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ACTIVITY OF PEROXIDASE AND CATALASE IN SOILS AS INFLUENCED BY SOME INSECTICIDES AND FUNGICIDES

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Abstract

In this study the levels of peroxidase and catalase activity in ecological soil treated with two insecticides and two fungicides over a 28 days period were evaluated. Peroxidases are extracellular enzymes with an important role in the oxidation of lignin and the removal of toxic substances from the environment. Catalase activity can be related to the metabolic activity of aerobic organisms and gives information about the soil fertility. The soil samples were analysed once every 7 days after the pesticides application in soil. Soil enzymes activities were measured using spectrophotometric methods. Overall, peroxidase and catalase activity was affected, positively or negatively, depending on the number of treatments applied.

Key words*: catalase, fungicides, insecticides, peroxidase.*

INTRODUCTION

Over the last 40 years, the rapid increase in population density and the rise of agricultural technology has led to an increasing release of xenobiotic compounds in the environment. Pesticides, including insecticides, fungicides and herbicides, have become an integrated part of modern farming systems. Pesticides are the main sources for applied xenobiotics, with an important role both in combating pests and diseases and in improving the quantity and quality of world production. However, by repetitive and excessive application of pesticides, they ultimately reach the soil, where qualitative and quantitative changes occur in biochemical processes driven by microorganisms (Dobre et al., 2016; Sharma and Ortiz, 2002). Microorganisms are the main source for soil enzymes, only a small fraction of the enzymes being derived from plants and/or animals. According to several authors, soil enzymatic activities are considered to be suitable indicators of soil quality due to their connection with nutrient cycles and biochemical (Buturuga et al., 2016) transformations and their influence on measuring the degree of soil degradation in both natural and agro-ecosystems. In addition, they can be measured easily, in comparison with other methods (Colombo et al., 2002; Nannipieri et al., 2002). One of the enzymes with an important role in soil fertility is catalase, which can be related to the metabolic activity of aerobic organisms (Shiyin et al., 2004; Trasar-Cepeda et al., 2007). Catalase (hydrogen peroxide oxidoreductase, EC 1.11.1.6) is an anti-oxidant enzyme that is responsible for the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen without causing free radicals. Catalase activity is very stable in soil and shows a significant correlation with the content of organic carbon decreasing with soil depth (Alef and Nannipieri, 1995).

Peroxidases (EC 1.11.1), as oxidative enzymes, use H_2O_2 as an electron acceptor in order to generate radical species, which then can act as catalysts for biological reactions (Passardi et al., 2007).Through this mode of action, peroxidases can be an important factor in lignin degradation, removal of hydrogen peroxide from the cell and oxidation of toxic substances (Erman and Vitello, 2002). Peroxidase activity in soil has been measured in a small number of studies. When present in soil environment, these enzymes mediate the biogeochemical processes of lignin degradation, carbon mineralization and

sequestration, and dissolved organic C export (Bach, 2013).
The aim of this study was to examine the influence of two types of insecticides and fungicides on the enzymatic activities of peroxidases and catalases in an ecological soil. The soil was subjected to multiple treatments over a 28 days period with recommended doses by the trader, at field application rate.

MATERIALS AND METHODS

Soil

This experimental study was conducted under greenhouse conditions and the soil was taken from the ecological department from Research Centre for the Quality Study of Food Products greenhouse. The soil used for the experiment was a mixture of soil with perlite and peat and was collected from the humus horizon (0-20 cm).

Pesticides

In order to determine their influence on soil enzymes activities, four types of pesticides were used: two fungicides and two insecticides, whose description is given in Table 1. All pesticides were purchased from Syngenta Romania.

Table 1. Chemical and biological classifications of pesticides

Biological classification	Common name	Chemical family
Fungicide	Ridomil Gold MZ 68WG (RG)	Fenilamide Dithiocarbamate
Fungicide	Bravo 500 SC (BV)	Organochlorine
Insecticide	Mospilan 20SG (MO)	Chlorothalonil
Insecticide	Vertimec 1.8% EC (VE)	Avermectin

Soils used in the present study

For this study, 1.5 kg of ecological soil was put in plastic pots of 10 cm diameter and 15 cm depth. 10 ml aqueous solution of the selected pesticides were applied at different time periods (days): 0, 7, 14 and 21.
For each treatment, soil samples from the 0-15 cm depth were with drawn at every time period and were subjected to different analyses. Soil not supplemented with pesticides was used as a control sample. Soil samples were air-dried at room temperature, cleaned by removing plant material and other debris, passed through 2 millimeter sieve and stored in sealed polyethylene bags at 4°C prior to analysis of enzymatic activities, in order to avoid the loss of moisture and inhibition of enzymatic reactions. In addition to enzymatic assays, other analyses included pH, conductivity, total dissolved solids (TDS), resistivity, dry substance and humidity. Soil pH was determined by using 1: 2.5 soil to water ratio pH meter, with glass electrode, pH range 0-14 ±1. For measuring soil moisture 1 g of soil was kept in the oven at 105°C for 3-4 hours, cooled in a desiccator and weighed. Drying continued for periods of one hour, followed by cooling in a desiccator and weighing until the sample reached the constant mass. The measurement was conducted in weighing bottles whose diameter was chosen so that the sample taken at work formed a layer of about 5 mm thick. The weighing bottle was brought to the constant mass under the same conditions as the determination (Scott and Maitre, 1988). Soil electrical conductivity (EC) is a measure of the amount of salts in soil and it is commonly expressed in units of milli Siemens per meter (mS/m) at 25°C. The electrical conductivity was determined using 20 g air-dried and passed through a 2 mm sieve soil sample mixed with 50 ml pure water (soil: water ratio - 1: 2.5). The samples were shaken for 4 hours in order to dissolve the soluble salts, filtered through Whatman No. 41 (11 cm) and measured with a platinum electrode (Rayment and Higginson, 1992).

Enzyme assays

Peroxidase activity was measured as the rate of substrate oxidation in the presence of added H_2O_2 (Burns et al., 2013). The measurement of peroxidase activity in soil was performed spectrophotometrically using pyrogallol (1, 2, 3-trihydroxybenzene) as substrate, in accordance with previous research (Floch et al., 2007; Riahi et al., 2007; Sinsabaugh et al., 2013). Sample suspensions were prepared by adding 0.1 g of fresh soil to 25 ml of 50 mMsodium acetate buffer, pH = 5. Suspension was homogenized with a Vortex Mixer (Ika Vortex 3) at high speed for 1 minute. Enzyme activities were measured by combining 1 mL of

soil suspension with 250 µL of substrate solution. Simultaneously were prepared: substrate + buffer, soil suspension + buffer, and buffer-used as blank. Each sample, including controls, received 5µL of 0.3% hydrogen peroxide. The samples were incubated in the dark at 20°C for 4 hours and determined spectrophotometrically at 460 nm. The results were calculated according to the method described by Bach (2013), and were expressed in µmol/g soil/h.

The catalase activity was measured by titrating residual H_2O_2 with $KMnO_4$ (Stępniewska et al., 2008; Roberge, 1978). One gram of soil sample was added to 5 mL distilled water with 1 mL of 3% hydrogen peroxide solution. The mixture was shaken and then 5 mL of 1.5 mol/L H_2SO_4 were added. After that, the solution was filtered and titrated using 0.05 mol/L $KMnO_4$. One enzyme unit was calculated as the amount of enzyme that catalysed the consumption of 1 µmol of H_2O_2 per g soil per hour (Vijayakumar et al., 2014). The evaluations of enzymatic activities were performed in triplicates, for better precision and reproducibility.

The results were processed using Microsoft Excel Tools. The relationship between pesticides influence on enzymatic activity and soil pH, humidity and conductivity was determined by calculating linear correlation coefficients.

RESULTS AND DISCUSSIONS

Regarding the enzyme tested and the applied xenobiotic dose, the effects on soil enzymes activities can be positive or negative. Enzymatic activity may be influenced by soil conditions such as pH, organic matter content, moisture, and temperature (Srinivasulu, 2014). The use of pesticides can provide various results, depending on the chemical structure of active ingredients, concentration of pesticides and the specific properties of soil (Jastrzębska, 2011).

As seen in Figure 1, the two insecticides (Mospilan 20 SG and Vertimec 1.8% EC) have a similar influence on soil catalase activity, exerting an increase of 58 %, respectively 74% in the first 14 days after which the catalase activity begins to decrease. At the same time a positive correlation can be observed between the influence of insecticides on catalase activity and pH (r = 0.94 for Mospilan 20 SG and r = 0.67 for Vertimec 1.8% EC).

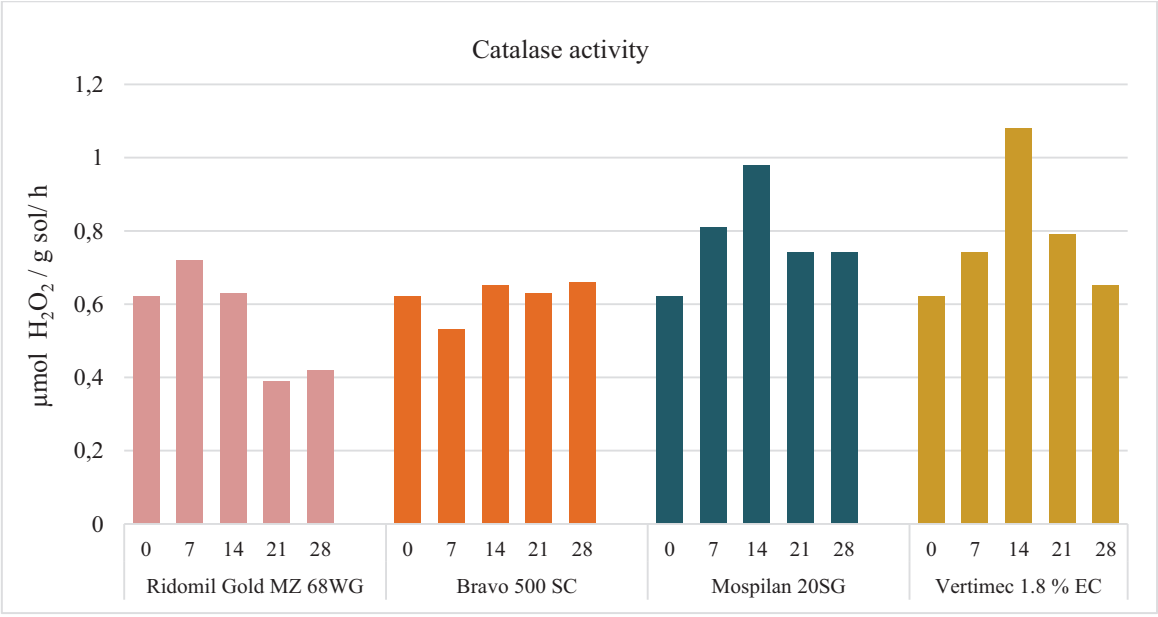

Figure 1. Effect of pesticides on the catalase activity in soil

In Table 2 a positive correlation can be observed comparing the catalase activity in soil samples and soil moisture (r = 0.86 for Ridomil Gold MZ 68WG, r = 0.85 for Bravo500 SC, r = 0.43 for Mospilan 20SG and r = 0.76 for Vertimec 1.8% EC). Borowik (2016) demonstrated higher enzymatic activity in soils with low moisture content (20% maximum

water capacity). No obvious influence on catalase activity after application of Bravo 500 SC fungicide at recommended doses was observed. In contrast, the use of fungicide Ridomil Gold lead to a decrease in catalase activity, starting at day 14, with approximately 70%. In previous studies, Shiyin et al. (2004) found that at different doses of pesticides soil catalase activities and their hydrolysates will recover from stimulative effect, and reach the level of the enzymatic activity of a blank soil sample after about 35 days. Peroxidase activity has positive association with the insecticides application in lower doses. Regarding the peroxidase activity, analysed after pesticides treatments for 28 days, no significant differences were obtained compared to control. A very significant correlation (r = 0.99) was observed at RG fungicide between the modification of peroxidase activity as a result of xenobiotic treatments and pH (Figure 2). A positive correlation can also be observed on the two insecticides applied. Baćmaga et al. (2016) concluded that the dose of pesticides recommended by the manufacturer generally stimulates enzyme activity. This stimulatory effect could be assigned to the adaptability of soil microorganisms, which can reduce the negative influence of chemical stressors under hostile conditions. These enzymes could also be protected by clay fractions or humic substances present in soil. The protective effect of those substances could reduce enzyme sensibility to pesticides (Baćmaga et al., 2012).

Table 2. Physico-chemical parameters of soil samples

Pesticides	Day	pH	Conductivity (mS/m)	TDS (ppm)	Resistivity ($E^{+3}\Omega \cdot cm$)	Humidity (%)
Fungicide Ridomil Gold MZ 68WG	0	7.1	0.35	174.2	2.87	13.8
	7	7.8	0.29	146.9	3.40	15.5
	14	8.1	0.18	88.8	5.63	14.6
	21	7.8	0.44	219.1	2.29	13.5
	28	7.8	0.23	116.0	4.31	12.4
Fungicide Bravo 500 SC	0	7.1	0.35	174.2	2.87	13.8
	7	7.9	0.23	116.8	4.27	12.2
	14	7.9	0.26	129.0	3.88	18.1
	21	8	0.25	125.8	3.98	16.3
	28	8	0.39	193.0	2.59	16.2
Insecticide Mospilan 20 SG	0	7.1	0.35	174.2	2.87	13.8
	7	7.6	0.45	225.7	2.22	14.4
	14	7.7	0.28	138.5	3.61	16
	21	7.4	0.26	129.5	3.86	15.7
	28	7.4	0.37	184.3	2.71	10.1
Insecticide Vertimec 1.8% EC	0	7.1	0.35	174.2	2.87	13.8
	7	7.3	0.50	248.1	2.02	16.7
	14	7.5	0.27	136.8	3.65	16.8
	21	7.2	0.28	137.9	3.63	15.3
	28	7.2	0.81	405.0	1.23	13.4

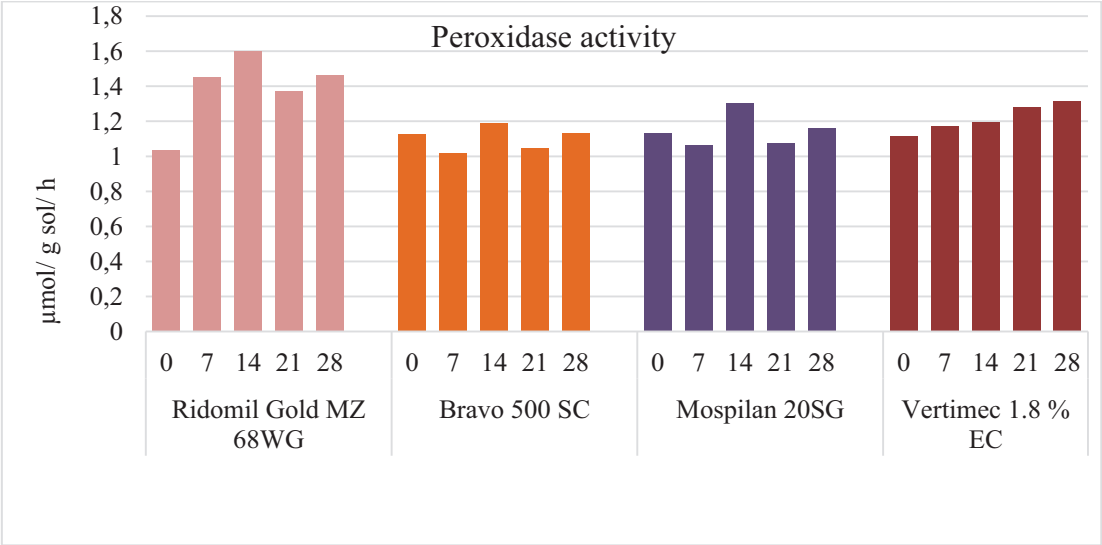

Figure 2. Effect of pesticides on the peroxidase activity in soil

CONCLUSIONS

The results obtained in the present study indicate that the two insecticides stimulate the catalase activity after the first two treatments. This increase can be related to the changes in soil pH. Instead, RG fungicide applications lead to a decrease of catalase activity.

Regarding peroxidase activity, results shown that were no significant differences compared to control after insecticide application. Compared to catalase activity, the presence of RG fungicide in soil lead to an increase in peroxidase activity.

There are a number of factors responsible for the various results obtained, such as chemical nature of pesticides, soil properties and biological function of soil. Even though pesticides applied in quantities recommended by the trader may cause slight changes in soil enzymatic activity, it is certain that long-term applications of pesticides may reduce soil fertility and enzymatic activity.

REFERENCES

Alef K., Nannipieri P., 1995. Catalase activity. In: Methods in Applied Soil Microbiology and Biochemistry. London: Academic Press, p. 362-363.

Bach C.E., Warnock D.D., Van Horn D.J., Weintraub M.N., 2013. Measuring phenol oxidase and peroxidase activities with pyrogallol.

Baćmaga M., Boros E., Kucharski J., Wyszkowska J., 2012. Enzymatic activity in soil contaminated with the Aurora 40 WG herbicide. Environ. Prot. Eng. 38 (1): p. 91-102.

Baćmaga M., Wyszkowska J., Kucharski J., 2016. The effect of the Falcon 460 EC fungicide on soil microbial communities, enzyme activities and plant growth. Ecotoxicology, 25: p. 1575-1587.

Borowik A., Wyszkowska J., 2016. Soil moisture as a factor affecting the microbiological and biochemical activity of soil. Vol. 62, 2016, No. 6: p. 250-255.

Burns R.G., DeForest J.L., Marxsen J., Sinsabaugh R.L., Stromberger M.E., Wallenstein M.D., Weintraub M.N., Zoppini A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216-234.

Buturugă M.D., Ştefanic G., Săndoiu D.I.,Bădulescu L., 2016. Ecological Methods of Pedo-Enzymatical Analysis for Soil Fertility Control. Romanian Biotechnological Letters, 21 (3), 11471.

Colombo C., Palumbo G., Sannino F., Gianfreda L., 2002. Chemical and biochemical indicators of managed agricultural soils. 17th World Congress of Soil Science Bangkok, Thailand 1740: p. 1-9.

Dobre A., Marin L.A., Manole C., Andrei N., Cornea C. P., 2016. E valuation of the capacity of different microorganisms to solubilize several compounds of phosphorous and zinc. Scientific Bulletin, Series F. Biotechnologies, Vol. XX, 2016.

Erman J.E., Vitello L.B., 2002. Yeast cytochrome c peroxidase: mechanistic studies via protein engineering. Biochimica et Biophysica Acta 1597: p. 193-220.

Floch C., Alarcon-Gutierrez E., Criquet S., 2007. ABTS assay of phenol oxidase activity in soil. J. Microbiol. Methods, 71, p. 319-324.

Jastrzębska E., 2011. The Effect of Chlorpyrifos and Teflubenzuron on the Enzymatic Activity of Soil. Polish J. of Environ. Stud. Vol. 20, No. 4, p. 903-910.

L-DOPA, and ABTS: Effect of assay conditions and soil type, Soil Biology & Biochemistry 67, p. 183-191.

Nannipieri P., Kandeler E., Ruggiero P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Enzymes in the environment.

Passardi F., Bakalovic N., Teixeira F.K., Margis-Pinheiro M., Penel C. and Dunand C., 2007. Prokaryotic origins of the non-animal peroxidase superfamily and organelle-mediated transmission to eukaryotes. Genomics 89: p. 567-579.

Rayment G.E., Higginson F.R., 1992. Australian Laboratory Handbook of Soil and Water Chemical Methods. Melbourne, Inkata Press. (Australian Soil and Land Survey Handbooks, Vol. 3).

Riahi S., Moghaddam A.B., Ganjali M.R., Norouzi P., 2007. Determination of the oxidation potentials of pyrogallol and some of its derivatives: theory and experiment. J. Theor. Comput. Chem. 6, p. 331-340.

Roberge M.R., 1978. Methodology of enzymes determination and extraction. In: Soil Enzymes Academic Press., p. 341-373.

Scott and Maitre, 1988. Interaction between vegetation and groundwater research priorities for South Africa. 730/1/98.

Sharma H.C., Ortiz R., 2002. Host plant resistance to insects: An eco-friendly approach for pest management and environment conservation. J. Environ Biol 23: p. 111-135.

Shiyin L., Lixiao N., Panying P., Cheng S., Liansheng W., 2004. Effects of pesticides and their hydrolysates on catalase activity in soil. Bulletin of Environmental Contamination and Toxicology, 72: p. 600-606.

Sinsabaugh R.L., Allison S.D., Donovan P.G., 2013. Measuring phenol oxidase and peroxidase activities with pyrogallol, L-DOPA, and ABTS: Effect of assay conditions and soil type. Soil Biology & Biochemistry 67 (2013), p. 183-191.

Srinivasulu M., Rangaswamy V., 2014. Enzymes and Pesticides, Enzymes in Agricultural Sciences. OMICS Group e Books, p. 1-10.

Stępniewska Z., Woli´nska A., Ziomek J., 2009. Response of soil catalase activity to chromium contamination. Journal of Environmental Sciences 21, p. 1142-1147.

Trasar-Cepeda C., Gil-Sotres F., Leiros M.C., 2007. Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. Soil Biology & Biochemistry, 39: p. 311-319.

Vijayakumar A.D., Subban M., Jmbulingam J., Annamalai P., Kalaichelvan P.T., 2014. A Rapid Sensitive Detection Method by Plate Assay for Catalase Activity from Bacterium *Acinetobacter calcoaceticus* AV6. RRJMB, Vol. 3.