EFFECTS OF DIFFERENT PESTICIDES ON THE ACTIVITY OF SOME ENZYMES IN THE RHIZOSPHERE OF BROWN FOREST SOIL

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Pesticides (herbicides, fungicides, zoocides, etc.) include all substances that reduce the harmful effects, or destroy pests that damage agricultural products or endanger humans in some way. Soil microbiotas produce countless enzymes, some of which can be induced or inhibited by certain chemical substances. Pesticides are made up of active ingredients, carriers and auxiliary substances. During their development, it is necessary to ensure that the active substance, which is the carrier of the biological activity, breaks down into biologically ineffective decomposition products after exerting the desired effect and does not have a harmful effect on the activity of the enzymes. In the present study, the pesticides used are: two herbicides (Ro-neet 6E, vernolat), two fungicides (fundazol 50 WP, Dithane M-45) and two zoocides (rogor L-40, phosphotion). Doses used during the study: (control; 1/2X, X (active ingredient: dose used in field) and 2X) of the active ingredient of each plant protection agent. After four weeks, the treated samples were analyzed in a controlled environment at 28°C and 45% soil moisture. The results showed the effect of different pesticides on the experiment, based on which it can be concluded that the order of toxicity was as follows: Dithane M-45 > vernolate > fundazol 50 WP > phosphotion > Ro-neet 6E > rogor L-40. The use of a double dose reduced the enzyme activity compared to the control. The use of the lowest concentration of pesticides showed a stimulating effect on the activity of the enzymes. Finally, it can be concluded that the used pesticides influenced the biological parameters of the examined brown forest soil in several ways, and these effects change the quality of the soil from different aspects. The plant protection agents used during the test must be chosen in such a way that they do not have a harmful effect on the biological functions of the soil. By analyzing the research, substances that have a positive or neutral effect on the biological functions of the soil can be used in agricultural practice.

***Keywords**: pesticides, activity of enzymes, rhizosphere, brown forest soil*

INTRODUCTION

Microbial properties like microbial diversity and biomass are useful for predicting changes in the soil functioning and for providing an integrated and relevant vision of soil quality. Little information is currently available for preventing the loss of microbial diversity and activity through the use of sustainable farming practices from the first cultivation year. Plants are able to select their own microbiome based on their specific root exudates or some other strategies such as root physical structure and mineral nutrients preference. The primary function of herbicides is to protect agricultural crops from infestation with weeds and to prevent arable soil from being overgrown by plant cover indigenous to the ecosystem. The chemicals known as pesticides are generally named synthetic organic compounds with broad molecular configurations having as a common property the ability of selectivity killing or inhibiting the growth of plants. All pesticides, including herbicides, play an important role in the environment by modifying the enzymatic activity of soils (Furczak and Gostkowska, [1], Nowak, [2] Strzelec, 3]).

Thus a higher enzyme activity indicates a greater intensity of mineralization of the organic matter (Benitez et al., [4], and Rossell et al., [5]). Soil microbiomes are very important for agroecosystem function and sustainability due to their contributions to nutrient cycling and to soil structure maintenance [6,7].
Bayoumi [8] studied the sensitivity of three strains of R. leguminosarum bv. viceae comparing with strains of R. leguminosarum bv. phaseoli, R. leguminosarum bv. trifolii, and R. loti to 8 herbicides. Among them, Paraquat was the most toxic one. Bayoumi et al. [9] reported that Acetochlor was the least inhibitor on tested Rhizobium strains. Bayoumi & Kecskés [10-13] in pot experiments established that microsymbionts were more sensitive to Trifluralin than macrosymbionts, whereas are sensitive to Paraquat, which reduced the size, number and dry weight of root-nodules, phytobiomass, height, and total N-content / plant. Eberbach & Douglas [14] found Paraquat and Trifluralin affected the nodulation potential of Rhizobium-legume.
Quantitative and representative recovery of microorganisms from environmental samples is essential in understanding ecosystem function. Microbial communities play essential roles in the earth's ecology. A powerful concept in modern biology is that of the ecosystem. Soil enzymes are present in important cycles, such as C (invertase), N (urease and protease), and P (phosphatase) cycles. Soil enzyme activities are used as indices of microbial activity and react quickly to environment change. Hydrolytic enzymes activities (phosphatase, β-glucosidase), oxidoreductase activities (dehydrogenase) and indole acetic acid production, were used as measures of soil perturbation. The hypothesis in this study is to estimate the effects of applied herbicides on some soil enzymes.

MATERIALS AND METHODS

Soil Samples

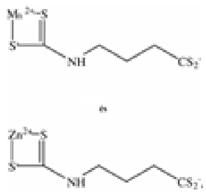
In this research work, the soil was collected from the garden of Rejto Sandor Faculty of Light industry and Environmental Engineering, Óbuda University. The garden is located to the west of the faculty building and the soil is Ramann's brown forest soil, which was not been exposed to any chemical or other agricultural treatments before. Using a garden shovel and plastic bag, around four kilograms of soil were taken from the topsoil, particularly from the depth of 0-25 cm. The collected soil was transferred to the laboratory and air dried for some weeks to remove excess moisture.
Plant residues, stones, and other debris or big particles were taken off by hand. A ≤ 2mm standard stainless-steel sieve was used for five minutes to homogenize the soil. Later, it was sent to Institute of Soil Science Centre for Agricultural Research, to measure some physicochemical characteristics of the soil. The results are shown in Table 1.

Table 1. Physicochemical characteristics of the tested soil

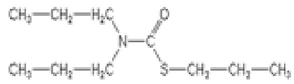
Tested parameters	Measurement units	Values
pH_{KCl}	-	7.13
EC	µS/cm	32.34
$CaCO_3$	m/m %	3.34
H	m/m %	1930
Total Nitrogen Content	mg/kg	18.76
NH_4-N	mg/kg	73.69
AL-P_2O_5	mg/kg	51.7
AL-K_2O	mg/kg	169
Al-Na	mg/kg	37.2
KCl-Mg	mg/kg	146
KCl-S	mg/kg	14.1

Pesticides used in the study

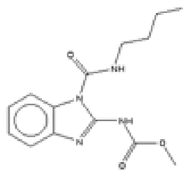
Six pesticides were applied to the brown forest soil of the wheat rhizosphere soil to investigate their side-effects on some biological caharacteristics of the tested soil. Pesticides were as mentioned below:



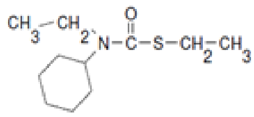
Dithane M-45
Mancozeb



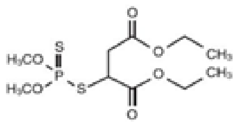
Vernolate



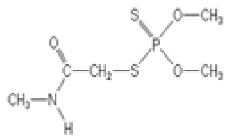
Fundazol 50 WP
Bromoxynil



Ro-neet 6E
Cycloate



Phosphotion
Malathion



Rogor L-40
Dimethoate

Figure 1. The pesticides used in the studa

According to the above plan, pesticides were each applied with three doses (1/2 X, X and 2X) doses as in Table 2 to the soil cultivated or non-cultivated (control) with wheat seeds (to study the effect of pesticides on the rhizospheric enzymatic activities) , and each cup was planted with seven wheat (common wheat: *Triticum aestivum* L.) seed. The seed was grown at 25±2°C for 28 days. The exact amount of water was given to the plants every two days to maintain the soil moisture at approximately 45%.

Table 2. The pesticides recommended applied rates

Pesticides	Field recommended applied dose (X)
Dithane M-45	1.2-1.5 l/ha
Vernolate	4 kg/ha
fundazol 50 WP	0.5-1 kg/ha
Ro-neet 6E	**4-6 l/ha**
Phosphotion	1.2-1.5 l/ha
Rogor L-40	**2 l/ha**

INVESTIGATION OF ENZYMATIC ACTIVITIES IN SOILS

β-glucosidase activity

The potential activity of β-glucosidase was calculated as following: Two ml of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of 50 mM *p*-nitrophenyl- **β** -D-glucopyranoside (PNG) were added to 0.5 g of soil sample. The rest of the method was the same as for phosphatase activity (Masciandaro, Garcia [15]). The PNP absorbance was measured at 400 nm. **β** -glucosidase activity is expressed as μmol of PNP per gram dry soil and incubation time (hours).

Phosphatase activity

The potential activity of phosphatase was done as following: Two ml of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of 0.115 M *p*-nitrophenyl phosphate (PNP) were added to 0.5 g of soil sample and incubated at 37°C for 90 min. The reaction was stopped by cooling to 2°C for 15 min., then 0.5 ml of 0.5 M CaCl2 and 2 ml of 0.5 M NaOH were added and the mixture was centrifuged to 4000 rpm for 5 min. The reaction product was filtrated and the filtrate (*p*-nitrophenol) was analyzed calorimetrically at 398 nm (Tabatabai and Bermner, [16]). Controls were made in the same way, although the substrate was added before the CaCl2 and NaOH. The PNP absorbance was measured at 398 nm. Phosphatase activity is expressed as □mol of PNP per gram dry soil and incubation time (hour).

Aryl-sulfatase activity

The potential activity of aryl-sulfatase (μmol nitrophenol/g dry soil/h) was determined according to Tabatabai & Bremner [17]). The activity was measured by absorption of p-phenol at 400 nm after incubation with PNP sulphate.

STATISTICAL ANALYSIS

As stated before, three duplicates in triplicate were used in the research work, which is placed in a randomized block design. To calculate the statistically significant differences between treatments, a correlation based on a single classification was implemented. The standard deviation in each value was calculated at $P < 0.05$.

RESULTS AND DISCUSSION

In vivo pot experiment which carried out randomly in three replicates using brown forest soil with 45% moisture content as well as cultivated with the seeds of wheat and after 28 days at 25±2℃ and the around 10-12 hours photoperiod, the following Figures from 1 to 6 show the effects of six pesticides on the three enzymatic activities (Phosphatase activity (expressed as μmol p-nitrofenol (PNP)/g dry soil/h, β-glucosidase activity (expressed as μmol p-nitrofenol/g dry soil/h), Aryl-sulphatase activity (expressed as μmol PNP/g dry soil/h) in the rhizosphere of wheat seedlings in comparison with non-cultivated soil as control. The pesticides applied doses were half and double recomeded rapplication rate of each pesticide in comparion with the reconneded dose for agricultural lands. The measurements of potential enzymatic activities of the mentioned enzymes were done using the spectrophotometrical method.

Figure 1 shows the effect of Cycloate on the three mentioned enzymes method. Comparing with the enzymatic activities of the three enzymes in non-cultivated soil, it was found that Cycloate can promote the three enzymes at half and normal recommended doses while the dould dose reduced the potential activities of the three enzymes but higher than the activites of the enzymes in control soils.

Figure 2 illustrates the differences between the potential enzymatic activivtes in rhizosphere of wheat seedlings and those in the non-cultivated soil samples. It wa found that Vernolate has similar effects as Cycloate did. Vernolate can promote rhe half and recommended doses but able to reduce the potential activities of the three enzymes also, it is clear that the reduction of the potential acivities of the enzymes not reach the harmful effect but similar relatively to the control samples.

Figure 3 domenestrates the stimulation effects of the half and double dodes of Benomyl on the three enzymes in similar way the above mentioned pesticides

Figure 4 represents the mode of action of the investigated pesticide Mancozeb on the potential acivities of phosphatase, aryl-suphatase and beta-glucosidase in cultivated and non-cultivated soil samples. It was found that Mancozeb also, carried out the similar mode of actions as done ba Cycloate, Vernolate and Benomyl but stimulate the potential activity of beta-glucosidase more than any other pesticids. Mancozeb is highly stimulating aryl-sulphatase even at double dose.

Figure 5 labells the clear stimulation of Dimethoate to the potential activity of aryl-sulphatase higher than the control even at double dose

Figure 6 shows the stimulation of Malathion onpotential activity of aryl-sulphatase even at double dose

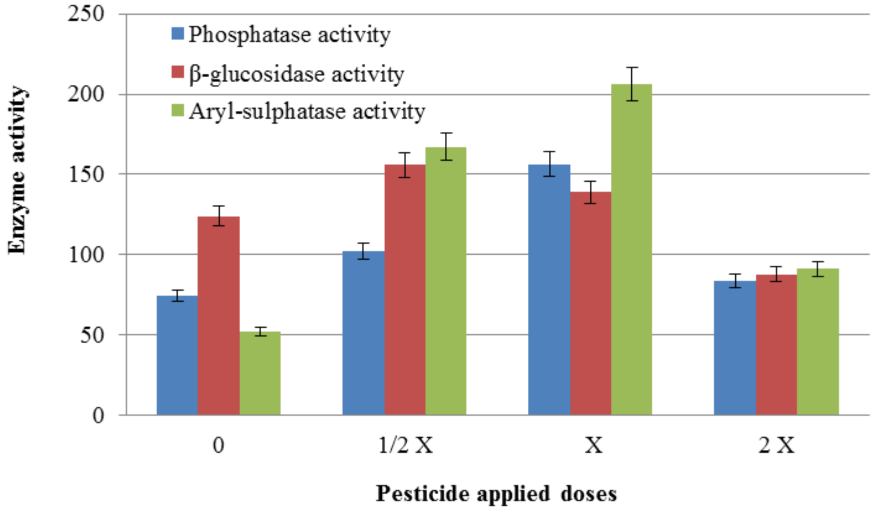


Figure 1. Effect of Cycloate on the enzymatic activies in brown forest soil

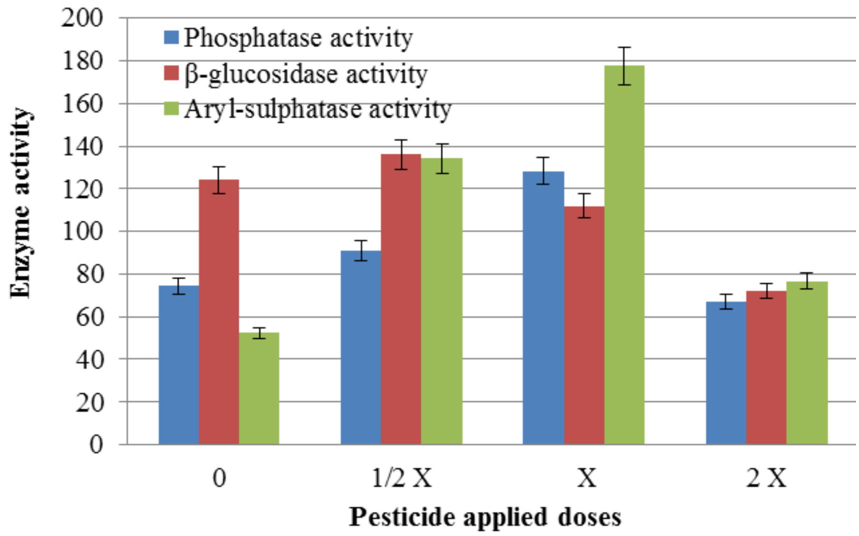


Figure 2. Effect of Vernolate on the enzymatic activies in brown forest

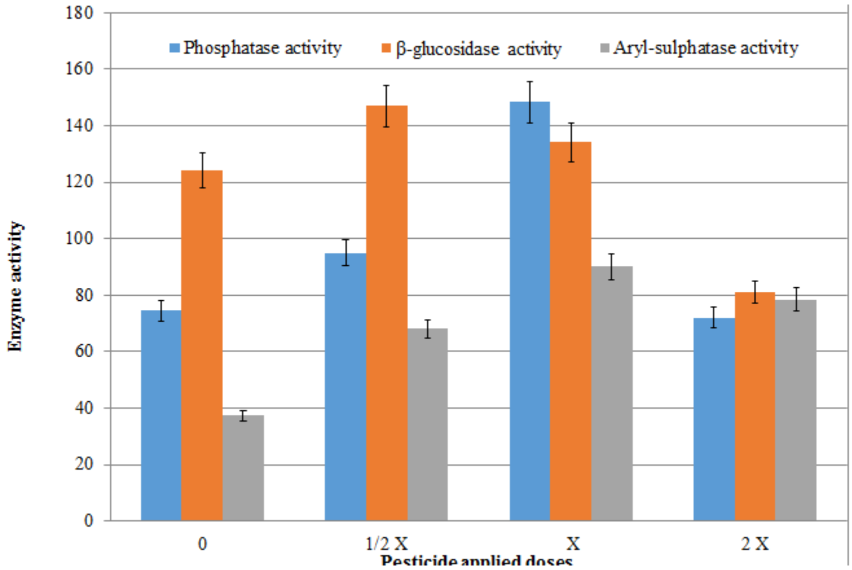


Figure 3. Effect of benomyl on the enzymatic activies in brown forest s

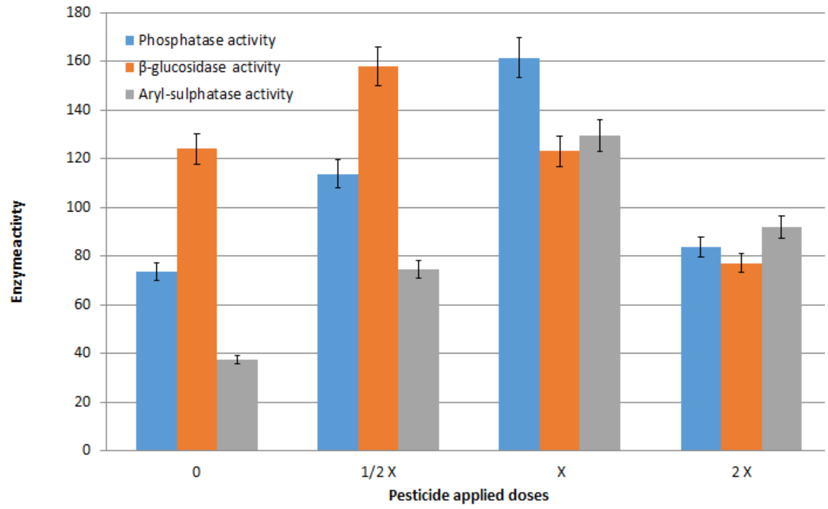


Figure 4. Effect of Mancozeb on the enzymatic activies in brown fore

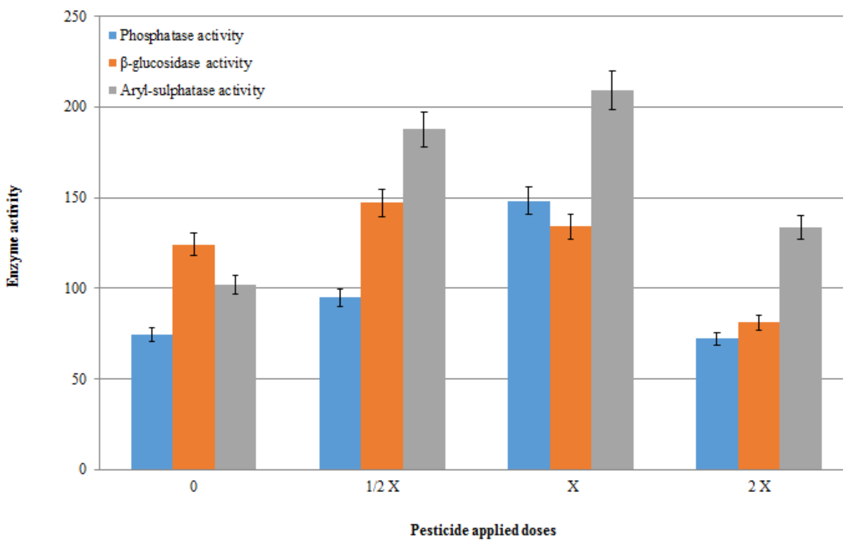


Figure 5. Effect of Dimethoate on the enzymatic activies in brown fore

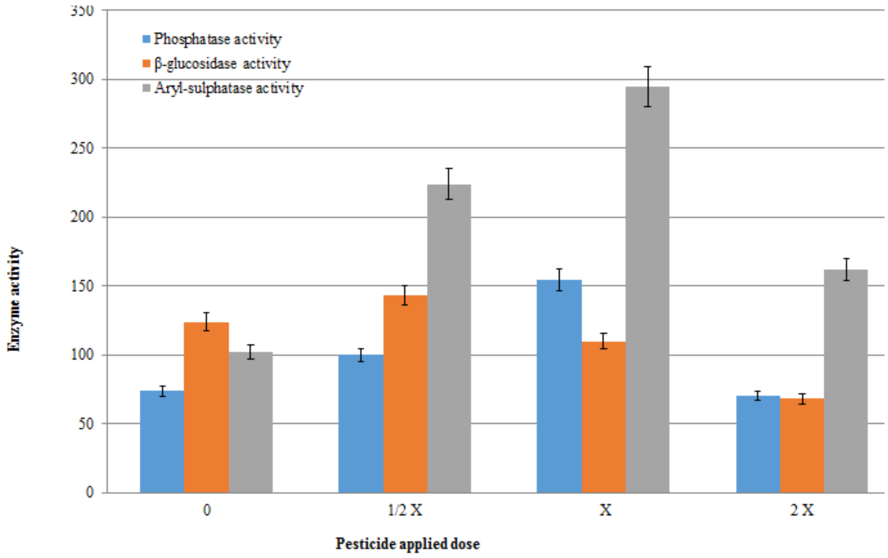


Figure6. Effect of Malathion on the enzymatic activies in brown forest s

CONCLUSION AND FURTHER TASKS

Pesticides are biologically active compounds, and an unintended consequence of its application may lead to significant changes in microbial populations and activities influencing microbial ecological balance affecting soil fertility. The fate of pesticides applied in agroecosystems is governed by the transfer and degradation processes, and their interaction with soil microorganisms. The increasing reliance of sustainable agriculture on pesticides has led to concern about their ecotoxicological effects influencing microbial populations and enzyme activities, which may serve as indicators of soil quality. In this study, the application of herbicides influenced the soil enzyme activity differently. From the present study it can be emphasized that the type of herbicide, its concentration and its time of exposure influence soil enzyme activities. Hence great care is required while applying herbicide to soil. The enzyme activities are considered to be sensitive to chemical pollutants/agrochemicals and have been proposed as potential indicators for measuring the degree of pollution of contaminated soil. The study confirmed that the pesticides may alter the affects the different soil enzymatic activities. Since the investigations were performed *in* vitro in pot experiment, and the effects of pesticides are highly transitory, it is particularly difficult to explain a change of soil enzymatic activities in response to certain factors or to establish the cause-effect relationships between the pesticide treatments and the various components contributing to the variation in overall soil enzyme activities. Among the applied pesticides, Mancozeb was the most toxic one. Further task, it is necessary to strengthen the scientific basis of modern agriculture, because herbicides may be advantageously used only if their persistence, bioaccumulation, and toxicity in agroecosystem are strictly controlled.

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