction of germination were observed only when 4-pyridineamidoxime at 1000 mg kg^{-1} d.w.s. was added to the soil

The analysis showed that the tested compounds inhibited or did not affect the growth of shoot and root of maize (Fig. 2 and 3). The impact was similar on the growth of both shoot and root. 2-Pyridineamidoxime caused the highest toxicity and the inhibition of root growth was observed at a concentration of 250 mg kg^{-1} d.w.s. and higher.

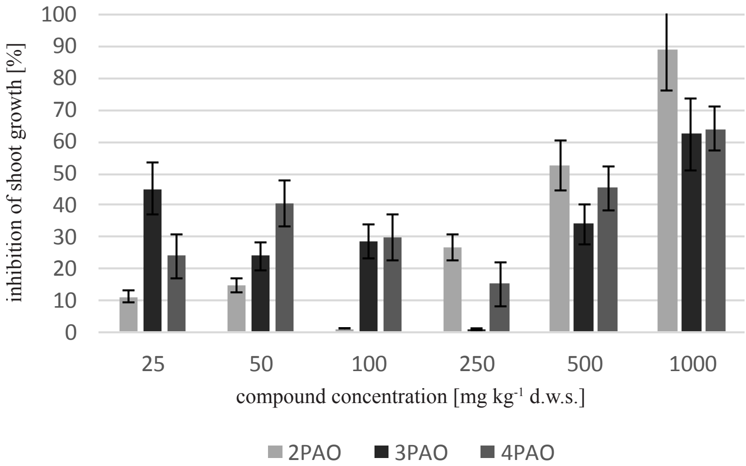

Figure 2. Shoot growth inhibition in maize after 6 days of exposure to pyridineamidoximes (average values with their SD and $p < 0.05$ – significant difference from control).

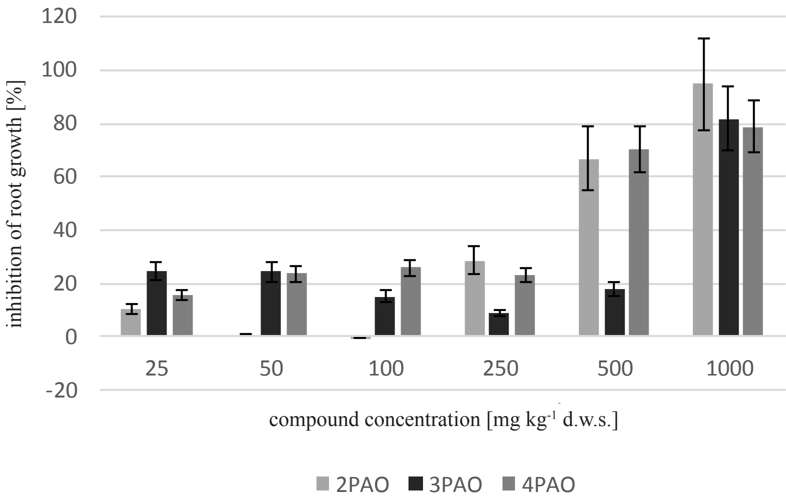

Figure 3. Root growth inhibition in maize after 6 days of exposure to pyridineamidoximes (average values with their SD and $p < 0.05$ – significant difference from control).

That compound did not reduce shoot growth at 100 and 250 mg kg^{-1} d.w.s.

The presence of the functional group in the 3 and 4 position in the pyridine ring resulted in increased toxicity. The reduction of the development of both shoot and root, was observed practically over the entire range of compound concentration. However, the presence of 3-pyridineamidoximes at 250 mg kg^{-1} d.w.s. did not affect maize shoot development. All pyridineamidoximes inhibited the growth of both the root and the shoot when applied at the highest concentration (500 and 1000 mg kg^{-1} d.w.s.).

Different effects of pyridienamidoximes were observed for sunflower (Fig. 4 and 5). When the functional group was located at the 2 or 3 position in the pyridine ring significant changes in toxicity relative to shoot and root was observed. The toxic effect was induced only if the concentration of the compounds were used at either extreme (minimum and maximum), which suggested that in the case of dicots there are defined ranges of effectively concentrations. However application of 4-pyridineamidoxime to the soil caused different results on sunflower. This compound inhibited root growth of sunflower by 33% at 25 mg kg^{-1} d.w.s., but the

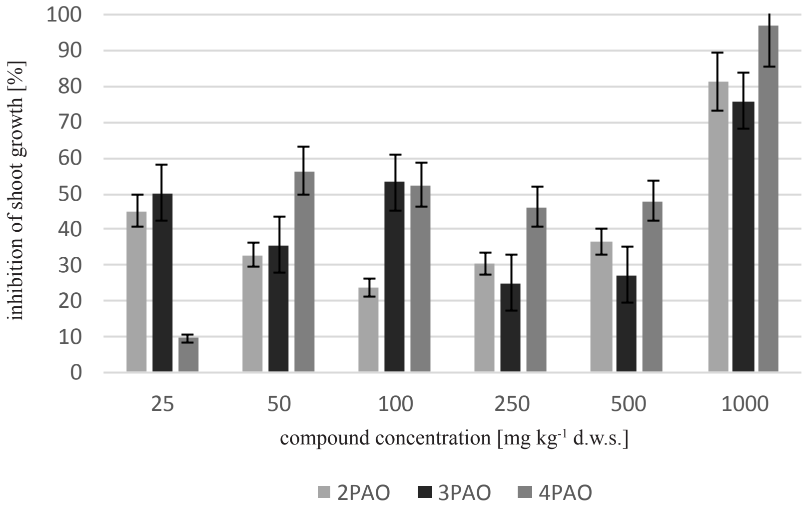

Figure 4. Shoot growth inhibition in sunflower after 6 days of exposure to pyridineamidoximes (average values with their SD and $p < 0.05$ – significant difference from control).

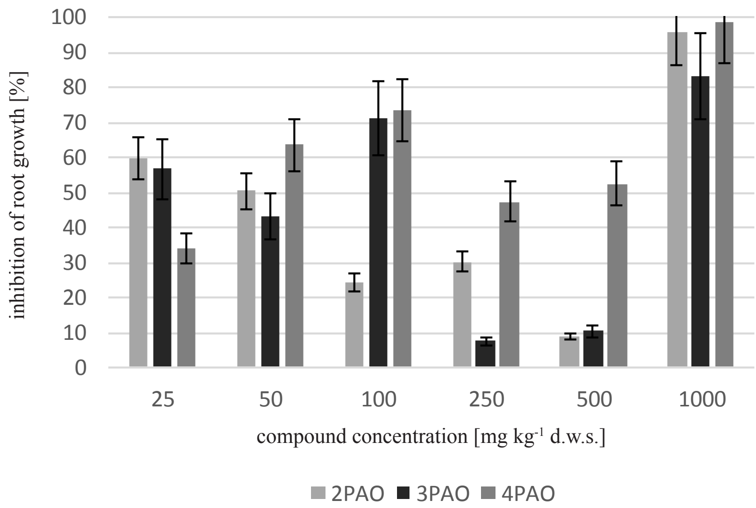

Figure 5. Root growth inhibition in sunflower after 6 days of exposure to pyridineamidoximes (average values with their SD and $p < 0.05$ – significant difference from control).

increasing of compound concentration in soil increased the inhibition of root development, and at 1000 mg kg^{-1} d.w.s. root growth was inhibited by over 90%. Similar effect was observed for shoot growth, however the concentration of 25 mg kg^{-1} d.w.s. reduced shoot growth by no more than 10%.

The highest tested concentartions of pyridineamidoximes reduced the GI values by up to 20% for both tested plants (Fig. 6 and 7).

IC_{50} values for GI are presented in Table 1. The pyridineamidoximes showed diverse toxicity towards the tested plants. In the case of maize 2PAO and 4PAO were more toxic (lower value of IC_{50}) than 3-pyridineamidoxime. They were approximately two times more toxic than isomer with functional group in 3 position of pyridine ring. A more complicated situation was for the germnation of sunflower seeds. The analysed compounds had three values of IC_{50}.

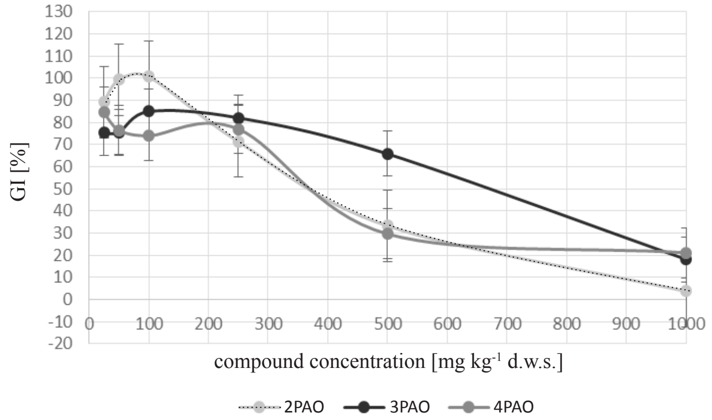

Figure 6. Dependence of germination index of maize on pyridineamidoxime concentration.

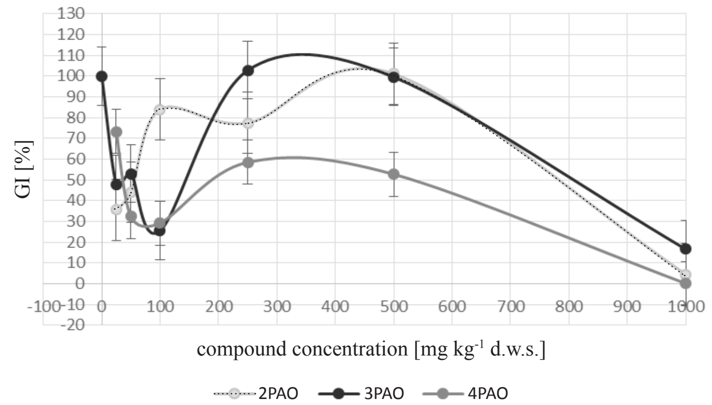

Figure 7. Dependence of germination index of sunflower on pyridineamidoxime concentration.

Table 1. IC_{50} values for root growth in maize and sunflower after 6 days of exposure to pyridineamidoxime.

Compound	Maize	Sunflower
2PAO	380	100 and 790
3PAO	670	25–50 and 800
4PAO	380	25, 200 and 500

DISCUSSION

Phytotoxicity of different compounds such as ionic liquids, quaternary ammonium salts and herbicides is a very popular subject of literature reports. Researchers described results about analysis of the impact of soils with varying contents of the clay minerals such as smectie and kaoline on the toxicity of ionic liquids with different anions and cations. Other investigators, like Bałczewski et al. (2007), Pernak et al. (2004), Studzińska and Buszewski (2009), Bubalo et al. (2014) also analyzed the effect of organic compounds on plants belonging to different units, monocots and dicots. The majority of those reports showed that increasing the concentration of compounds under investigation resulted in increased toxicity. For example, Bałczewski et al. (2007) described that barley was a more resistant plant which fairly well tolerates test ionic liquid concentration up to 200 mg kg^{-1} of soil; whereas, for radish, the growth and development inhibiting concentration is 100 mg kg^{-1} of soil. Pernak et al. (2004) reported the 1,3-dialkoxmethylimidazolium tetrafluoroborate salts introduced to the soil at concentration of 1000 or 100 mg kg^{-1} d.w.s, were found to exert a phytotoxic effect on monocotyledonous plants. Studzińska and Buszewski (2009) proved that hazardous effects of imidazolium ionic liquids were closely connected with organic matter content in soil. But on the other hand, the hazardous character of analyzed compounds was strongly connected with hydrophobicity, indicating that the more hydrophobic ionic liquids were, the higher the decrease of seed germination was. Bubalo et al. (2014) studied the effect of different anions and alkyl chains lengths on the barley seedlings. They reported that the inhibitory effect depended on the ionic liquids concentration and also on the chemical structure, whereby the most toxic compound contained 10 carbon atoms in chain, and the next less toxic was chain with 7C and a short chain containing 4C. The observed side chain effect could be explained by the model of toxic action which takes place through membrane disruption due to ionic liquids structural similarities to detergents, pesticides or antibiotics (Docherty et al., 2005).

In the literature, the effect of pyridine, pyridine derivatives such as carboxylic acids and their amides on plant growth was described (Berglund et al., 1993; Taguchi et al., 1992). Those studies were concerned with the impact of the toxic compounds on the morphology and metabolism of plants (including wheat). They used lower concentrations (up to 10 mg L^{-1}) than those employed by us. Among other things, we showed a positive effects on plant development of the presence of nicotinic acid amide, but a negative one of 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 investigators also observed inhibition of plant growth in the presence of pyridine acids and amides concentration of 0.001 M above (Berglund et al., 1993; Taguchi et al., 1992).

The results of the effect of pyridineamidoximes confirm the hypothesis that the phytotoxicity was dependent on the concentration of the compound in soil, but significant effects of toxicity phenomenon depended on the structures of analyzed compounds and, specifically, on the position of the functional group in the pyridine ring. But this study also indicated that the toxic effect was dependent on the type of plant. The compound with the functional group situated in 2 and 4 position of the pyridine ring caused higher toxicity than 3-pyridineamidoxime. Compounds such as nicotinamide (3-pyridinecarboxylic amide) and nicotine acid (3-pyridinecarboxylic acid) are precursors of two active biological compounds (NAD^+ and $NADP^+$) (Mohamed et al., 1989). The lower toxicity effect of compounds with the functional group in position 3 of the pyridine ring could be explained by these compounds being able to switch on the metabolic pathway. Also the monocot plant (maize) was more resistant than the dicot (sunflower). The plants used in this project belong to different taxonomic ranks and have different transport mechanisms. Stimulation of the shoot and root growth in maize was observed for lower concentrations and, in the case of sunflower, at medium concentration compared to the control. These phenomena can be explained by the partial degradation of the tested compounds and their use by plants to increase growth of biomass. By another hypothesis, the plants used in the experiments do have mechanisms allowing to stop the transport or have systems of detoxification of pyridine derivatives. In such a situation, when the concentration extremes (maximum) were used, it can be assumed that the defense mechanisms are switched on only after reaching a limit concentration. For example, herbicides belonging to the group of synthetic auxins used at low concentrations cause acidification of the cell wall by stimulating the activity of ATPase proton pump associated with the cell membrane. pH reduction contributes to increased enzymatic activity resulting in loosening cell walls, which leads to disturbances of in the transport and functionalization of the plant. It can also cause stimulation mimicking the RNA polymerase, resulting in increased synthesis of DNA, RNA and protein synthesis. Abnormal growth processes may lead to uncontrolled cell division and growth, thereby resulting in the destruction of the vascular tissue. In turn, high concentration of the herbicide inhibits cell growth and division usually in the region of meristems. In turn, the high concentration of the herbicide inhibits cell growth and division is usually in the region of meristems. Herbicides can accumulate in the phloem, causing disturbances in the functioning of the plant (All, 2002; Taguchi et al., 1992).

It is also possible that the presence of these compounds can influence gene expression, and the defense mechanism ceases to operate effectively above the limit concentration. Berglund et al. (1993) described the impact of nicotinamide on gene expression.

CONCLUSIONS

1. The phytotoxicity tests utilizing maize (*Zea mays*) and sunflower (*Helianthus annuus*) showed that the two different plants varied for their tolerance of pyridineamidoximes.
2. The results demonstrated a higher susceptibility of sunflower vs maize to the tested compounds.
3. Phytotoxic response in maize occurred typically at the highest concentrations (more than 500 mg kg^{-1} d.w.s.), while in the sunflower symptoms of growth inhibition due to the presence of the test compounds occurred at the lowest (25 and 50 mg kg^{-1} d.w.s.) and the highest concentration (1000 mg kg^{-1} d.w.s.).
4. Results of phytotoxicity effect caused by pyridineamidoximes on the maize showed that the tested compounds influenced on development of shoots and roots length. 2-pyridineamidoxime was the most toxic – the roots growth inhibition was observed even at a concentration of 250 mg kg^{-1} d.w.s.
5. All analyzed compounds inhibited maize development at the highest concentration (500 and 1000 mg kg^{-1} d.w.s.). 3-pyridineamidoxime showed the lowest toxicity, and it limited the growth of the roots only at the highest concentration (1000 mg kg^{-1} d.w.s.).
6. Pyridineamidoximes produced the toxic effect on sunflower only if the concentration extremes (minimum and maximum) were used.

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