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INFLUENCE OF COMBINED FUNGICIDES AND ADJUVANTS APPLICATION ON ENZYMATIC ACTIVITY AND ATP CONTENT IN SOIL

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ABSTRACT

In laboratory experiment the influence of different dosages of fungicides on the activity of acid and alkaline phosphatase, dehydrogenase, urease, β-glucosidase and ATP content in loamy clay soil was determined. The fungicides were applied alone and with additions of an adiuvants applied at field rate and 10-fold and 100-fold higher. The following combinations were used: Miedzian 50 WP (a.i. copper oxychloride), Miedzian 350 extra SC (Miedzian 50 WP + Polybutene emulsion) and Miedzian 50 WP applied together with Rapol 90 EC. The soil sample was submitted to analysis in 1, 3, 7, 14, 28, 56 and 112 days after the pesticides treatment. The investigations showed that soil enzymes activity after use pesticides with adiuvants was lower from activities these enzymes in soil after use of fungicides without adiuvants, it was especially visible in case of dehydrogenase and β-glucosidase. Also ATP content in soil with addition all of three concentrations Miedzian 350 extra SC in the course first 60 days of experiment was lower then after use Miedzian 50 WP.

Key words: fungicide, adjuvant, enzymatic activity, ATP, soil

INTRODUCTION

One of many agricultural practices is plants protection by pesticides use. However, due to progressing degradation of the natural environment and rapid growth of pollutants in air, water and soil, to reduction pesticide use to an admissible minimum has become highly recommended. Among pro-ecological methods, aimed to reduce pesticide application, are of genetically resistant varieties, biological measures, allelopatic interaction and integrated systems for plant protection [16, 18]. On the other hand, negative results of agriculture chemicalisation are to be mitigated by new highly efficient and selective chemicals, characterised by a quick and environmentally friendly decomposition, such as the newest photodynamical products [21]. Nevertheless, the high application cost of such modern chemicals in conjunction with a relatively inefficient non-chemical treatment, stimulates the search for other solutions [24]. A wide range of adjuvants, surface-active compounds, mineral or plant oil in character, as well as silicon chemicals that at the same time, increase pesticides efficiency and reduce the required amount of a biologically active agent, can provide such a solution.

Activity measurements of enzymes responsible for both, the direction and intensity of specific biochemical transformations in soil can serve as a tool to evaluate changes in the soil environment induced by chemicals [7]. Similarly, ATP content that was shown to be strongly correlated with selected soil enzymes activities, can be interpreted in terms of interface between various metabolic processes in a soil environment, provides valuable information on trends in pesticides transformations in soils [6].

Negative impact of pesticides on soil hydrolases and oxidoreductases activities has been widely reported in the literature [5, 8, 17, 23]. However, the knowledge on influence of adjuvants combined with pesticides on metabolic processes in soil are limited [14]. Although adjuvants are belived to be neutral for the soil environment, they are capable of slowing the pace and trends of pesticides degradation in the soil [19]. Hence, it seems worth considering whether a combined usage of adjuvants and pesticides can affect activities of selected enzymes, namely acid and alkaline phosphatase, dehydrogenase, urease and β-glucosidase, as well as ATP content in soils. The presented research focuses on such considerations.

MATERIALS AND METHODS

Laboratory studies performed on samples collected from arable-humus horizon (0 - 30 cm) of black earth at Gumieniecka Plain. The soils grain composition is typical for light silty clays of low organic carbon content (1.2-1.8%) and slightly acid or neutral reaction, highly abundant in available phosphor and medium to high abundance of available magnesium and sodium. They are classified as groups II, III a and III b of a bonitation class for arable lands. To sallow parts of the prepared soil material water solutions of the following compounds:

1. Miedzian 50 WP, i.e. a fungicide containing 50% of copper oxichloride,
2. Miedzian 350 Extra SC, i.e. a fungicide containing 350 g of copper oxichloride per 1 dm^3 with admixture of Polibutene emulsion (patent PL 175693) as adjuvant,
3. Miedzian 50 WP combined with Rapol 90 EC an adjuvant containing 90% of plant oil and 10% of non ionic surface-active substances,

were added in doses recommended by the manufacturer, ten times higher and hundred times higher. Field doses were calculated for the amount to be applied for 1 kg of soil; the data are presented in Table 1.

Table 1. Doses of specimen applied in the experiment

No.	specimen	applied doses					
		$dm^3 \cdot ha^{-1}$ / $kg \cdot ha^{-1}$			$mm^3 \cdot kg^{-1}$ / $mg \cdot kg^{-1}$		
		I	II	III	I	II	III
1.	Miedzian 50 WP	5	50	500	6.52	65.2	652
2.	Miedzian 350 extra SC	5	50	500	6.52	65.2	652
3.	Miedzian 50WP Rapol 90 EC	5 1.5	50 15	500 150	6.52 1.96	65.2 19.6	652 196
4.	Polibutene emulsion	0.8	8	80	1.04	10.4	104

Having applied the emulsion, soil moisture was balanced at 60% maximum water capacity, the soil was thoroughly mixed and stored in tight polyethylene containers at optimal temperature of 20°C. Soil to which no

investigated compounds were added served as a reference sample (control). At few days intervals at fist, and few weeks intervals later on (on days 1., 3., 7., 14., 28., 56. and 112.) the measurements of soil enzyme activities were performed with suitable methods and equipment. Namely, for acid and alkaline phosphatase a Lambda Bio spectrophotometer manufactured by Perkin Elmer and the methods of Tabatabai and Bremner [22] as well as Eivazi and Tabatabai [3] modified by Margesin [11] were employed; for dehydrogenase Malkomes's [9] method was applied, whereas for urease and β -glucosidase measurements methods of Bonmati at al. [2] and Hoffman and Dedecen's [4] were used, respectively.

In addition on days 1., 30., 60., and 90. of the experiment measurements of ATP content in the soil contaminated with various concentrations of Miedzian 50 WP, Miedzian 350 extra SC and Polibutene emulsion were made with a Lumat LB 9507 luminometer manufactured by Berthold according to Margesin's method [12] that employs an enzymatic luciferin/lucifarase system.

The obtained data have been processed statistically. The smallest significant differences were calculated according to Tukey's test at the significance level $\alpha = 0.05$.

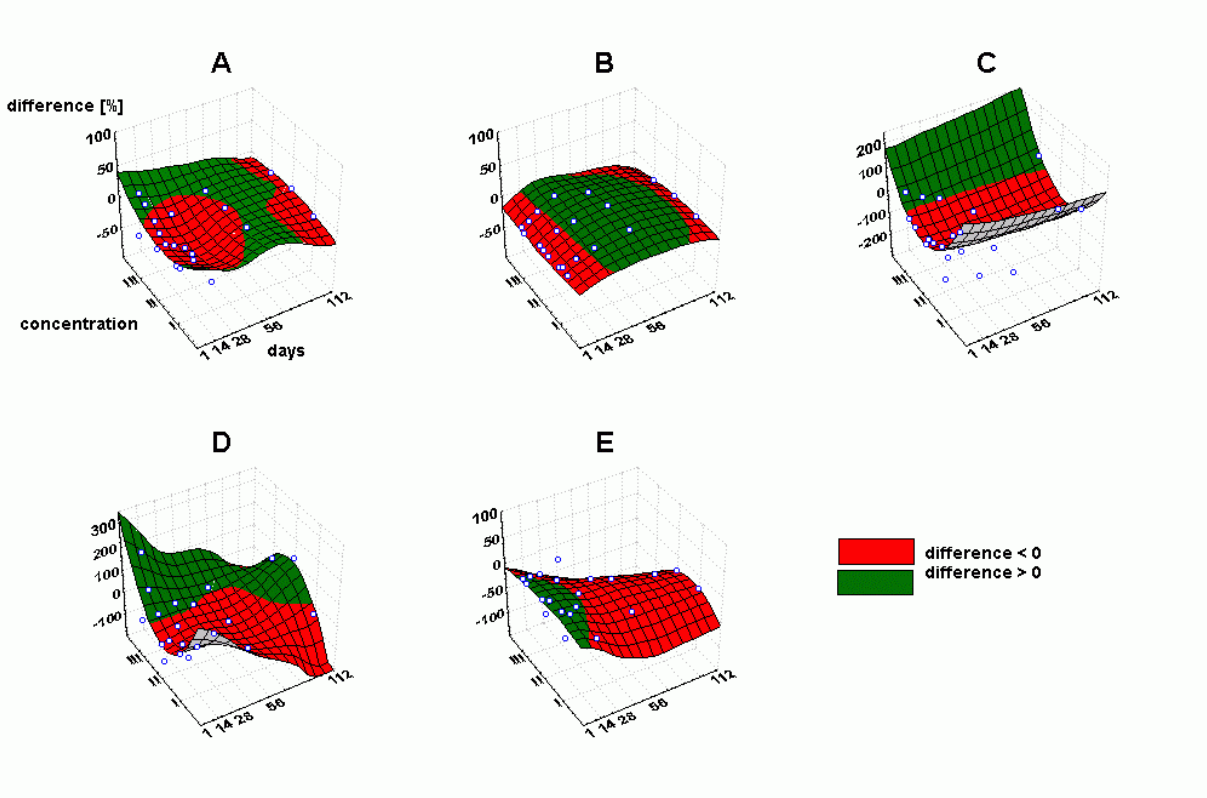
RESULTS

The results of the conducted research are presented as surface plots illustrating differences in enzymes activities in soil induced by applied respectively with Miedzian 50 WP and Miedzian 350 extra SC, and finally with Miedzian 50 WP mixed with Rapol 90 EC. Real activity was recalculated and quoted as percentage of the enzyme activity in the control soil, whose activity was assumed as 100%. Differences greater than zero are marked on the plots with green. It indicates beneficial influence of the applied fungicides that have been investigated with reference to Miedzian 50 WP. Red colour corresponds to the reverse relationship, i.e. a negative influence of Miedzian 350 extra SC and Miedzian 50 WP combined with Rapol 90 EC when related to fungicide with no adjuvant, that is Miedzian 50 WP.

ATP content in soil after applying various concentrations of Miedzian 50 WP, Miedzian 350 extra SC combined with a Polibutene emulsion are given as $\mu g\ ATP \cdot g^{-1}_{d.m.\ of\ soil}$ and presented as bar charts.

Disadvantageous influence of the applied adjuvants upon acid phosphatase activity in soil was particularly visible after application Miedzian 350 extra SC in concentrations II and III, and all three concentrations of Miedzian 50 WP combined with Rapol 90 EC (Fig. 1A and 2A). The biggest differences in the activity of that enzyme reaching even –50% were observed for concentration II and III of Miedzian 50 WP - Rapol 90 EC combination applied on day 28, while a medium differences at the level of –20% were recorded over the first month of the experiment for all three Miedzian 350 extra SC concentrations applied. In the final stage of the experiment the observed differences in activities were small and amounted to more few percent.

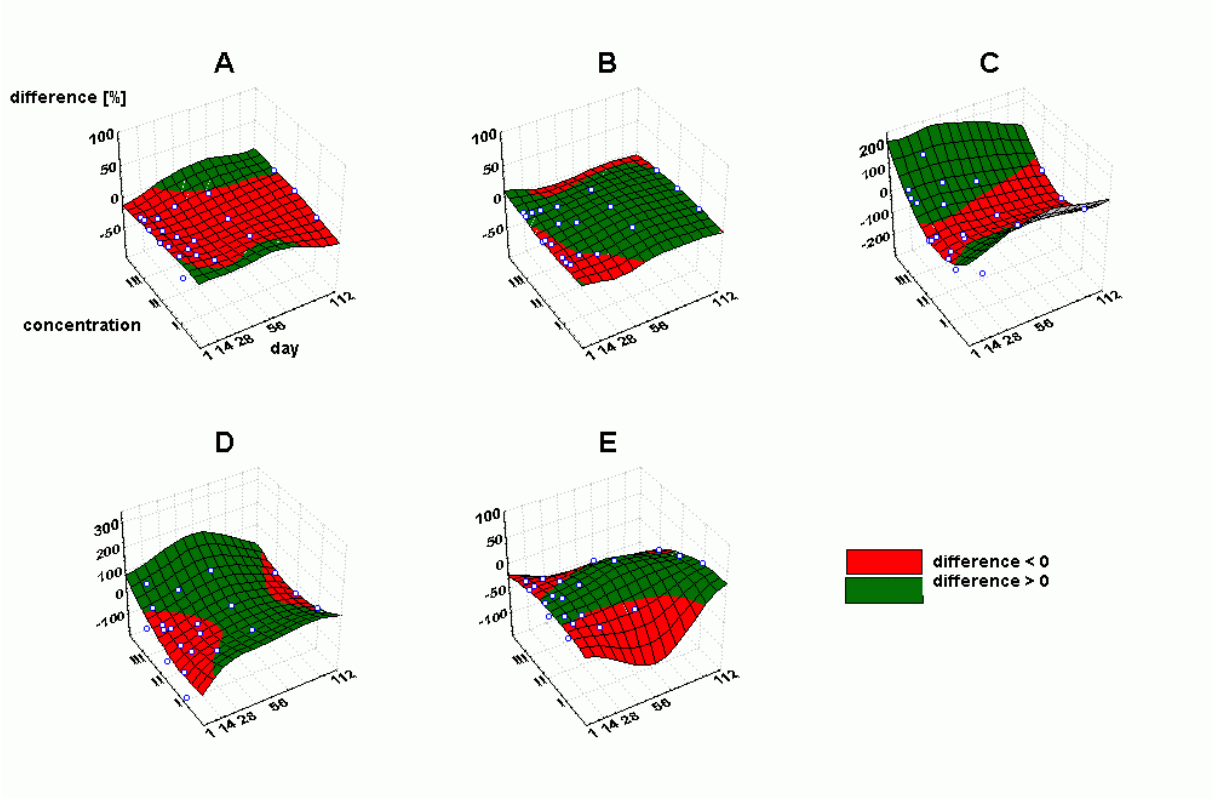
Fig. 1. Percentage differentions of activity: A – acid phosphatase, B – alkaline phosphatase, C – dehydrogenase, D – urease, E - β-glucosidase, after applied of Miedzian 50 WP and Miedzian 50 WP with Rapol 90 EC



Alkaline phosphatase activities in soil were quite different in character (Fig. 1B and 2B). At the beginning and final stages of the experiment after all concentrations of Miedzian 50 WP - Rapol 90 EC combination applied, activity of that enzyme was lower than after application of Miedzian 50 WP alone. In the analysed period differences in the enzyme activities reached nearly –30%. For the rest of the experiment time scale admixture of Rapol 90 EC into Miedzian 50 WP caused an insignificant activation of alkaline phosphatase with activity differences not higher than 10%. Yet another trend in the enzyme activity was noted after Miedzian 350 extra SC was applied. Activity lowered by about 10% was recorded only over the first week that followed fungicide – adjuvant combined application in concentrations I and II, when compared to the one after application of Miedzian 50 WP alone. At all the other times polibutene emulsion admixture to Miedzian 50 WP caused the enzyme to be slightly activated.

Practically, all the applied doses of Miedzian 50 WP - Rapol 90 EC combination detrimentally affected soil dehydrogenase activity throughout the entire duration of the experiment (Fig. 1C). The most pronounced differences that reached –200% were noted in the first half of the experiment after concentrations II and III had been applied, whereas in the second half of the experiment the concerned differences diminished. Similar dependencies were found after application of Miedzian 350 extra SC, though at the highest concentration applied the agent influence was definitely more beneficial when compared to Miedzian 50 WP (Fig. 2C).

Fig. 2. Percentage differentions of activity: A – acid phosphatase, B – alkaline phosphatase, C – dehydrogenase, D – urease, E - β-glucosidase, after applied of Miedzian 50 WP and Miedzian 350 extra SC

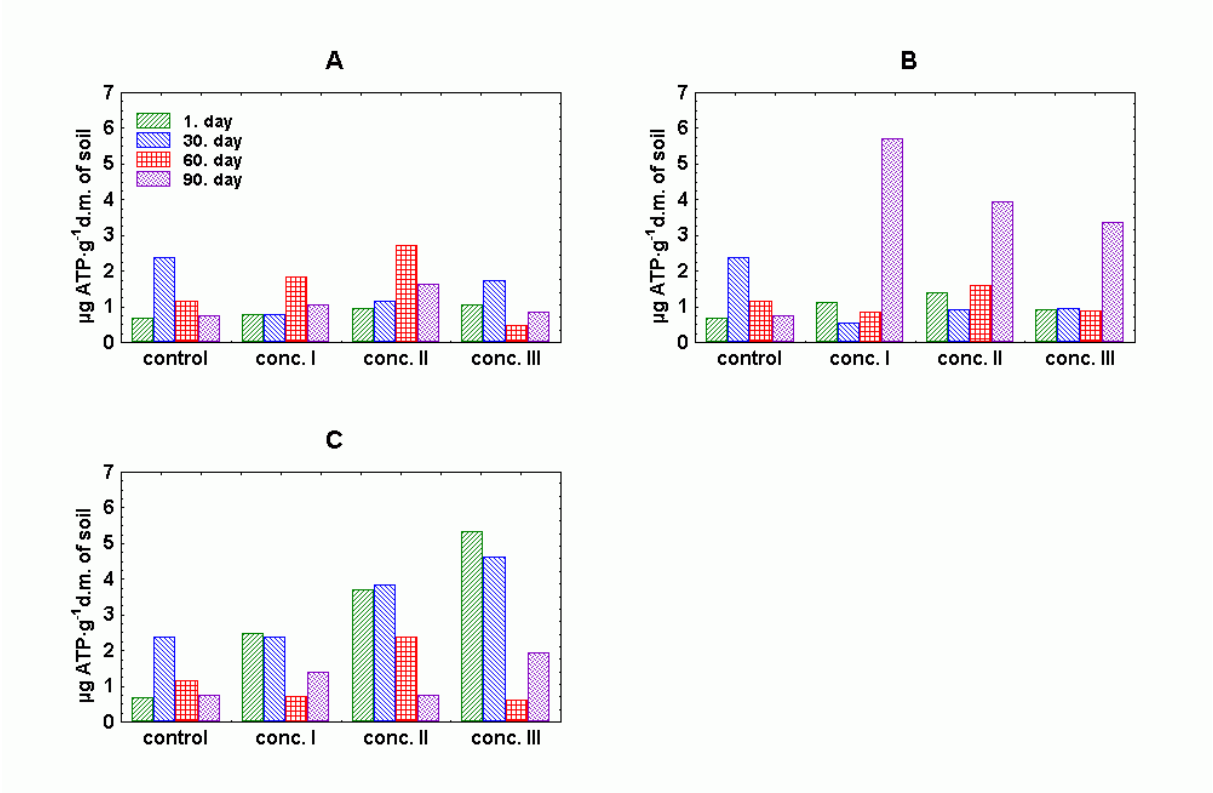


For the first 28 days of the experiment following application of Miedzian 350 extra SC in concentrations I and II, soil urease activity was lower than the enzyme activity in the soil to which respective concentrations of Miedzian 50 WP were added (Fig. 2D). Registered activity differences in that period reached –190% at the beginning of the experiment, to drop down steadily as time passed to reach –20% after a month. During last days of the experiment all three concentrations of Miedzian 350 extra SC yield less beneficial effects than Miedzian 50 WP applied on its own. Throughout the entire experiment duration a disadvantageous influence of Rapol 90 EC admixture to Miedzian 50 WP was observed for the applied concentrations I and II; registered activity changes varied from –30% to –70% (Fig. 1D).

Great differences in β-glucosidase activity were noted after application of Miedzian 50 WP and Miedzian 50 WP used with Rapol 90 EC (Fig. 1E). From day 28 on, all the applied concentrations of the concerned fungicide-adjuvant combination affected the enzymes activity more detrimentally that application of Miedzian 50 WP alone. In particular, differences in activity reached nearly –80% after the highest dose application. In fact, when compared to Miedzian 50 WP, Miedzian 350 extra SC had less detrimental effect on β-glucosidase activity over the entire time span of the experiment solely for concentration III, whereas concentration I was less detrimental in the first half of it. Nevertheless, activity differences were small and reached merely –20% (Fig. 2E).

Over the entire experiment ATP content in the control soil remained stable at the level of 1 µg, with the exception of day 30 when its content reached 2.4 µg. On the first day of the experiment after application of both Miedzian 50 WP and Miedzian 350 extra SC, ATP content in the soil was similar to the one in the control soil (Fig. 3a and 3B). Also on days 60 and 90 after applying Miedzian 50 WP in concentrations I and II, ATP content was higher than in the control soil. After Miedzian 350 extra SC application in all concentrations ATP content in soil on day 60 was at the level of 1 µg, but on day 90 it was found to rise rapidly up to 5.7 µg ATP with the output dose. After applying all the concentrations of Polibutene emulsion a significant increase in ATP content was observed on the first day of the experiment, notably from 2.5 µg for concentration I to 5.3 µg after application of the highest dose (Fig. 3C). With time, ATP content in the soil with the concerned adjuvant tended to decrease to reach the value of 1-2 µg ATP on the day 90 of the experiment.

Fig. 3. Influence of Miedzian 50 WP (A), Miedzian 350 extra SC (B) and Polibutene emulsion (C) on ATP content in soil



On the basis of NIR values calculated with Tukey's test for a one-factor experimental setup, observed differences in the activity of the studied enzymes after application of fungicide without and with an adjuvant, were in most cases found significant. Insignificant differences occurred sporadically and usually were close to the limit values.

DISCUSSION

Human activities have been always accompanied by emission of compounds that naturally do not occur in the environment. Pesticides can be included into the group of such compounds. In literature on the subject numerous contradictory statements related to their influence on the biological activity of soil, and in particular on enzymatic activity and ATP content, are reported. Hence, it is quite likely to find papers on negative pesticides influence upon enzymatic activity of soil [5, 8, 17, 23], though research results claiming that no such influence occurs, or even that soil enzymes activity and ATP content are raised by chemical plants protection agents [13, 20]. Such a wide discrepancy of published results may result from pesticides multifunctionality as well as from diversity and numerous stages of the processes taking place in soil that are frequently overlapped [1, 10]. Usually undesirable interactions were observed after overdosed application of pesticides, significantly higher than recommended by the manufacturer. The results we obtained indicate great diversification in intensity of processes catalysed by the studied enzymes after chemical protection plant agents introduced into soil. Detrimental impact of adjuvants to pesticides admixture confirm the scarcely reported research in that field [14, 15]. Unfavourable influence of combined fungicides-adjuvants use was customarily intensified after the highest dose application, though they were also observed for standard field doses. With such a view to the subject, Malkomes's [10] suggestion to include research on adjuvant impact upon soil ecosystem into the canon of toxicological environmental surveys gains gravity and significance.

CONCLUSIONS

4. The biggest differences in soil enzymatic activities after application of fungicides combined with adjuvants were found for dehydrogenase; the studied hydrolases exhibited smaller respective differences.
5. Combined fungicides and adjuvants usage can significantly contribute to reducing activity of some soil enzymes, particularly when doses considerably exceed the recommended ones.

6. A substantiated necessity to monitor adjuvants influence on the natural environment has been identified and reported.
7. After longer deposition of preparation with adjuvant – Miedzian 350 extra SC in soil, caused increase in ATP content in soil, but increase of fungicide concentration caused decrease in ATP content.

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