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Influence of fungicides, carbendazim and metalaxyl on soil enzymatic activity and microbial population under laboratory conditions

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ABSTRACT

Laboratory experiment was conducted to investigate the impact of different dosages of two fungicides, Carbendazim and Metalaxyl on the activity of soil enzymes dehydrogenase, urease, acid invertase and rhodanese as well as total counting of bacteria and fungi in sandy clay loam soil. The fungicides were applied at 1, 5 and 10 mg ai kg^{-1} dry soil. The soil sample was submitted to analysis at 0, 7, 14, 28, 35, 42, 49, 56 and 63 days after the fungicides treatments. The investigations showed severe inhibitory effect of these fungicides on dehydrogenase activity. While the activity of urease enzyme was fluctuated form decrease to increase during all the periods of the experiment. Application of all doses of tested fungicides significantly decreased the activity of acid invertase enzyme once added to soil and the inhibition was reversible over time at low doses of the fungicides particularly Carbendazim. Carbendazim and Metalaxyl at 1 mg kg^{-1} significantly enhanced the rhodanese activity once added to soil and this effect continued until the end of the experiment with percentages of activation 11.23% and 9.66%, respectively. All dosages of Carbendazim and Metalaxyl significantly decreased the total number of fungi throughout all the experimental periods except the low dose 1mg kg^{-1} of Carbendazim which gave some increase at zero, 35 days and 42 days after treatment. As mean of all periods of the experiment, Metalaxyl ranked the first in decreasing the total number of fungi. The effects of the Carbendazim and Metalaxyl at low doses were converted since these doses gave a highly significant increase in the total number of bacteria throughout all the incubation periods of the experiment. As a collective mean of all incubation periods of the experiment, Metalaxyl and Carbendazim at 1 mg kg^{-1} gave 107.72% and 47.28% increase of total number of soil bacteria, respectively.

Keywords: *Carbendazim; Metalaxyl; Soil enzymes activity; Dehydogenase; Urease; Invertase; Rhodanese ; Microorganisms population.*

INTRODUCTION

Soil is a complex environment, where microorganisms are an important biological component of the soil ecosystem and play vital roles in soil fertility and quality through their roles in nutrient cycling and organic matter decomposition, thereby, plant productivity (Truu *et al.*, 2008 and Kaschuk, *et al.*, 2010).

Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system. They are important in catalyzing several important reactions necessary for the life processes of micro-organisms in soils and the stabilisation of soil structure, the decomposition of organic wastes, organic matter formation, nutrient cycling, soil fertility and plant productivity (Bohme *et al.*, 2005; Fließbach *et al.*, 2007; Makoi and Ndakidemi, 2008 and Xiaoqiang *et al.*, 2008). Moreover, an analysis of soil enzymatic activity is one of microbiological indicators of soil quality (Winding *et al.*, 2005). Enzymatic activities as caused by soil microbial activities were sensitive indicators to detect changes occurring in soils (Rahmansyah *et al.*, 2009). Soil contains many types of enzymes with different functions.

Dehydrogenase enzyme is known to oxidize soil organic matter and its activity reflects the total oxidative activity of the microbial biomass (Tripathi *et al.*, 2007). Since these processes are part of respiration pathways of soil microorganisms, studies on the activities of dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility. Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil, as well as a direct measure of soil microbial activity (Monkiedje *et al.*, 2002 and Cycoń *et al.*, 2005).

Urease (urea amido hydrolase), is responsible for the hydrolysis of urea fertilizer applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH, a process considered vital in the regulation of N supply to plants after urea fertilization. (Samborska *et al.*, 2004 and Makoi and Ndakidemi, 2008). So, information on the nature of urease activity in soil was beneficial to develop and employ strategies for nitrogen management.

Invertase is also of particular importance due to their role in the soil phosphorus and carbon cycles (Wang *et al.*, 2009). Soil invertase (saccharase) involves the transformation of carbohydrates in soil, and hydrolyzes sucrose into glucose and fructose, which can be used by plant and soil microorganism (Makoi, and Ndakidemi 2008 and Li *et al.*, 2010).

Thiosulfate sulfurtransferases referred to rhodanese catalyze the transformation of $S_2O_3^{-2}$ and CN to SCN and SO_3^{-2} (Lettl, 1987 and Cipollone *et al.*, 2004) and also it is related to the microbial oxidation of S to SO_4^{-2} (Ray *et al.*, 1984). Crop plants generally use SO_4^{-2} as S source. So that, it is desirable that elemental –S or $S_2O_3^{-2}$, which is added as fertilizer be rapidly oxidized to SO_4^{-2}. So, rhodanese involved in S plant nutrition and S mobilization in agricultural soils (Kertesz and Mirleau, 2004 and Saidu, 2004).

A lot of research works have been conducted for assessing the dissipation of fungicides in soil and their effects on soil microorganisms and these studies reported conflicting results (Smith *et al.*, 2000; Monkiedje and Spiteller 2002; Sigler and Turco, 2002; Kinney *et al.*, 2005; Cycon *et al.*, 2006; Bending *et al.*, 2007; Xiaoqiang *et al.*, 2008; Aurelia, 2009 and Yunlong *et al.*, 2009). Fungicides might affect microorganisms by reducing their numbers, biochemical activity, diversity and changing the microbial community structure (Martinez-Toledo *et al.* 1998; Smith *et al.* 2000, Chen *et al.* 2001, Cycoń and Kaczyńska 2004 and Cycoń and Piotrowska-Seget, 2007).

Carbendazim [methyl 2-benzimidazole carbamate] is a systemic benzimidazole fungicide and used to control a broad range of diseases on arable crops (e.g., cereals, oil seed rape), fruits, vegetables, ornamentals and medicinal herbs. It is also a main metabolic product of some other systemic fungicides, such as benomyl and thiophanate methyl. The behavior of Carbendazim in the soil environment and its effect on soil microbial activities have been well investigated (Burrows and Edwards, 2004; Sousa *et al.*, 2004; Xiuguo *et al.*, 2009 and Yunlong *et al.*, 2009).

Metalaxyl [N-(2, 6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester] is a systemic acylanilide fungicide. It is effective against Oomycetes, especially *Peronosporales* such as *Phytophthora spp., Pseudoperonospora spp., Peronospora spp., Sclerospora spp., Bremia spp., Pythium spp.*, and other spp. causing downy mildews, late blight, damping off, and root, stem

and fruit rots in many crops (Sukul, 2006). Numerous studies have documented microbiological and enzymatic activities changes in soil ecosystem as a result of Metalaxyl application (Sukul and Spiteller, 2001; Monkiedje *et al.,* 2002; Demenaou *et al.,* 2004; Jastrzębska and Kucharski, 2007).

The present investigation has been set to study the effect of Carbendazim and Metalaxyl at different concentrations on soil enzymatic activities taking dehydrogenase, urease, acid invertase and rhodanese under consideration. In addition, effect of these fungicides on total soil microbial population.

MATERIALS AND METHODS

Soil treatments: Laboratory studies performed on soil samples collected from the farm of Faculty of Agriculture Alex University in Abbis region (0 - 20 cm). Soil physical and chemical characters were described in Table 1.

Table: 1. Chemical and physical characters of the used soil.

Soil parameters	Value
EC (dSm^{-1})	8.52
pH(1:2.5) $meqL^{-1}$	8.43
HCO_3^- $meqL^{-1}$	20.4
Cl^- $meqL^{-1}$	29
Ca^{++} $meqL^{-1}$	40
Mg^{++} $meqL^{-1}$	30
K^+ $meqL^{-1}$	2.95
Na^+ $meqL^{-1}$	41.3
$CaCO_3$%	8.6
Total N%	0.14
Available N mg kg^{-1}	406
NH_4^+ mg kg^{-1}	168
NO_3^- mg kg^{-1}	234
Available P mg kg^{-1}	70.3
Available K mg kg^{-1}	480
Soil Text.:	
Sandy Clay Loam	
Sand%	60.63
Silt%	13.12
Clay%	26.25

The technical grades of Carbendazim (98%) and Metalaxyl (96%) were obtained from Syngenta Co. The calculated volumes of the application

solution which give rates of 1, 5 and 10 mg kg^{-1} dry soil were dispensed on to portions of 10 gm air-dried soil in a porcelain dish. The treated subsample of soil was thoroughly mixed manually until complete evaporation of the acetone solvent. The subsample was subsequently added to the total soil mass, 100 gm. This was followed by the adjustment of the moisture content of the soil to 60% of the maximum holding capacity, to allow optimal conditions for activity of aerobic soil microorganisms to occur. The soil was stored in tight polyethylene containers at optimal temperature of 25°C in the dark. Soil to which no investigated compounds were added served as a reference sample (control). Each treatment including the control was performed in four replicates. Soil water content was maintained by addition of water at 2 days intervals. Soil samples were taken from all treatments after 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 incubation days. Samples were stored at 4 °C until use for enzymes analysis and microbial count.

Soil enzymes activities determination: Dehydrogenase activity was determined using the reduction of 2,3,5- triphenyltetrazolium chloride (TTC) according to Tabatabai, 1994. A sample of 1 gm soil and 10 mg $CaCO_3$ were mixed thoroughly. 1 ml of 3% TTC and 2.5 ml of distilled water were added. The samples were mixed on a vortex and incubated at 37°C. After 24 hours the triphenylformanzan (TPF), a product from the reduction of TTC, was extracted by adding 10 ml methanol and shaken for 1 min. The color intensity of the filtrate was measured at 485 nm with methanol as a blank and the enzyme activity expressed as nmole TPF gm dry soil/ hour.

Urease measurement adapted from Kandeler *et al.*, (1999) method. One gram of soil sample was incubated with 2.5 ml of phosphate buffer (pH 6.7), 2.5 ml of 1% urea solution, and 125 µl of toluene were added. After incubation at 37°C for 24 hours the contents were centrifuged, and an aliquot 1 ml from the supernatant was treated with 1.5 KCl (1 N). The contents were left aside for 10 min. and then 0.5 ml of this extract was added to one ml of *p*-dimethylamino benzaldehyde reagent and this mixture was diluted to total volume 10 ml by distilled water. Absorbance of developed color was measured against a reagent blank at 420 nm, and compared with the standard curve of urea which was carried out using standard urea solution. The data of enzyme activity was calculated as mmole urea gm^{-1} dry soil per hour.

Invertase activity was determined according to Srinivasulu and Rangaswamy (2006). 5 gm of soil sample was transferred to 100 ml Erlenmeyer flasks, 1 ml of toluene and 6 ml of 0.2 M acetate phosphate buffer (pH 5.5) containing 18 mM sucrose were added to the soil samples and the flasks were closed with cotton plugs and held for 24 h at 37°C. Soil extracts were passed through Whatman No.1 filter paper and glucose in the filtrate was assayed (Nelson, 1994). The enzyme activity expressed as mg glucose gm^{-1} dry soil per hour.

Rhodanese activity: The method described by Tabatabai and Singh (1976) is based on calorimetric determination of the SCN produced by rhodanese activity when Soil sample (2 gm) was incubated with 4 ml of tris (hydroxymethyl) aminoethane (THAM) buffer, 0.5 ml of 0.1 M $Na_2S_2O_3$, 0.25 ml of toluene and 0.5 ml 0. 1 M. KCN were added, at 37°C. After one hour, 2.5 ml. of $CaSO_4$-formaldehyde solution was added. The SCN-produced was extracted by filtration with adding to 0.5 ml. of the ferric nitrate reagent. The formed of reddish brown color from the Fe-SCN complex was measured with a spectrophotometer at 460 nm. The data were recorded as µ mole SCN gm^{-1} dry soil per hour.

Total count of fungi and bacteria: The numbers of colony forming units (CFU) in the selective media were determined by means of the serial dilution technique and the spread plate method (Carvalhal *et al.*, 1991). Analyses were performed in four replicates. The determination of total number of bacteria was determined using nutrient agar (3 gm beef extract, 5 gm peptone, 15 gm agar per liter). The inoculated plates were incubated at 28°C for 2 days, before the colonies were counted. Viable counts for fungi were performed using a Rose Bengal-Streptomycin agar containing (per liter): 10 gm glucose; 5 gm peptone; 1 gm K_2HPO_4; 0.5 gm $MgSO_4.7H_2O$; 0.033 gm Rose Bengal; 15 gm agar. Streptomycin was added after autoclaving at final concentration of 30 mg ml^{-1}. The plates were incubated at 22°C and colonies were counted after 4 days (Pepper *et al.* 1995).

Statistical analysis: Effect of fungicides doses and period of exposure on tested parameter and interactions between these parameters were treated statistically by two -way ANOVA. The statistical significance (P <0.05) of differences were assessed. Comparison of means was done using least significant differences ($LSD_{0.05}$) by Student–Newman–Keuls (SNK) test, Cohort Software Inc. (1986).

RESULTS AND DISCUSSION

In literature on the effects of pesticides in general and particularly fungicides, numerous contradictory statements related to their influence on the biological activity of soil, and in particular on enzymatic activity are reported. Hence, it is quite likely to find papers on negative pesticides influence upon enzymatic activity of soil (Sousa *et al.*, 2004; Sukul, 2006 and Xiuguo *et al.*, 2009), though research results claiming that no such influence occurs, or even that soil enzymes activity and microbial population are raised by chemical plants protection agents (Monkiedje *et al.* 2002; Srinivasulu and Rangaswamy, 2006 and Cycon *et al.*, 2006). 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 (Kłódka and Nowak, 2004). 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 enzymes activities and microbial population after Carbendazim and Metalaxyl introduced into soil.

Soil enzymes activities:

Dehydrogenase activity: Sixty three days following the application of Carbendazim and Metalaxyl, the activity of dehydrogenase in all cases of treated samples was significantly less than that of the untreated samples (control), indicating a severe inhibitory effect of these fungicides on dehydrogenase activity (Table 2). This inhibition remained irreversible at all sampling periods except in the treatments of 7 and 28 days after application of low doses of the fungicides and followed a dose-response pattern, with higher doses exhibiting a more severe inhibitory effect on the activity of the enzyme. Similar effects was observed with respect to Carbendazim which induced a negative effect on dehydrogenase activity (Burrows and Edwards, 2004; Sousa *et al.*, 2004 and Xiuguo *et al.*, 2009).Metalaxyl had the most inhibitory effect on the activity of this enzyme either with high or low dose since 10 mg kg^{-1} of it gave a value of 0.098 ηmole TPF/gm dry soil/ h compared to 0.267 ηmole TPF/gm dry soil/ h for the control treatment with a percentage of 63.3% decrease. Also, our results in this point are in agreement with Monkiedje and Spiteller 2002; 2005 and Sukul, 2006, they reported higher doses of Metalaxyl exhibiting a more severe inhibitory effect on the activity of dehydrogenase enzyme.

Urease activity: The activity of this enzyme was fluctuated form decrease to increase during all the periods of the experiment (Table 3). Urease was strongly active after three weeks incubation in soil with all the doses of the two used fungicides, this result was similar to the results of Rahmansyah *et al.*, 2009. The final result as a mean of all the periods, Carbendazim at all its doses was significantly decreased the activity of urease activity and the inhibition did not follow the dose response pattern. Also Metalaxyl but only at 5 and 10 mg kg^{-1} followed the same trend. Concerning to the effect of the time on the activity of this enzyme, it was found that the activity was significantly decreased at all the periods of the experiment, treatments of 56 days and 63 days after fungicides application took the first places in this effect. Uyanoz *et al.*, 2005 and Sukul, 2006 showed the same effects particularly with Metalaxyl.

Acid invertase activity: Invertase enzyme activity was expressed as the amount of glucose formed from the substrate, sucrose in soils incubated for different period till to 63 days after application of the selected fungicides. Results on enzymatic activity are depicted in Table 4. Generally, the application of all doses of tested fungicides significantly decreased the activity of the enzyme once added to soil and the inhibition was reversible over time at low doses of the fungicides particularly in Carbendazim. The inhibition of Metalaxyl at its high dose was remained irreversible during all sampling periods.

Table 2: Effect of the fungicides Carbendazim and Metalaxyl on the activity of soil enzyme dehydrogenase (ηmole TPF/gm dry soil/ h.) at different time intervals.

Treatments	Dose mg/kg	Days after treatments										
		0	7	14	21	28	35	42	49	54	63	Mean
Control		0.568	0.318	0.410	0.289	0.284	0.240	0.127	0.135	0.157	0.148	0.267^{j}
Carbendazim	1	0.565	0.328	0.219	0.264	0.346	0.225	0.102	0.144	0.075	0.082	0.235^{e}
	5	0.446	0.187	0.091	0.132	0.245	0.096	0.087	0.110	0.064	0.061	0.152^{d}
	10	0.429	0.122	0.023	0.130	0.174	0.028	0.065	0.080	0.045	0.038	0.113^{b}
Metalaxyl	1	0.487	0.600	0.187	0.236	0.451	0.138	0.123	0.100	0.051	0.050	0.242^{e}
	5	0.317	0.199	0.139	0.069	0.300	0.082	0.107	0.096	0.040	0.046	0.14^{c}
	10	0.218	0.153	0.072	0.053	0.237	0.047	0.075	0.057	0.037	0.034	0.098^{a}
Mean		0.433^{g}	0.272^{e}	0.163^{d}	0.168^{d}	0.291^{f}	0.122^{c}	0.097^{b}	0.103^{b}	0.067^{a}	0.065^{a}	

All values are means of four replicates samples, means of treatments and periods followed by the same letter are not significantly different according to LSD ($P < 0.05$) two way ANOVA (SNK) test.

Table 3: Effect of the fungicides Carbendazim and Metalaxyl on the activity of soil enzyme urease (mmole urea /gm dry soil /hr.) at different time intervals.

Treatments	Dose mg/kg	Days after treatments										
		0	7	14	21	28	35	42	49	54	63	Mean
Control		49.48	34.32	33.65	28.93	24.85	32.18	34.78	28.18	24.86	24.66	31.59^{d}
Carbendazim	1	46.92	32.30	26.44	33.16	28.92	24.85	28.86	24.78	24.93	24.76	29.59^{b}
	5	34.54	35.53	32.53	38.85	24.87	24.90	24.78	33.05	24.92	24.85	29.88^{b}
	10	28.41	24.86	32.13	41.42	31.88	31.30	30.98	32.55	24.94	31.89	31.04^{c}
Metalaxyl	1	38.14	30.13	34.07	46.05	31.90	24.85	32.63	33.51	24.84	24.84	32.09^{e}
	5	33.61	26.44	28.34	31.09	31.88	24.84	32.54	24.90	24.87	24.87	28.34^{a}
	10	33.60	28.90	28.58	36.55	24.86	28.21	29.12	24.88	24.82	24.85	28.44^{a}
Mean		37.82^{g}	30.35^{e}	30.82^{e}	36.58^{f}	28.45^{d}	27.30^{c}	30.54^{e}	28.83^{d}	24.88^{a}	25.82^{b}	

All values are means of four replicates samples, means of treatments and periods followed by the same letter are not significantly different according to LSD ($P < 0.05$) two way ANOVA (SNK) test.

Table 4: Effect of the fungicides Carbendazim and Metalaxyl on the activity of soil enzyme acid invertase (mg glucose/gm dry soil/ h.) at different time intervals.

Treatments	Dose mg/kg	Days after treatments 0	7	14	21	28	35	42	49	54	63	Mean
Control		0.65	0.62	0.52	0.51	0.72	0.69	0.54	0.46	0.48	0.47	0.57^{d}
Carbendazim	1	0.57	0.88	0.79	0.85	0.93	1.03	0.73	0.59	0.51	0.29	0.72^{e}
	5	0.35	0.70	0.57	0.76	0.71	0.96	0.63	0.50	0.42	0.24	0.58^{d}
	10	0.33	0.44	0.40	0.68	0.63	0.92	0.62	0.28	0.28	0.17	0.48^{c}
Metalaxyl	1	0.62	0.57	1.17	0.66	0.98	0.46	0.48	0.29	0.20	0.41	0.58^{d}
	5	0.56	0.36	0.75	0.54	0.92	0.14	0.40	0.18	0.19	0.32	0.43^{b}
	10	0.42	0.34	0.43	0.43	0.64	0.12	0.28	0.15	0.18	0.30	0.33^{a}
Mean		0.50^{c}	0.56^{e}	0.66^{G}	0.63^{f}	0.79^{h}	0.62^{f}	0.53^{d}	0.35^{b}	0.32^{a}	0.32^{A}	

All values are means of four replicates samples, means of treatments and periods followed by the same letter are not significantly different according to LSD ($P < 0.05$) two way ANOVA (SNK) test.

Table 5: Effect of the fungicides Carbendazim and Metalaxyl on the activity of soil enzyme rhodanese (μ mole SCN/gm dry soil / hour) at different time intervals.

Treatments	Dose mg/kg	Days after treatments 0	7	14	21	28	35	42	49	54	63	Mean
Control		0.47	0.47	0.41	0.45	0.50	0.44	0.55	0.50	0.47	0.18	0.44^{e}
Carbendazim	1	0.53	0.63	0.50	0.50	0.56	0.48	0.62	0.59	0.36	0.18	0.49^{f}
	5	0.53	0.52	0.46	0.48	0.33	0.32	0.41	0.43	0.13	0.13	0.38^{d}
	10	0.35	0.38	0.33	0.44	0.32	0.32	0.33	0.40	0.13	0.11	0.31^{b}
Metalaxyl	1	0.51	0.50	0.59	0.49	0.68	0.47	0.59	0.54	0.40	0.11	0.49^{f}
	5	0.43	0.36	0.53	0.41	0.51	0.34	0.35	0.28	0.14	0.10	0.35^{c}
	10	0.43	0.32	0.51	0.36	0.33	0.28	0.31	0.27	0.10	0.07	0.29^{a}
Mean		0.46^{ef}	0.45^{e}	0.48^{G}	0.45^{e}	0.46^{ef}	0.38^{e}	0.45^{e}	0.43^{d}	0.25^{b}	0.12^{a}	

All values are means of four replicates samples, means of treatments and periods followed by the same letter are not significantly different according to LSD ($P < 0.05$) two way ANOVA (SNK) test.

Comparison of the mean values of fungicides treatments with control treatments revealed that there was no significant statistical difference between 5 mg kg^{-1} of Carbendazim, 1 mg kg^{-1} of metalaxyl and control treatment in relation to invertase activity. But interestingly, Carbendazim at its low dose 1mg kg^{-1}, showed a significant stimulatory effect on the enzyme activity, with 26.72% increase compared to control treatment. Similar results on the activation of invertase activity were obtained with other fungicides as captan; tridemorph and copper-based fungicides (Srinivasulu and Rangaswamy, 2006; Wang *et al.*, 2009). In soil samples receiving 1.0 – 10 mg $kg^{-1}$of the fungicides, the accumulation of reducing sugar was pronounced more at 7, 14, 21, 28, 35 and 42 days, and the activity of the invertase was drastically decreased with increasing period of incubation up to 49, 54 and 63 days without a significant difference between the last two periods.

Rhodanese activity: This enzyme belongs to the family of transferases. Data of Table 5 showed the effects of the three doses of Carbendazim and Metalaxyl on the activity of this enzyme. It was clear that Carbendazim and Metalaxyl at 1 mg kg^{-1} significantly enhanced the rhodanese activity once added to soil and this effect continued until the end of the experiment with percentages of activation 11.23% and 9.66%, respectively. The effect of doses 5 and 10 mg kg^{-1} of the tested fungicides oscillated between the increase and decrease at intervals of experiment. There is severe shortage of recent research associated with the impact of pesticides in general and fungicides in particular on the enzyme activity of rhodanese in soil.

Total count of microorganism: The all used dosages of Carbendazim and Metalaxyl significantly decreased the total number of fungi throughout all the experimental periods except the low dose 1mg kg^{-1} of Carbendazim which gave 14.33%, 18.46% and 28.21% increase at zero, 35 days and 42 days after treatment, respectively (Table 6). Treatments of 56 days and 42 days after fungicides application were significantly the same and take one order lower in decreasing the total counting of fungi as compared to the control treatment. As mean of all periods of the experiment, Metalaxyl ranked the first in decreasing the total number of fungi with percentages of 67.24%, 95.51% and 100% decrease for its three doses in comparison to the control treatment. Analysis of variance showed significant effect of all the treatments of the used fungicides and exposure time on the total number of fungi (2-way ANOVA, $P < 0.05$). The interactions between these factors were also found. Similarly, decrease in the total number of soil fungi

in response to fungicides application observed in some studies (Abdel-Fattah *et al.*, 1982 and Rahmansyah *et al.*, 2009). To the best of our knowledge, the effect of the pesticides, particularly fungicides on fungi as soil microorganism did not take the same attention which the soil bacteria took it.

Concerning to the effect of the used fungicides on the total number of bacteria (Table 7), we found that the high dose 10 mg kg^{-1} of the two fungicides appeared the same trend as in fungi since it significantly decreased the total number of bacteria particularly in Carbendazim which gave as a mean of all periods of the experiment a percentage of 68.18% decrease compared to the control treatment. Surprisingly the effects of the Carbendazim at 1mg kg^{-1} and Metalaxyl at 1 and 5 mg kg^{-1} were converted since these doses gave a highly significant increase in the total number of bacteria throughout all the incubation periods of the experiment. As a collective mean of all the incubation periods of the experiment, Metalaxyl at 1mg kg^{-1} ranked first since it gave 107.72% increase of total number of soil bacteria compared to control treatment, while Carbendazim with 1 mg kg^{-1} gave 47.28% increase and take second place. Another opposite observation, the total number of bacteria was on a rise with the time and reached to the maximum at the period of 35 and 49 days after treatment with the fungicides.

Table 6: Effect of the fungicides Carbendazim and Metalaxyl on the total count of soil Fungi (X 10^4) at different time intervals.

Treatments	Dose mg/kg	Days after treatments										
		0	7	14	21	28	35	42	49	54	63	Mean
Control		2.56	2.47	3.37	2.93	4.00	2.16	1.67	2.48	1.67	2.67	2.59^{d}
Carbendazim	1	2.93	1.77	1.96	1.11	3.33	2.56	2.14	2.22	1.33	1.67	2.10^{c}
	5	2.30	0.81	1.29	0.00	2.00	1.00	0.00	1.00	0.00	0.33	0.87^{b}
	10	0.00	0.00	1.02	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.24^{a}
Metalaxyl	1	1.53	2.14	1.70	1.85	1.29	0.00	0.00	0.00	0.00	0.00	0.85^{b}
	5	0.00	0.00	0.00	0.00	1.17	0.00	0.00	0.00	0.00	0.00	0.12^{a}
	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00^{a}
Mean		1.33^{c}	1.03^{bc}	1.33^{c}	0.84^{ab}	1.87^{d}	0.82^{ab}	0.54^{a}	0.82^{ab}	0.43^{a}	0.67^{a}	

All values are means of four replicates samples, means of treatments and periods followed by the same letter are not significantly different according to LSD ($P < 0.05$) two way ANOVA (SNK) test.

Table 7: Effect of the fungicides Carbendazim and Metalaxyl on the total count of soil bacteria (X 10^6) at different time intervals.

Treatments	Dose mg/kg	Days after treatments										
		0	7	14	21	28	35	42	49	54	63	Mean
Control		17.46	12.84	10.81	10.40	24.67	12.55	11.00	20.68	7.00	4.33	13.17^{d}
Carbendazim	1	18.70	24.76	21.90	23.08	34.00	12.67	11.24	25.00	11.67	11.0	19.40^{e}
	5	16.54	21.82	11.21	11.00	20.67	1.43	6.00	10.11	2.33	3.67	10.48^{c}
	10	1.86	10.00	1.04	8.89	8.00	1.00	2.14	5.33	1.00	2.57	4.19^{a}
Metalaxyl	1	11.13	19.54	21.92	37.90	30.83	59.24	20.33	47.62	19.00	6.13	27.37^{f}
	5	5.04	15.35	19.96	24.17	12.86	40.00	17.01	35.00	7.67	2.33	17.94^{e}
	10	0.00	11.01	14.44	14.73	2.33	26.00	3.04	4.33	2.33	0.67	7.89^{b}
Mean		10.10^{c}	16.48^{d}	14.47^{d}	18.59^{e}	19.05^{e}	21.84^{F}	10.11^{c}	21.15^{f}	7.29^{b}	4.39^{A}	

All values are means of four replicates samples, means of treatments and periods followed by the same letter are not significantly different according to LSD ($P < 0.05$) two way ANOVA (SNK) test.

The inhibitory effect of the fungicide Carbendazim was noticed and it was dose dependent (Burrows and Edwards, 2004; Sukul, 2006; Aurelia, 2009 and Xiuguo *et al.*, 2009). Monkiedje *et al.* 2002 and Cycoń and Piotrowska-Seget, 2007 found that the growth of microorganisms was stimulated by addition of the fungicide Metalaxyl. The phenomenon of increase of soil bacteria number could be expected as a result of utilization of applied pesticides as a source of carbon, energy and others nutrient elements by some soil microorganisms (Cycoń and Piotrowska-Seget, 2007).

It could be seen from this study that normal doses of the used fungicides not only has no effects on soil enzymes and microorganisms but also had an enhanced effect on these parameters of soil. The inhibitory effect increased with the concentration of the fungicides so, avoiding overdose is useful. This study was conducted in the laboratory and included a single application of fungicides without plants. Therefore, it may be unrealistic to extrapolate laboratory results to the natural environment. So, further studies must be carried out in the field, including plants and different soil types may provide additional informations.

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تأثير المبيدات الفطريه الكاربندازيم والميتالاكسيل على نشاط بعض إنزيمات التربه وعلى التعداد الميكروبى تحت الظروف المعمليه.

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تمت دراسه معمليه لتقدير تأثير إثنان من المبيدات الفطريه (كاربندازيم و ميتالاكسيل) بتركيزات مختلفه (1 – 5 – 10 مليجرام / كجم تربه) على نشاط بعض إنزيمات التربه وهى (الديهيدروجيناز – اليورياز – الانفرتاز – الرهودانيز) بالإضافه الى تقدير التأثير على التعداد الميكروبى للفطريات والبكتريا الموجوده فى التربه وذلك بعد المعامله بالمبيدات بـ (0, 7, 14, 21, 35, 42, 49, 65, 63 يوم) , وقد أظهر تحليل التربه انها (تربه طينيه رمليه لوميه).

وكان من اهم النتائج:

1- حدوث انخفاض حاد فى نشاط انزيم الديهيدروجيناز نتيجه المعامله بالمبيدان بالتركيزان 5 و 10 مليجرام/ كجم تربه .
2- نشاط انزيم اليورياز كان يتأرجح بين الإنخفاض والزياده وذلك خلال فتره التحضين مع المبيدات المختبره.
3- كل التركيزات المستخدمه من المبيدات المختبره أحدثت خفض معنوى لنشاط انزيم الإنفرتاز وذلك فى بدايه التجربه وهذا الخفض كان عكسى وإستعاد الانزيم نشاطه بعد ذلك وذلك مع أقل تركيز من الكاربندازيم .
4- كلا المبيدان بتركيز 1 مليجرام / كجم تربه أحدثا زياده معنويه فى نشاط انزيم الرهودانيز, إستمرت هذه الزياده حتى نهايه التجربه وقد أحدث الكاربندازيم زياده قدرها 11.23 % بينما أحدث الميتالاكسيل زياده قدرها 9.66 %.
5- حدث انخفاض معنوى للتعداد الكلى للفطريات وذلك عند معامله التربه بالمبيدان بإستثناء المعامله بأقل تركيز من الكاربندازيم والذى أحدث زياده فى بدايه التجربه وكذلك بعد 35 و 42 يوم من المعامله . وكمتوسط عام للتجربه فإن الميتالاكسيل كان له التأثير الاقوى فى خفض التعداد الكلى للفطريات . أما عن التعداد العام للبكتريا فإن المبيدان الفطريان بالتركيزات المنخفضه أحدثا زياده معنويه وذلك خلال فتره التجربه وعموما فإن الميتالاكسيل اعطى 107.72 % زياده بينما اعطى الكاربندازيم 47.28 % زياده فى التعداد العام للبكتريا.