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COMPARISON OF HERBICIDES CONTAINING ISOPROTURON, 2.4-D AND DICAMBA ON PHOSPHATASE ACTIVITY IN THE SOIL AND IN SPRING WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

The pot experiment examined the effect of varied doses of herbicides, namely Izoturon 500 SC (500 g isoproturon), Aminopielik Super 464 SL (344 g 2,4-D and 120 g dicamba) and Rokituron D 470 SC (250 g isoproturon, 200 g 2,4-D and 20 g dicamba) on the activity of acid and alkaline phosphatases in the soil and in spring wheat plants. The pots were filled with black earth of the granulometric composition of light lessive clay containing 1.2 – 1.8% of humus. Water emulsions of the herbicides studied were introduced into the soil at the standard, 5-fold and 25-fold higher doses, after which spring wheat was sown. The measurements of soil and plant phosphatase activities were made at respective wheat development phases. The results obtained revealed that the herbicides used significantly stimulated the activity of acid phosphatase in the soil and, at the beginning of vegetation period – also in wheat plants, as well as alkaline phosphatase in the soil. The higher the doses of Izoturon 500 SC and Rokituron D 470 SC were used, the greater was the inhibition of alkaline phosphatase activity in wheat. The correlation coefficients indicated no relationships between the changes in the activity of soil and plant phosphatases following the application of the standard and 5-fold higher dose of Izoturon 500 SC and Rokituron D 470 SC. However there was observed a significant correlation between the phosphatases activity in the soil after the application of those herbicides as well as Aminopielik Super 464 SL at the dose 25-fold higher than the standard. The activity of alkaline phosphatase in the soil following Aminopielik Super 464 SL application at all the doses applied was positively correlated with the activity of the alkaline phosphatase in wheat.

Key words: acid and alkaline phosphatase, isoproturon, 2,4-D, dicamba, soil, spring wheat.

INTRODUCTION

Herbicides can enhance food production as weed control facilitates higher plant productivity. However, frequently not meeting the treatments and waiting period deadlines and not complying with the adequate herbicide doses can result in environment contamination.

All the herbicides, even those used as foliar treatments, get into the soil which is the main reservoir accumulating their residues [1]. Evaluating the effect of pesticides on the soil ecosystem, the soil enzymatic activity is a frequently used indicator [7]. Soil phosphatases react fastest to the pesticide stresses and so they are the most frequently investigated enzymes [2]. Acid EC 3.1.3.1 and alkaline EC 3.1.3.2 phosphatases catalyze hydrolytic breakdown of phosphomonoesteres, therefore there exists a high correlation between soil phosphatase activity and the content of phosphorus in the soil [3].

Phosphorus, one on the most important nutrients for the plant growth, takes place in energy flow, forms many chemical compounds and its deficiency limits the plant growth [12]. Phosphorus uptaken from soil is mostly part of inorganic bonds [11]. However the organic phosphorus compounds accumulated in vacuoles, such as phytine, lecithin and phospholipids, are also a very valuable source of phosphorus for plants [19]. These compounds are hydrolyzed in plants also by acid and alkaline phosphatases. Besides, phosphatase together with kinases can, by reversible covalent modifications, regulate many processes in plants. It is a kind of 'safety valve' thanks to which, due to kinases phosphorylation and phosphatase dephosphorylation, one can increase or decrease the catalytic effectiveness of other enzymes [10]. The plant phosphatase activity can therefore affect both the content of phosphorus in wheat grain and straw and the rate of many processes in plants.

The aim of the present research was to compare the effect of three herbicides containing different active substances (isoproturon, 2,4-D and dicamba) used at three doses on the soil and plant activity of phosphatases.

MATERIALS AND METHODS

The pot experiment was carried out May 4 to August 20, 2004 as completely randomized blocks in greenhouse of the Szczecin Agricultural University. Spring wheat was sown into the pot filled with black earth sampled from the Gumieniecka Plain of the granulometric composition of light lessive clay containing 1.2 – 1.8% of humus. Soil was sampled from 0 – 30 cm and screened through the 2 mm sieve and divided into 3-kg samples. Water emulsions of Izoturon 500 SC, Aminopielik Super 464 SL and Rokituron D 470 SC herbicides were introduced into the soil at the standard dose (I) and 5-fold (II) and 25-fold (III) higher doses, after which spring wheat was sown (Table 1). The preparation doses were calculated against the standard dose assuming the arable layer 1 dm deep and 1.53 $g \cdot cm^{-3}$ soil compactness. Table 1 shows the content of active substances in respective preparations in $mg \cdot kg^{-1}$ soil. The experimental soil moisture was maintained at 60% of maximum water capacity.

Table 1. Doses of the herbicides applied

Herbicide	Active substance	$g \cdot dm^{-3}$	Dose applied						Active substance introduced into soil		
			$dm^3 \cdot ha^{-1}$			$mm^3 \cdot kg_{soil}^{-1}$			$mg \cdot kg_{soil}^{-1}$		
			I	II	III	I	II	III	I	II	III
Izoturon 500 SC	isoproturon	500	2	10	50	1.30	6.50	32.50	0.65	3.25	16.25
Aminopielik Super 464 SL	2,4-D	344	1	5	25	0.65	3.25	16.25	0.22	1.10	5.50
	dicamba	120							0.08	0.40	2.00
Rokituron D 470 SC	isoproturon	250	4	20	100	2.60	13.00	65.00	0.65	3.25	16.25
	2,4-D	200							0.52	2.60	13.00
	dicamba	20							0.05	0.25	1.25

The measurements of soil and plant phosphatases activities were made at successive developmental phases: 3-leaf (34^{th} day of the experiment), shooting (79), flowering (95) and milk maturity (113). The activity of soil phosphatases was determined with the Tabatabai and Bramner [16] colorimetric method, modified by Margesin [7], while that of plant phosphatases – according to Bessey and Lowry [5] method. The phosphatase activity in soil and in plants untreated with herbicides constituted the control sample.

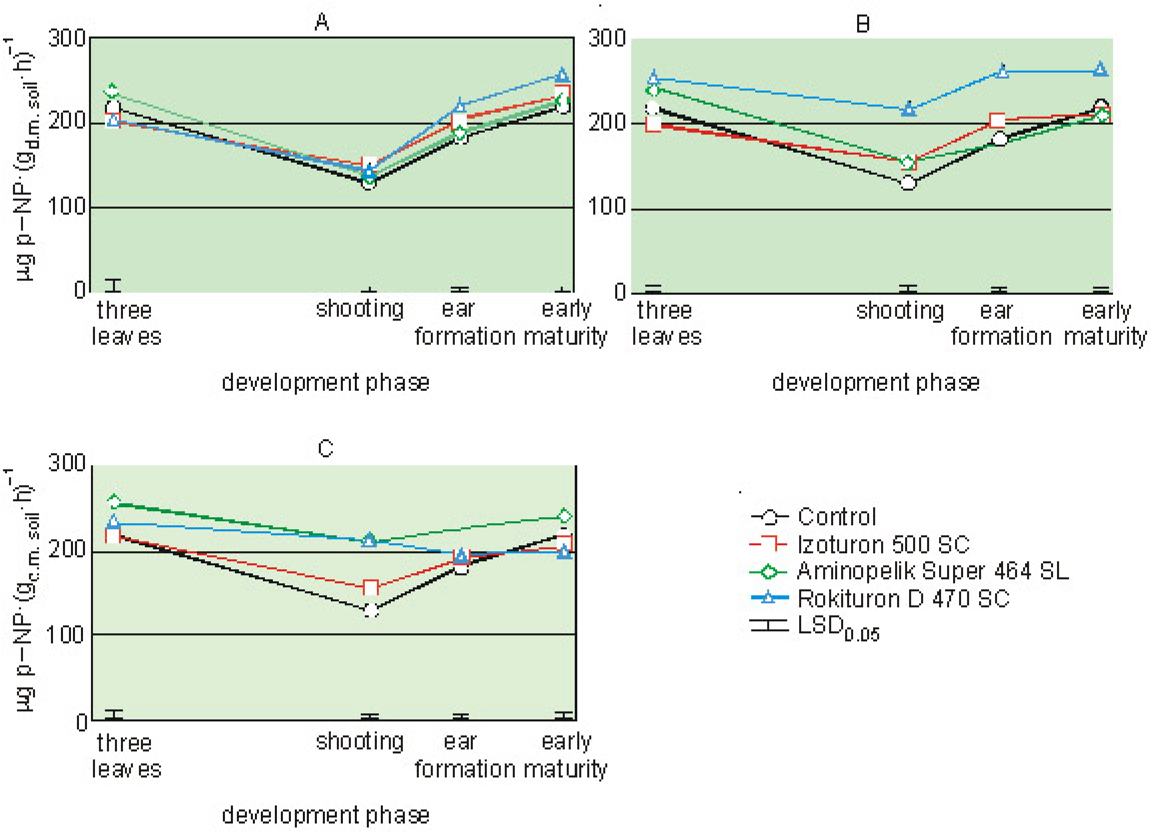
The results obtained were verified statistically with the single-factor variance analysis and the lowest significant differences were calculated with the Tukey test at α = 0.05. The analyses were made independently for each measurement period.

The activity of soil phosphatases is given in mg p-NP g of dry matter of soil $\cdot h^{-1}$, while that of plant in μM p-NP in 100 g of fresh weight of plant $\cdot 0.5\ h^{-1}$. To compare the effect of the herbicides studied on the activity of soil and plant phosphatases, mean enzymatic activity values were calculated for each dose and all measurements, which were then converted into percentage of the control, where 100% represented mean control phosphatase activity.

RESULTS AND DISCUSSION

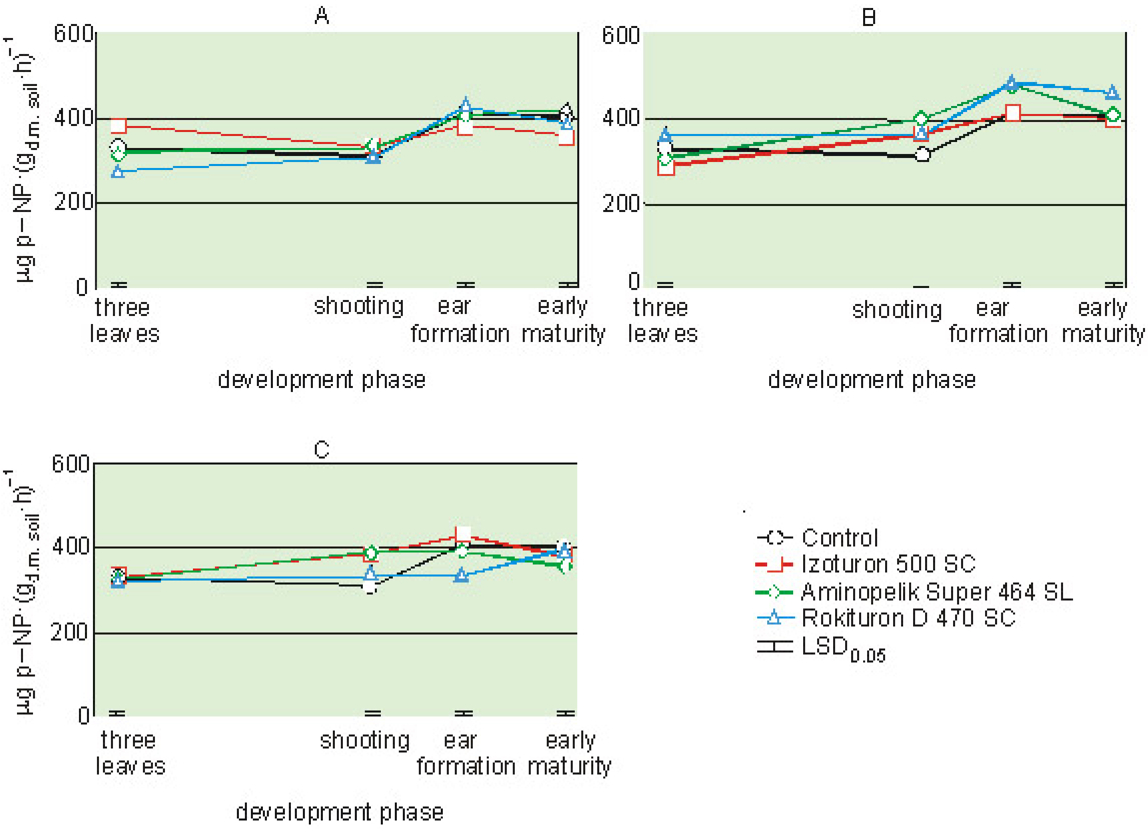
The activities of soil and plant phosphatases are given in a form of linear curves in Figs 1-4, whereas Figs 5-7 illustrate the effect of respective herbicides on the averaged percentage of the enzymatic activity against the control. Having applied herbicides into soil, in most cases there was recorded a significant increase in the activity of acid phosphatase in soil (Fig. 1). A slight inhibition of the activity of that enzyme was observed only at the 3-leaf phase in wheat when Izoturon 500 SC was applied at the dose 5-fold higher than the standard as well as at the milk maturity phase following the treatment with Izoturon 500 SC and Rokituron D 470 SC at the dose 25-fold higher than recommended.

Fig. 1. Effect of different doses of the herbicides used on the activity of acid phosphatase in soil: A – standard dose, B – 5-fold higher dose, C – 25-fold higher dose



In most cases there was also noted a significant activation of alkaline phosphatase in soil treated with the herbicides studied (Fig. 2). A significant inhibition of the activity of this enzyme at the 3-leaf phase was only found when Izoturon 500 SC was applied at the phase of flowering and milk maturity and at the standard dose at the 3-leaf phase.

Fig. 2. Effect of different doses of the herbicides used on the activity of alkaline phosphatase in soil: A – standard dose, B – 5-fold higher dose, C – 25-fold higher dose



A significant inhibition of alkaline phosphatase activity in wheat at the 3-leaf phase was observed as a result of Izoturon 500 SC application at the dose 5-fold higher than the standard and the application of Rokituron D 470 SC standard dose, whereas at the flowering phase – of Izoturon 500 SC standard dose and the dose of Rokituron D 470 SC 25-fold higher than recommended. Also at the milk maturity phase, the activity of alkaline phosphatase in soil was considerably inhibited when the plants were treated with the standard dose of Izoturon 500 SC and with Aminopielik Super 464 SL used at the dose 25-fold higher than recommended.

There was observed a stimulation of the soil phosphatases due to herbicides application. Similar results indicating an increase in soil phosphatases activity or a slight effect of pesticides on the activity of those enzymes were reported by Tu [17] following the application of nitropyrin and pyroxyfur, by Nowak and Kłódka [8] investigating Decis 2.5 EC (delthametrin) and Shukla [13] after the application of butachlor, fluchloralin and oxyfluoren. Shukla [13] also observed an inhibiting effect of 2.4-D on soil phosphatases activity. Nowak et al. [9] stated a decrease in soil phosphatase activity following Lontrel 300 SL (clopyralid), Mustang 306 SE (florasulam) and Solar 200 EC (cinidon ethyl), while Wyszkowska and Kucharski [18] – after Granstar 75 WG (tribenuron methyl) application.

At the beginning of development, e.g. at 3-leaf phase, all the herbicides doses, except the Aminopielik Super 464 SL dose 25-fold higher than the standard resulted in a considerable increase in acid phosphatase in wheat (Fig. 3), while 25-fold higher dose at this phase inhibited it. Over successive development phases both Izoturon 500 SC and Rokituron D 470 SC, at all the doses, inhibited the activity of this enzyme. The standard dose of Aminopielik Super 464 SL decreased the activity of acid phosphatase in wheat when applied at shooting and flowering phases, while the dose 5-fold higher than the standard – only when applied at the shooting phase. The dose 25-fold higher than recommended was activating acid phosphatase in wheat from the shooting phase to the end of the experiment.

The activity of wheat alkaline phosphatase was only slightly stimulated by Izoturon 500 SC and Rokituron D 470 SC used at the standard dose and by Aminopielik Super 464 SL at the dose 25-fold higher throughout the experiment (Fig. 4). However, at the shooting phase, this activation following the standard dose of Izoturon 500 SC was not significant. Izoturon 500 SC and Rokituron D 470 SC at the dose 5-fold higher than the standard did not affect the activity of alkaline phosphatase in wheat, either. The application of Izoturon 500 SC and Rokituron D 470 SC at the dose 25-fold higher than the standard decreased the activity of alkaline phosphatase throughout the experiment, while Aminopielik Super 464 SL stimulated it.

Fig. 3. Effect of different doses of the herbicides used on the activity of acid phosphatase in wheat plants: A – standard dose, B – 5-fold higher dose, C – 25-fold higher dose

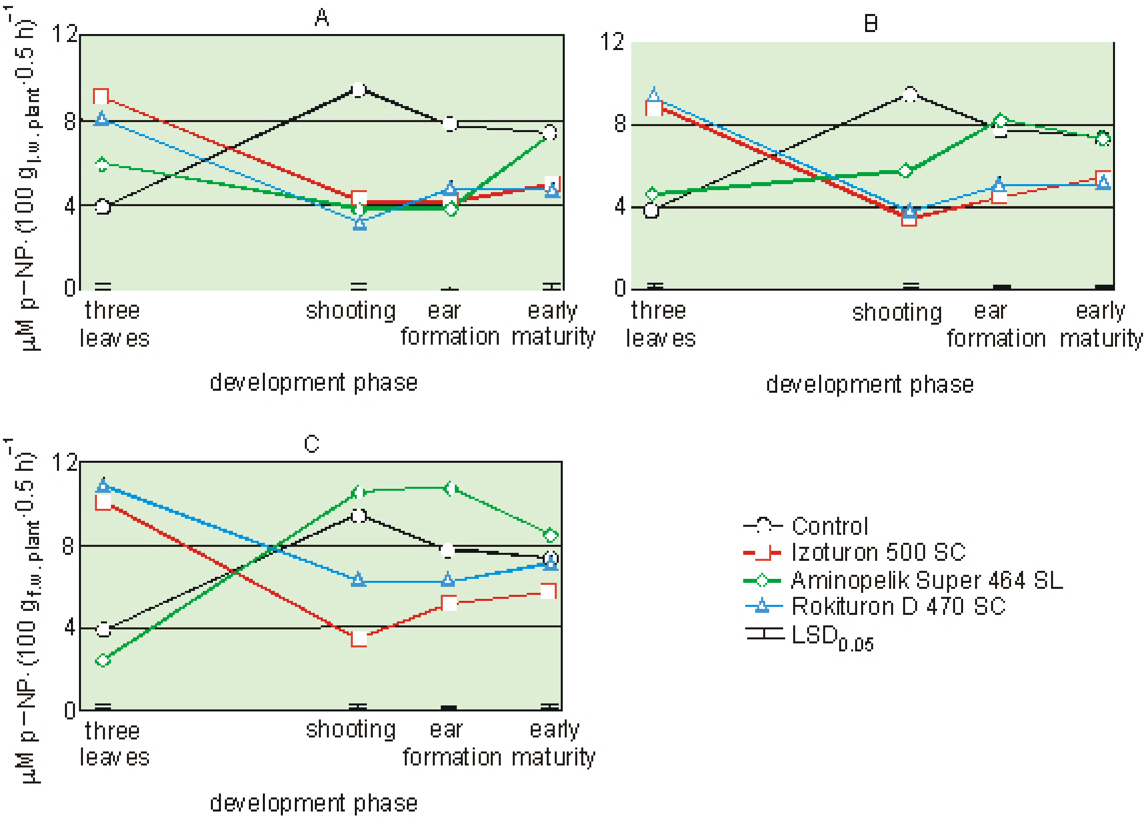
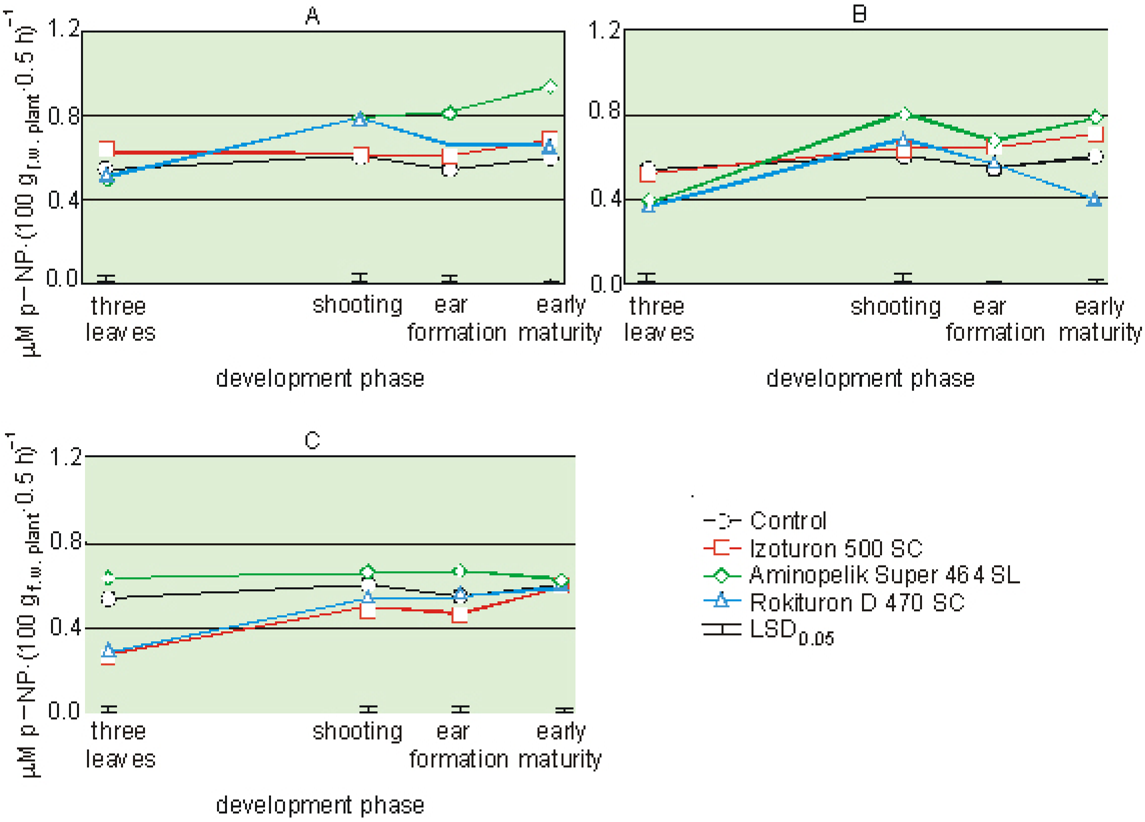


Fig. 4. Effect of different doses of the herbicides used on the activity of alkaline phosphatase in wheat plants: A – standard dose, B – 5-fold higher dose, C – 25-fold higher dose

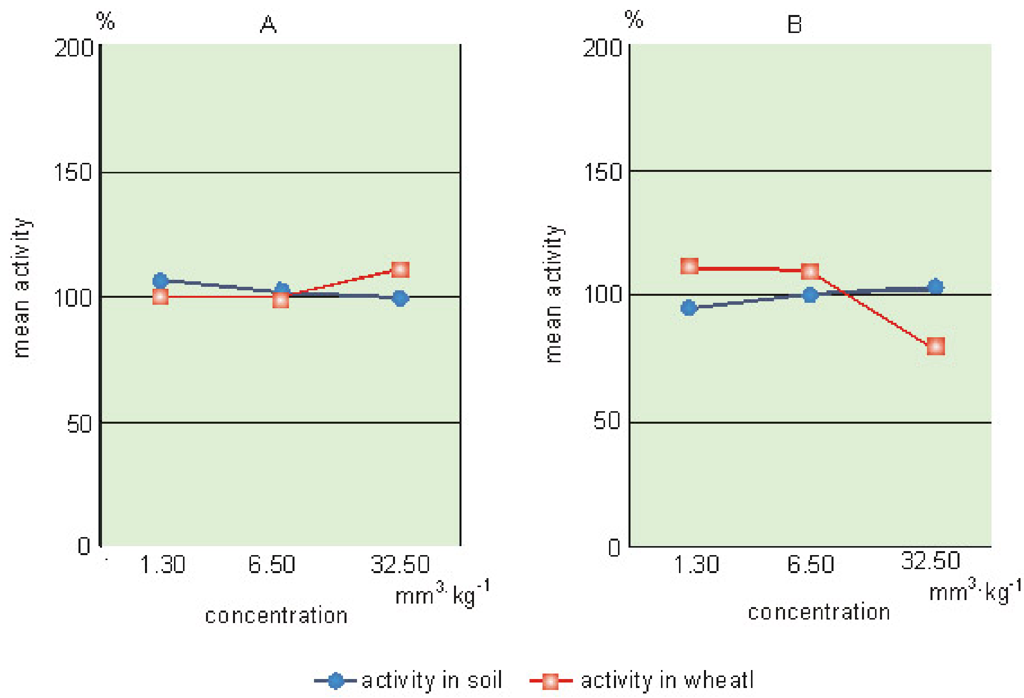


The activity of acid phosphatase in wheat was mostly inhibited, while of alkaline phosphatase – by higher doses of Izoturon 500 SC and Rokituron D 470 SC and was activated following Aminopielik Super 464 SL application. Nowak and Telesiński [10] also reported an inhibiting effect of Izoturon 500 SC at the standard, 5-fold and 100-fold higher doses on the activity of alkaline phosphatase and a stimulating effect on acid phosphatase activity in wheat seedlings. They also observed that Izoturon 500 SC stimulated the activity of catalase and peroxidase and inhibited the activity of nitrate reductase in spring wheat seedlings. Kłódka et al. [4] reported on an inhibiting effect of different concentrations of Miedzian 50 WP and Miedzian 350 Extra SC on the activity of peroxidase in radish, while Smolik and Nowak [15] demonstrated a stimulating effect of phosphatase activity after the application of 0.5 $mmol \cdot kg^{-1}$ Cu^{2+} in wheat. Also Skupień – Wysocka and Cholewiński [14] observed an enhanced activity of phosphatases in pea seedlings grown in soil treated with NaF.

The comparison of averaged values of enzyme activities expressed as a percentage of the activity in the control soil and plants allows for determining the enzyme activity trend depending on the herbicides doses applied. Analyzing the average values of plant and soil phosphatase activities, there was observed a slight activation of acid phosphatase in the treated with standard Izoturon 500 SC dose (Fig. 5), however at higher doses – no effect was noted. In wheat Izoturon 500 SC activated acid phosphatase when applied at the dose 25-fold higher than recommended. No such influence was found following standard and 5-fold higher dose of that herbicide.

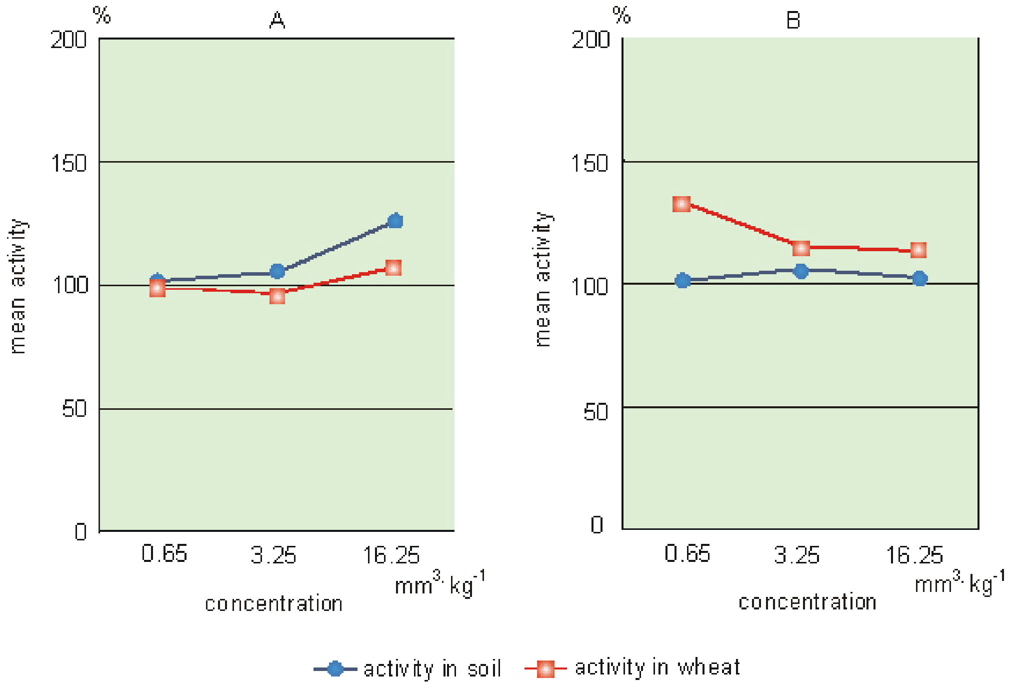
Izoturon 500 SC applied at all the doses slightly modified also the activity of alkaline phosphatase in soil, while used at the doses both standard and 5-fold higher than the recommended stimulated the alkaline phosphatase activity in wheat plants, whereas 25-fold higher dose – inhibited it.

Fig. 5. Effect of different doses of Izoturon 500 SC on the phosphatase activity in soil and in wheat plants: A – acid phosphatase, B – alkaline phosphatase



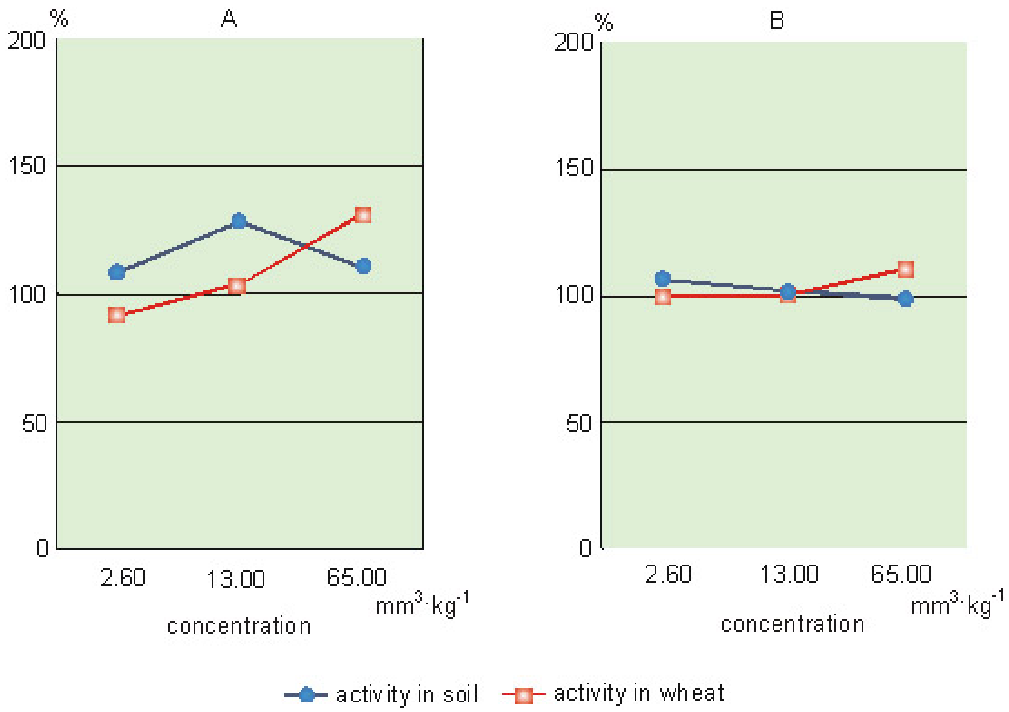
Aminopielik Super 464 SL used at the standard dose changed considerably the activity of acid phosphatase neither in soil nor in wheat (Fig. 6). Similarly the 5-fold higher dose did not alter the activity of acid phosphatase in wheat but stimulated it in soil. Only at the dose of as much as 25-fold higher than the standard was the activity of acid phosphatase both in soil and wheat enhanced. All the doses of Aminopielik Super 464 SL applied also activated alkaline phosphatase in wheat. The highest increase in the activity (135%) was noted at the standard dose, and the increase in the Aminopielik Super 464 SL dose decreased this activation up to 110%. None of the doses applied changed the activity of soil acid phosphatase.

Fig. 6. Effect of different doses of Aminopielik Super 464 SL on the phosphatase activity in soil and in wheat plants: A – acid phosphatase, B – alkaline phosphatase



The standard and 25-fold higher dose of Rokituron D 470 SC (Fig. 7) stimulated the activity of acid phosphatase in soil up to 110% of the control, while the 5-fold higher dose resulted in an even higher stimulation (up to 130%). In wheat Rokituron D 470 SC slightly inhibited the activity of this enzyme showing an increase tendency due to the use of 5-fold dose up to 105% and 135% when the 25-fold dose was applied.

Fig. 7. Effect of different doses of Rokituron D 470 SC on the phosphatase activity in soil and in wheat plants: A – acid phosphatase, B – alkaline phosphatase



Rokituron D 470 SC at the standard dose slightly decreased the activity of alkaline phosphatase in soil, while the higher doses of this herbicide increased it. The standard dose of Rokituron D 470 SC, however, resulted in a clear increase in the alkaline phosphatase activity, whereas higher doses inhibited it in wheat plants.

At the standard and 5-fold higher doses of the herbicides used the correlation coefficients (Table 2) in most cases showed a relationship between the changes in the activity of acid and alkaline phosphatase neither in soil nor in wheat. However, the dose 25-fold higher than the standard significantly enhanced the activities of both acid and alkaline phosphatases in soil and in wheat plants. Aminopielik Super 464 SL was the only one which when used at the 25-fold dose higher than recommended decreased the activity of both phosphatases studied in soil and in wheat. There was also demonstrated a significantly positive correlation between the activity of alkaline phosphatase in soil and in wheat following the standard Aminopielik Super 464 SL dose and following the 5-fold higher dose of Aminopielik Super 464 SL and Izoturon 500 SC.

Table 2. Correlation coefficients between the doses of herbicides and phosphatases activities in soil and wheat plants

Herbicide	Dose	Correlation coefficients between activity of	
		acid phosphatase in soil and in wheat plants	alkaline phosphatase in soil and in wheat plants
Izoturon 500 SC	standard dose	0.236	0.106
	5-fold standard dose	0.546	0.868*
	25-fold standard dose	0.857*	0.711*
Aminopielik Super 464 SL	standard dose	0.464	0.747*
	5-fold standard dose	-0.505	0.622*
	25-fold standard dose	-0.845*	0.624*
Rokituron D 470 SC	standard dose	0.189	0.237
	5-fold standard dose	0.441	0.339
	25-fold standard dose	0.837*	0.648*

* significant at $\alpha = 0.05$

CONCLUSION

1. Izoturon 500 SC, Aminopielik Super 464 SL and Rokituron D 470 SC significantly stimulated the activity of alkaline phosphatase in soil and of acid phosphatase in soil and at the juvenile development phases – in wheat.
2. The greater the dose of Izoturon 500 SC and Rokituron D 470 SC were used, the greater was the inhibition of the alkaline phosphatase in wheat.
3. The dose of Izoturon 500 SC and Rokituron D 470 SC 25-fold higher than recommended resulted in a positive correlation between the activities of soil and plant phosphatases.
4. Following Aminopielik Super 464 SL application, there was observed a positive correlation between alkaline phosphatase activity in soil and in wheat plants.

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