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ECOTOXIGOLOGICAL IMPACT OF SOME FUNGICIDES USED IN AGRICULTURE ON SOIL USEFUL MICROBIOTA AND ENZYMATIC ACTIVITIES IN VITRO

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***Abstract**: One of the main factors that affect the distribution and activity of microorganisms in the soil is the usage of practices. Nowadays, when the global population is increased and the agricultural land is decreased and also due to the climatic changes, humans increasingly applied the pesticides in agriculture to increase the crop production for humanity and controlling various plant diseases. As a result of these applications, the amount and number of pesticides used is increasing.in which are intensively polluting the agricultural soil environment. The effects of different fungicides on microbial content and enzymatic activity in the soil need to be studied, also taking into account sustainable agriculture and environmental protection aspects. The aim of the study in pot experiment was to evaluate the effects of four concentrations (control, ½X, X and 2X) of two fungicides (folpet, thiuram) on soil microbial populations such as aerobic heterotrophic bacteria, filamentous fungi, cellulose-decomposing microbes, phosphate-solubilizing microbes, soil respiration (CO_2 release) and four soil enzymatic activities of dehydrogenase, urease, phosphatase, β-glucosidase in brown forest soil, using pot experiment. The results indicated that the application of thiuram ha smore ecotoxicological effects on the microbial populations and enzymatic activities than folpet at the concentrations X and 2X. The soil respiration was increased by increasing the concentrations of fungicides. For further research, it is important to use other soil types and other pesticides, it is recommended to set up further experiments to get an even more complete and comprehensive picture of the soil biological affected by pesticides as soil pollutant agents.*

Keywords: Ecotoxigological impact; fungicides; agriculture soil; useful microbiota; enzymatic activities in vitro

INTRODUCTION

Misuse or overuse of pesticides degrades the soil and can damage the community of organisms living in the soil. Some pesticides are more toxic to soil organisms than others. Some pesticides degrade rapidly when applied to the soil, while others may persist for a longer period of time. Both soil and pesticide type can affect pesticide persistence. The contaminating effect of pesticides consists of the effect of plant protection products on non-target species. Pesticides are chemical preparations used to kill fungal or animal pests. More than 98% of sprayed insecticides and 95% of herbicides reach destinations other than their target species because they are sprayed or spread throughout the agricultural area. The runoff releases pesticides into the aquatic environment, while the wind can transport them to other fields, grazing areas, human settlements, and undeveloped areas that may affect other species. Other problems arise due to poor production, transportation and storage practices. Over time, repeated application will increase pest resistance, while its effects on other species may facilitate pest regeneration.

The assessment of the biocidal activity of the active substance of folpet demonstrates that it has a sufficient level of efficacy against the target organism(s) and the evaluation of the summary data provided in support of the efficacy of the accompanying product, establishes that the product may be expected to be efficacious. The behaviour of folpet in the aquatic environment was investigated in two dissimilar water/sediment systems (silty clay and sandy loam) in a study conducted at 20°C according to SETAC 1995/BBA Part IV, 5-1 guidelines. In each test, folpet was rapidly degraded in both the overlying water and the whole system.
Folpet was metabolised to CO_2 as a principal metabolite. Low recoveries were obtained at most sampling intervals, partly due to the loss of CO_2 during sample processing. Folpet is rapidly degraded in a range of aerobic soil types at temperatures of 20 and 25°C under laboratory conditions. The degradation of folpet under aerobic conditions at a lower temperature of 10°C was measured to be 3.8 days in a silt loam soil (corresponding value at 20°C was 0.8 days). The equivalent range of soil degradation rates at the EU average temperature of 12°C can be estimated to be 0.4 to 14.3 days. However, the data suggest that factors other than temperature (e.g. soil pH) have a significant influence on soil degradation. Levels of bound residues initially accumulated to a level of 31.2% AR at day 14, but subsequently declined resulting in substantial mineralisation to CO_2. Overall, folpet is not considered to be persistent in soil. Two studies with inocula from domestic catchment sewage treatment plants have tested the effects of folpet on microbial processes involved in aerobic biological waste-water treatment. The first investigated the effect of folpet on the rate of O_2 uptake (total respiration) by activated sludge and the second specifically addressed effects on nitrifying microorganisms, which are generally the most sensitive group. [1]
Thiram is an organic disulfide that results from the formal oxidative dimerisation of N,N-dimethyldithiocarbamic acid. It is widely used as a fungicidal seed treatment. It has a role as an antibacterial drug, an antiseptic drug and an antifungal agrochemical. In studies with ruminant animals fed corn treated with thiram, rumen microorganisms are degraded thiram to carbon disulfide (CS_2) and probably hydrogen sulfide (H_2S) and dimethylamine. Thiram is moderately toxic by ingestion, but it is highly toxic if inhaled.
Microbial degradation is an important step in the disappearance and, in most cases detoxification of pesticides. Many soil applied pesticides are degraded more rapidly following repeated application at the same site. These types of pesticides, adversely affect fungi and bacteria which may lead to the break down of food chains and consequently the disturbance of the natural balance. Some factors, like temperature and humidity influence pesticides effect on microorganisms. The inhibitory effect of pesticides on the growth of bacteria and actinomycetes was reported to be at high temperatures. Thiram degrades by microbial action or by hydrolysis more rapidly in acidic soils and in soils high in organic matter. In a humus sandy soil, at pH 3.5, Thiram decomposed after 4 to 5 weeks, while at pH 7.0, Thiram decomposed after 14 to 15 weeks. Thiram persisted for over two months in sandy soils, but disappeared within one week from a compost soil. The major metabolites of Thiram in the soil are copper dimethyl-dithiocarbamate, dithiocarbamate, dimethylamine and carbon disulfide. Minor degradative products included nitrite ions (nitrate reduction) and dimethylnitrosamine. It was reported more than 95% conversion of Thiram to metabolites by soil microorganisms after 55 days of incubation. The harmful effects of pesticides are generally studied for many communities and individuals in the ecosystem, but very few studies were devoted for investigating the effects of soil microorganisms on the degradation of pesticides in soil. [2] The objectives of the study was to investigate the ecotoxicological effects of the two fungicides on soil biological activities.

MATERIALS AND METHODS

The long-term, regular use of fungicides has a detrimental effect on the environment, flora and fauna, human health and, last but not least, reduces the sustainability of agricultural production. Nevertheless, there is an increase in the amount of fungicides used in several countries, while biological pest control methods are becoming less common. Table (1) shows some of the properties of the used fungicides. The experimental soil sample was collected from the garden of the Sándor Rejtő Faculty of Light Industry and Environmental Engineering of the University of Óbuda.
The sample used was obtained from the upper 0-25 cm of the soil layer. This soil is a slightly alkaline Ramann's brown forest soil that is not previously cultivated. Table (2) shows some of the physico-chemical properties of the studied soil sample.
After homogenization of soil samples, both fungicides concentrations were pipetted separately onto 200 g of soil surface and then homogenized for 30 min in a rotary mixer where soil samples treated with different concentrations (1/2 X, X and 2 X) of fungicide (Table 3) were added to each plastic pot. Untreated soil

served as a control. The pols were kept in a controlled environment, in the laboratory (LABOR 8, Institute of Environmental Engineering and Natural Sciences), at 25±3°C. 45% of the soil moisture remained.
The size of the microbial population was determined by the plate method. I used a spectrophotometer to determine the enzyme activity.

Table (1) Some properties of the pesticides used.

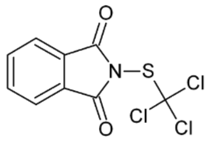
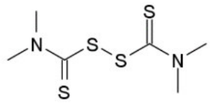
Active substance	Folpet	Thiuram
Active substance group	phthalimide	Thiocarbamates
Active substance	N-(trichloromethylthio)-phthalimide	bis-(dimethylthiocarbamate)-disulfide
Field application rate	>940 g/kg	3 kg/ha
Chemical structure		

Table (2) Some physicochemical characteristics of the soil

Tested parameters	Units	Values
pH_{KCL}		7.13
EC	µS/cm	483
$CaCO_3$	m/m %	32.34
H	m/m %	3.34
Total N content	mg/kg	1930
NH_4-N	mg/kg	18.76
NO_3-N	mg/kg	13.69
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

Table (3) Rate of field application of selected fungicides

Fungicides	Experimental field application rate		
	½X	X	2X
Thiuram	1,5 kg/ha	3 kg/ha	6 kg/ha
Folpet	250 g/kg	500 g/kg	1000 g/kg

The study was performed on soil microbial populations such as aerobic heterotrophic bacteria, filamentous fungi, cellulose-decomposing microbes, phosphate-solubilizing microbes, soil respiration (CO_2 reéease) and determination of soil enzymatic potencial activities such as dehydrogenase, urease, phosphatase, β-glucosidase.
Soil respiration was determined [3] by trapping the evolved CO_2 in NaOH and back titrated with HCl. Furthermore, the total mesophilic aerobic heterotrophic bacterial and fungal populations were counted according to Szegi [4]. Cellulose decomposers were detected according to Hendricks et al. [5] and phosphate solubilizers were determined according to Goldstein [6].
Enzymatic activities were determined for following: Dehydrogenase activity was measured by the method of García et al. [7]. Urease activity was determined according to the method of Nannipieri et al. [8]. Phosphatase activity was measured according to Tabatabai and Bremmer [9] and β-glucosidase activity according to Masciandaro et al. [10]
Experimental statistical analysis was compiled in a randomized block design in three parallel studies with three replicates. The mean and correlation were calculated as well as the regressiones between the treatments

RESULTS AND DISCUSSION

Pesticides are used worldwide for agricultural purposes to increase yields and mitigate losses. They can

destroy pests and insects. They can eradicate undesirable herbs and grasses between crop plants or in other areas. They have increased our reliance on them because they can improve agricultural production. Despite their beneficial effects, these pesticides also have enormous impacts on the soil, water, and other natural resources, leading to many environmental and animal health issues. [11].
The following Figures (from 1 to 9.) show the effect of the two fungicides on soil respiration, microbial populations and the potential activitities of some enzymes in the treated brown forest soil. Here, it is clear significant difference between the effect of 2X dose of fungicides and the control. The double dose significantly increased CO_2 emissions, resulting in toxic effects and the partial biodecomposition of both fungicides. Figure (1) shows the effect of the two fungicides on CO_2 emissions from brown forest soils

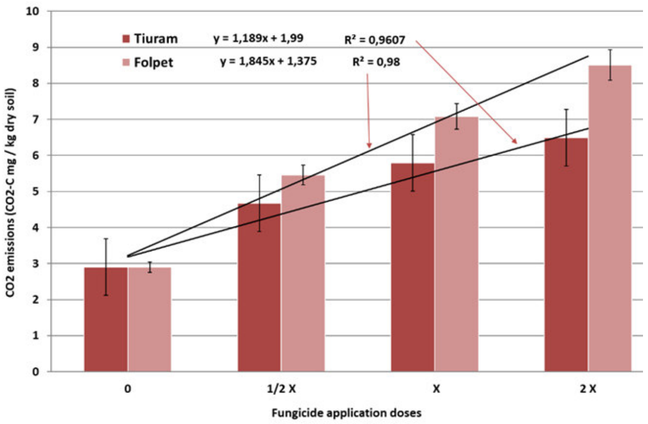


Figure (1)
Effect of fungicides on CO_2 emissions from brown forest soils

The applications of differenet doses such as control, ½X and X dose were not significant between the two fungicides regarding to the emissions of CO_2. However, in each case, the use of the double dose significantly reduced the number of bacteria as shown in Figure (2).

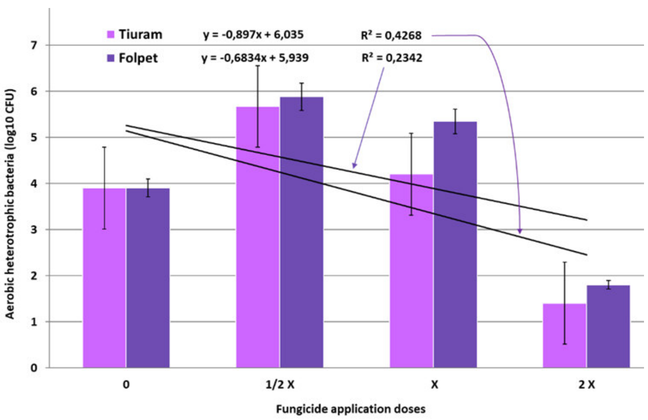


Figure (2)
Effect of fungicide on heterotroph aerobic bacteria in brown forest soil

A significant difference can be observed for Folpet, yet Thiuram can be said that it has a stronger ecotoxicőlogical effect of the fungicide on aerobic mesophil heterotrophic bacterial count. The use of double doses for both fungicides also has an ecotoxicological effect (Figure 2). Similar observations were recognized with the enumeration of filamentous fungi as in Figure (3).

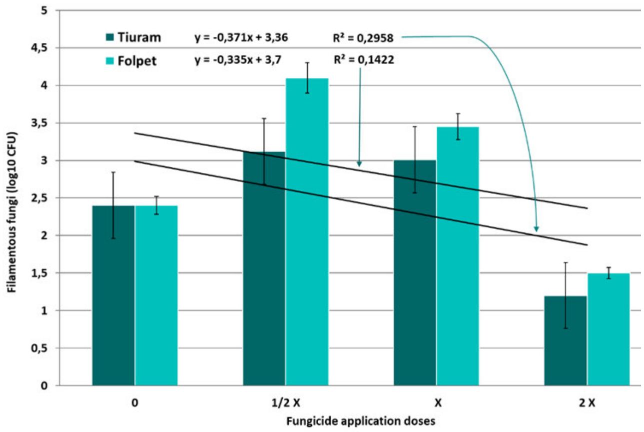


Figure (3)
Effects of fungicides on filamentous fungi in brown forest soil

Figure (4) illustrates that fungicides applied to the brown forest soil samples at doses ½X and X had a stimulating effect on cellulose-decomposing microbes, while 2X significantly reduced the number of microbes. Thus, it can be said that cellulose breakers also have an ecotoxicological effect with the use of all pesticides, as a significant difference is observed at double doses.

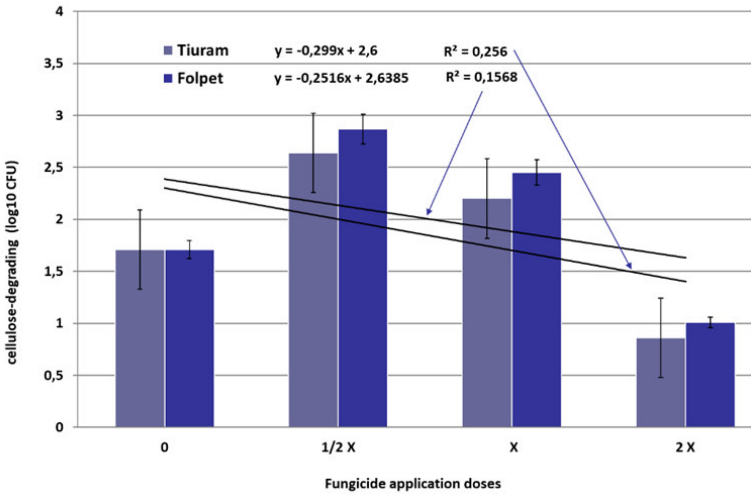


Figure (4)
Effect of fungicide on cellulose decomposers in brown forest soil

Figure (5) demonstrates the relationship between the applications of the fungicides on the phosphate solubilizers in the brown forest soil. I was found that as the Figure shows that the two fungicides had a similar effect compared with the microbial counts. Compared to the control soil sample, the phosphate solubilizers was increased in 2 cases (½X and X), and the double dose of each fungicide indicates a toxic effect.
Organic matter increase soil fertility in several ways and compost as an important source of organic matter has primary impacts on important soil properties. Based on the high nutrient mineralization potential and humus formation capacity, Fungicides application can distrib the nutrient supply and organic matter content of soils. Organic matter plays also an important role in decreasing heavy metal availability. These results have the main roles of soil biotas which have the ability to convert the organic matter to inorganic and in this case increase the soil fertility of the soil.

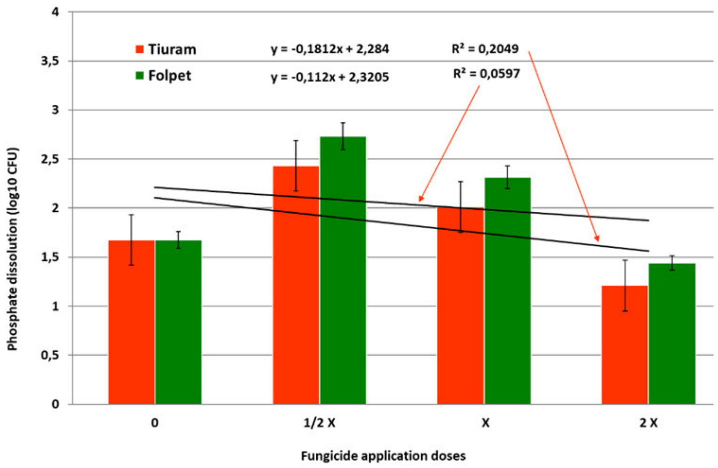


Figure (5)
Effect of fungicide on phosphate dissolution in brown forest soil

In the case of dehydrogenase activity as shown in Figure (6), the amount of potencial activity of the enzyme in the soil showed a significant increase in both fungicides under normal (X) and half dose (½X). However, the effect of the double dose was not significant for any of the two fungicides for this parameter, but the enzyme activity in the soil decreased to a certain level.

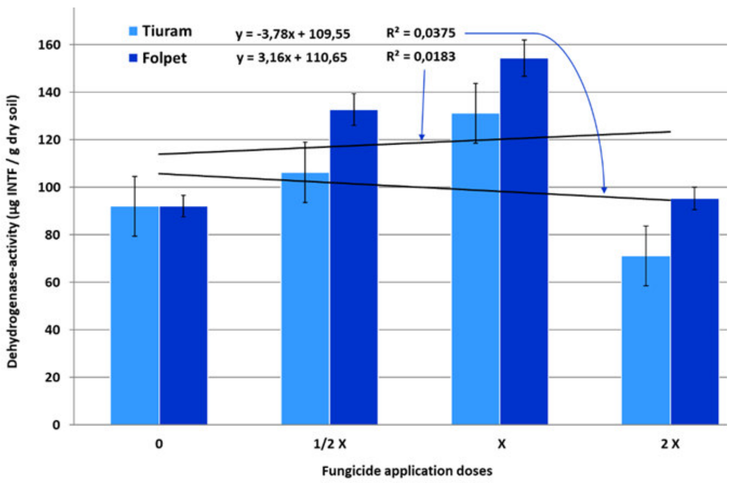


Figure (6)
Effect of fungicide on dehydrogenase activity in brown forest soil

Considering the control sample, the increase can be said to be significant for the half-dose (½X) and the normal applied dose (X) when examining urease as in Figure (7). At 2X doses of fungicides, the potencial activities were not significantly between them. But the effect of the application of the 2X dose of each fungicide to the brown forest soil is highly reduced the potencial enzyme activity compared to the control soil and other soil amended with low and normal applied doses.

Consequently, the soil receives the bulk of complex agrochemical compounds such as fungicides, several of which are poisonous to the activity of non-target beneficial soil microorganisms. Microorganisms in the soil are exclusively important because they impact soil structure, functions, and fertility. These organisms are primarily decomposers of organic matter, but also perform many other functions such as provide nitrogen, phosphorus, potassium, etc., through fixation and mineralization.

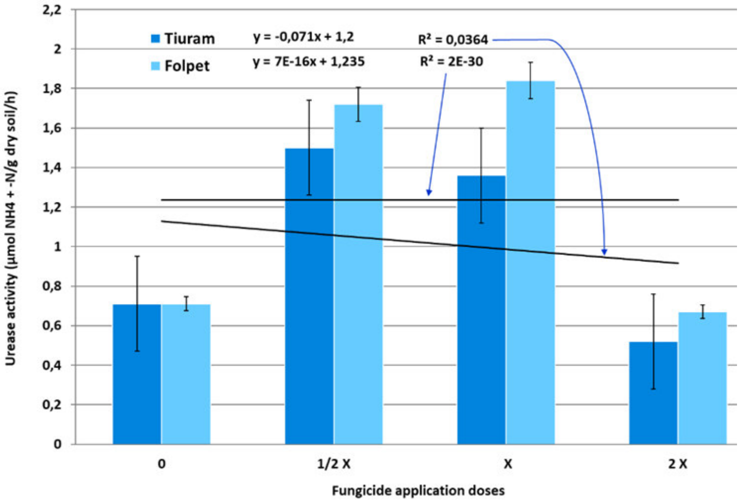


Figure (7)
Effect of fungicide on urease activity in brown forest soil

The nature of phosphatase potencial activity can be observed in Figure (8). There is a significant difference between the two and fungicides at the normal and double doses, but this is not significant compared to the control. Nevertheless, due to decreased enzyme activity, the use of 2X can be said to be toxic.

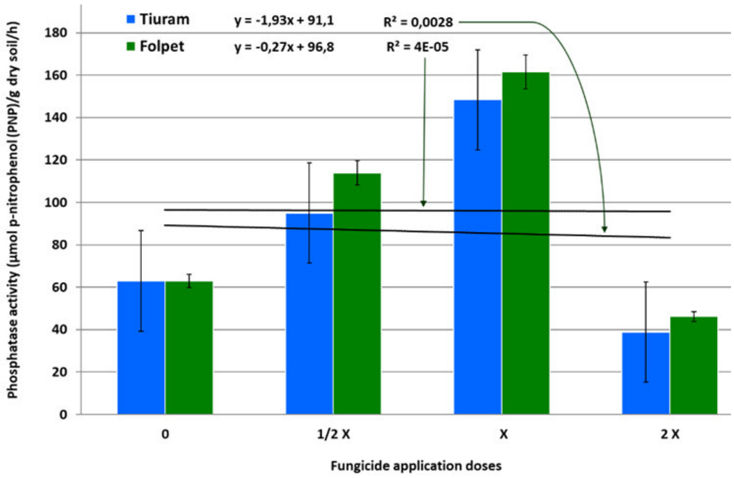


Figure (8)
Effect of fungicide on phosphatase activity in brown forest soil

In the following Figure (9), it can be observed that β-glucosidase potencial activity is much higher at normal applied doses of fungicides administered to the brown forest soil than the half applied doses. Thus, it can be said to be significant compared to the control soil sample. Double dosing also reduced soil activity in this enzyme activity, but was significant only in the case of Folpet.

There are no adequate test methods to evaluate the soil microbiological and biochemical effects of fungicides. This is due on the one hand to the diversity of fungicides and soil types and on the other hand to the relatively short history of fungicides. The results for urease and β-glucosidase show the process of carbon and nitrogen cycles. The test procedures presented above were not originally developed in this area, but are widely used to evaluate the microbiological and biochemical effects of fungicides on soil.

The use of other soil types and other fungicides is important for further research, it is recommended to set up further experiments to get an even more complete and comprehensive picture of the soil biological effects of fungicides as soil contaminants. It is suggested that further investigation of the mechanism of action of various fungicides on non-target microorganisms at the level of molecular genetics.

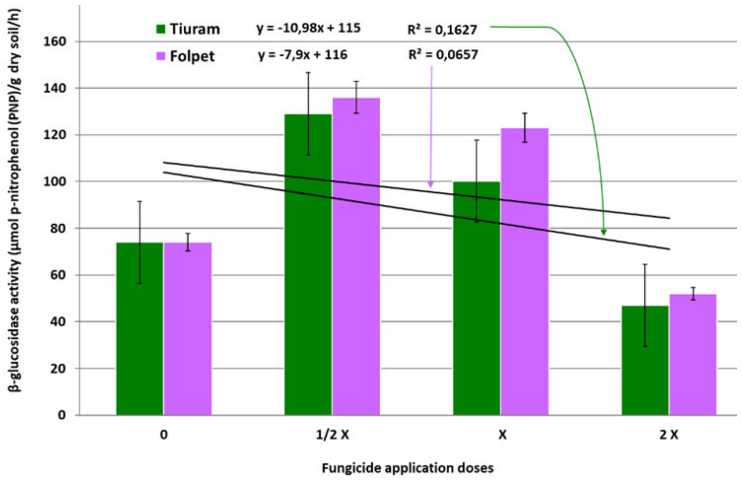


Figure (9)
Effect of fungicide on β-glucosidase activity in brown forest soil

Most copper based fungicides have a deleterious effect on the population of N-fixing bacteria. Fungicidal residues, for example, apron, arrest, captan, tend to remain in soil reacting with living organisms and affecting the N-fixation in legume-Rhizobium association [12]. Both mancozeb and chlorothalonil can decrease the process of nitrification and denitrification at an incubation period of 48 h [13]. The negative impact of the long-term application of organomercurial Verdean on cellulolytic fungal species has also been reported [14]. Several studies have reported the harmful effects on soil microbial growth, survival, and activity [15]. Fungicide bavistin has an inhibitory effect on several soil microbial populations, but the impact is non-significant [16]. Several biochemical processes in soil are closely linked with enzymatic activities which are adversely affected by residues and toxic elements left after application of fungicides [17]. Fungicides benomyl, mancozeb, and tridemorph inhibit the soil enzymatic activity of dehydrogenase, urease, and phosphatase [18]. Activities of phosphomonoesterase and urease enzymes are also inhibited in soils treated with captan, trifloxystrobin, and thiram fungicides [19],[20]. Yet, captan and thiram are classified as soil and seed protectant fungicides, respectively. However, the fungicide ridomil has a non-significant impact on the activity of a phosphatase enzyme [21]. These enzymes may be protected from degradation by adsorption on clays or humic substances in soil [22],[23]. The smaller the size of the clay particle, the greater is the protection against the added fungicides [24]. The synthesis of amino acids of certain bacteria is repressed by some glucopyranosyl antibiotic fungicides [25],[26]. The use of Cuin combination with mefenoxam can disturb soil microbial diversity as determined by structural and metabolic profiling. The population of ammonium oxidizing bacteria is decreased by the application of mefenoxam and mefenoxamp Cu fungicides after 60 days of application [27].

CONCLUSION

There are no adequate test methods to evaluate the soil microbiological and biochemical effects of fungicides. This is due to the diversity of fungicides and soil types on the one hand, and the relatively short history of fungicides on the other. The results for urease and β-glucosidase show the process of carbon and nitrogen cycles. The test procedures presented above were not originally developed for this field, but are widely used to evaluate the microbiological and biochemical effects of fungicides on soil.
In general, the concentrations of the two pesticides I studied (Folpet and Thiuram) had an increasing or decreasing effect on both soil respiration and soil microbiological populations and enzyme activities for all treated soil samples. The pesticides to be used in the study should be selected so as not to adversely affect the biological functions of the soil. Thus, by analyzing research, agents that have a positive or neutral effect on soil biological functions can be used in agriculture. The use of other soil types and other fungicides is important for further research, it is recommended to set up further experiments to get an even more complete and comprehensive picture of the soil biological effects of fungicides as soil contaminants. It is suggested that further investigation of the mechanism of action of various fungicides on non-target microorganisms at the level of molecular genetics.

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