

Impacts of three soil applied pesticides on soil enzymes activity and soil respiration under controlled conditions

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Abstract

The experiment was conducted in a laboratory to investigate the impact of soil applied pesticides belonging to three different types; herbicides (Metribuzin), fungicide (Carbendazim) and insecticide (imidacloprid) on the activity of enzymes in soil, alkaline phosphatase, Urease, dehydrogenase and Rhodanese as well as soil respiration in two types of soil, silty and silty loam. The pesticides were applied in 0, 0.1, 10, and 100 mg kg⁻¹ dry soil. The measurable microbiological processes in soil were estimated after 0, 1, 2, 7.14, 21, 45.60 and 120 days from the application of pesticides. The study showed that based on the enzyme activity and soil respiration in the estimated time after treatment with pesticides. It was clear that after the application of pesticides to increase the rate of activity for 24 hours and then drop to the lowest level after 48 hours. Highest values of the activity of processes studied were recorded in 14 days of soil treatment with pesticides. Curb these operations again after 45 days and return to normal activity with the passage of time after 120 days. Herbicides, metribuzin was an highest inhibitor for enzymes activity and soil respiration, followed by insecticide, Imidacloprid and less impact by Fungicide, carbendazim was noticed depending on the concentration of pesticides. Phosphatase was more sensitive to pesticides, and the less sensitive in the two types of soil was urease. Biological processes studied were more sensitive to pesticides in the silty soil than the silty loam one.

Keywords: Pesticides; Soil enzymes activity; Soil Respiration.

Introduction

It is well known that soil is an open but self-regulating ecosystem with a large variety of microbial populations (Kizilkaya et al., 2004). The living dynamic nature is one of the important features for soil quality and often used as a bio-indicator for soil health (Gianfreda et al., 2005; Sukul, 2006). Soil enzymes, that represent the major living organism activity, are involved in catalyzing various reactions necessary for organic matter metabolism, nutrient cycling, energy

transfer and crop productivity (Kizilkaya et al., 2004; Truu et al., 2008; 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, soil enzymatic activity and respiration can be utilized as indicator for changes in soil quality (Winding et al., 2005; Yao et al., 2006; Hua et al., 2009; Rahmansyah et al., 2009). Several important soil enzymes in matter cycle were selected to test in the study.

Alkaline Phosphatase Phosphatases are a broad group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid. In soil ecosystems, these enzymes are believed to play critical roles in P cycles as evidence shows that they are correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system (Dick et al., 2000; Schneider et al., 2001; Makoi and Ndakidemi, 2008; Rahmansyah et al., 2009; Wang et al., 2009)).

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; Makoi and Ndakidemi, 2008). So, information on the nature of urease activity in soil was beneficial to develop and employ strategies for nitrogen management.

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; Cycon et al., 2005).

Rhodanese (Thiosulfate sulfurtransferases) catalyzes the transformation of $\text{S}_2\text{O}_3^{2-}$ 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 $\text{S}_2\text{O}_3^{2-}$ 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; Saidu, 2004).

Pesticides have been designed to specifically affect certain enzyme activities in order to restrict impacts to target weeds, insect and plant pathogens, but Active substances found in many pesticides may hamper the rate of a series of biochemical processes, interfering with the soil enzymatic activity and microbial growth. Modifications in the activity of microorganisms may lead to upsetting the biological equilibrium of soil, which in turn depresses its fertility. All these considerations emphasize the importance of studies on the effect of pesticides on the biological activity of soil, and particularly on soil enzymes and soil respiration, which can serve as good

indicators of the impact of pesticides on soil metabolism (Smith et al., 2000; Monkiedje and Spiteller 2002; Sigler and Turco, 2002; Wyszowska and Kucharski 2004; Kinney et al., 2005; Singh and Singh, 2005; Cycon et al., 2005; Yao et al., 2006; Bending et al., 2007; Vinther et al., 2008; Xiaoqiang et al., 2008; Aurelia, 2009; Hua et al., 2009; Niemi et al., 2009; Yunlong et al., 2009; Singh and Ghoshal 2010, and Uqab et al., 2016).

Metribuzin (4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one) is selective soil applied herbicide which have high mobility in soil, and thus, with great potential to leach and pollute surface and groundwater (Niemi et al., 2009; Fernandez-Perez et al., 2010).

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; Yunlong et al., 2009).

Imidacloprid [1-[1-(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine], a new promising insecticide, has been commercially introduced to the market recently. It is a systemic insecticide used for seed treatment, soil and foliar applications. Imidacloprid belongs to the group of nicotine-related insecticides

referred to as neonicotinoids (Ingram et al., 2005; Jeschke and Nauen 2008).

The present investigation has been set to study the effect of three systemic formulated soil applied pesticides which are used in vegetables and other crops in Saudi Arabia, Metribuzin as herbicide, Carbendazim as fungicide and Imidacloprid as insecticide at 1, 10 and 100 mg ai kg⁻¹ soil on soil enzymatic activities taking dehydrogenase, urease, alkaline phosphatase and rhodanese as well as soil respiration under controlled consideration. The results will be useful for assessing the possible effect on soil quality and may be used to monitoring the pesticides and toxicant residues in soil.

Materials and Methods

Pesticides

Formulations of metribuzin (Herbacor 70% WP), Carbendazim (Carben, 50% WP) and Imidacloprid (Imiguard 20% SL) were used in this study.

Tested soil

Laboratory studies performed on two types of soil since the impact of pesticides on soil-enzyme activities depend on the type of soil (Ingram et al., 2005). Samples was collected from Qassim region, (0-20 cm) previously cultivated with grazing fodder, was air dried and sieved. Soil sample (100 g) were placed in brownish glass bottles and treated with mentioned doses of each pesticides for enzymes activity and (50 g) for soil respiration. The moisture content was adjusted at 60% W.H.C. Soil enzyme activity is variable in time (Rahmansyah et al., 2009), soil samples were taken from all treatments after 0, 1, 2, 7, 14, 21, 45, 60, and 120 incubation days. Each

treatment was replicated three times in a complete randomize design and all treatments were incubated at 37°C. Soil physical and chemical characters were described in Table 1.

Determination of soil enzyme activity

Alkaline Phosphatase activity: alkaline phosphatase activities were determined according to the method of Monkiedje et al., (2007), using p-nitrophenylphosphate as substrate, with slight modifications. Soil samples (1 g) were mixed with modified universal buffer (MUB) at pH 11 and 0.05M p-nitrophenyl phosphate (1 ml). After incubating the mixture for 1 h at 37°C, 0.5M CaCl₂ and 0.5M NaOH (4 ml) were added to it and the mixture was centrifuged at 1500g for 10 min. The p-nitrophenol (PNP) in the supernatant was determined colorimetrically at 420 nm.

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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.

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 CaCO₃ 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 triphenylformazan (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 ηmole TPF/gm dry soil/hour.

Rhodanese activity

The method described by Tabatabai & 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 TAHM buffer, 0.5 ml of 0.1 M Na₂S₂O₃, 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 CaSO₄-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.

Table 1. Chemical and physical characters of the two types of used soil.

EC (dSm-1)	1.3	5.6
pH(1:2.5) meqL ⁻¹	8.03	7.9
CO ₃ ⁻ +HCO ₃ ⁻ meqL ⁻¹	2	1.5
SO ₄ ⁻ meqL ⁻¹	6.47	1.8
Cl ⁻ meqL ⁻¹	13.6	60
Ca ⁺⁺ meqL ⁻¹	5	15.5
Mg ⁺⁺ meqL ⁻¹	3	14.5
Na ⁺ meqL ⁻¹	10	32.5
Total N%	79	1.4
Available N mg kg ⁻¹	10	15.8
NH ₄ ⁺ mg kg ⁻¹	12.6	14.6
NO ₃ ⁻ mg kg ⁻¹	9.3	21.3
Organic matter %	1.3	1.6
Soil Text	silty	Silty loam
Sand%	7.9	25.3
Silt%	83.8	55.9
Clay%	8.3	18.8

Determination of soil respiration

CO₂ production was measured. After the incubation of the samples with NaOH, excess BaCl₂ was added to the NaOH solution to precipitate carbonate and the remaining NaOH was titrated with HCl using phenolphthalein as an indicator. Three replicates of each sample were tested. Data are expressed as mg CO₂/g dry soil/h. (Wang et al., 2003).

Statistical analysis

Effect of the three pesticides, doses and period of exposure on tested parameter and interactions between these parameters were treated statistically by three -way ANOVA. The statistical significance (P <0.05) of differences was assessed. Comparison of means was done using least significant

differences (LSD0.05) by Student–Newman–Keuls (SNK) test, Cohort Software Inc. (1986).

Results and Discussion

In literature on the effects of pesticides, 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; Tejada, 2009; Xiuguo et al., 2009), though research results claiming that no such influence occurs, or even that soil enzymes activity is raised by chemical plants protection agents (Monkiedje et al. 2002; Srinivasulu and Rangaswamy, 2006; 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 soil respiration after Metribuzin, Carbendazim and Imidacloprid introduced into soil.

Effects on alkaline phosphatase activity

Results on enzymatic activity are depicted in Tables (1 and 3). Generally, the application of all doses of the three tested pesticides significantly increased the activity of alkaline phosphatase activity once added to soil. The activity of this enzyme was fluctuated form decrease to increase from one day incubation period and during all the

periods of the experiment till to 120 days, the highest activation of the enzyme reached at 14 days incubation period with a value of 147.661 $\mu\text{g Pi/g dry soil/h}$ compared to 74.326 $\mu\text{g Pi/g dry soil/h}$ for the control treatment. Comparison of the mean values of the tested pesticides treatments with control treatments revealed that the herbicide Metribuzin was the most effective pesticides in decreasing the activity of the enzyme followed by the insecticide Imidacloprid, while the fungicide Carbendazim ranked the last in decreasing the activity of this enzyme. Inhibition of the activity significantly decreased with the three used doses, but the inhibition at the low doses 1 and 10 mg kg^{-1} did not follow the dose dependent pattern since the lowest one had not the least inhibitory effect. Concerning to the activity of the enzyme in the silty loam soil, data in Tables (2 and 4) revealed that, Metribuzin ranked the first in inhibition of the enzyme activity followed by Carbendazim. All the tested three doses significantly decreased the activity of this enzyme and it was not surprise that the higher concentrations of the pesticide were added into the soil, the higher decrease in alkaline phosphatase activity was ascertained. The result was also found in the treatments of silty soil. The result is in agreement with the results of Cycon et al., (2010). The same trend of the activity during the incubation periods in silty soil was noticed in silty loam soil since, the activity of the enzyme significantly increased with the time of incubation till to 21 days and after that it was decreased with the time and the highest value was noticed at 14 days incubation

period. The results of Scelza et al., (2008) supported this point of our work.

Effects on urease activity

Urease is an extracellular enzyme responsible for the hydrolysis of urea to ammonium, which can be assimilated by microbes and plants. Urease activity is a useful indicator to evaluate the soil pollution situation (Wang et al., 2010). Taking a look at Tables (5 and 6), all the interaction treatments reduced the activity as $\text{mmole urea /g dry soil /h}$. of urease enzyme either in silty or silty loam soil. As collective mean of the effects of the incubation periods, pesticides and doses, Tables (1 and 2) showed that all the incubation periods in the two types of soil had lower values of urease activity as than the treatment of zero time. Carbendazim added in silty loam soil and Imidacloprid in silty soil had the most inhibitory effect on the activity of the enzyme. Concerning to the effects of doses, there is no clear difference between the three doses especially in silty soil samples. Many studies reached to the same results that activity of urease either declined or did not affected with the application of the different types of pesticides since is well known that urease is strongly bonded to the organic matter and mineral particles of soil; hence, it is protected from degradation and denaturation. The soil enzyme complexes formed by urease may be more stable and resistant to degradation than those formed by other enzymes (Uyanoz et al., 2005; Sukul, 2006; Scelza et al., 2008; Cycon et al., 2010; Wang et al., 2010).

Effects on dehydrogenase activity

Since dehydrogenase activity reflects the physiological state of microorganisms, so this enzyme has been proposed as accurate

measure of potential microbial activity in soil treated with agrochemicals (Rossel and Tarradellas, 1991). The values and behavior of dehydrogenase activity, another key enzyme in the C cycle, were decreased as a result of interaction between the tested factors in the majority of the treatments in silty and silty loam soils (Tables 7 and 8). Values close to zero were measured at each time. Dehydrogenase recorded highly significant increasing in the activity after 48 hours from application of the pesticides with values reached to 0.585 η mole TPF g⁻¹ dry soil/ h and 1.305 η mole TPF g⁻¹ dry soil/ h for silty and silty loam soil, respectively. Tables (1 and 2) showed that the insecticide Imidacloprid had the lowest inhibitory effect on the activity of this enzyme while the fungicide Carbendazim and the herbicide Metribuzin had the same effect and ranked the last either in silty or silty loam soil. Similar effects was observed with respect to Carbendazim (Burrows and Edwards, 2004; Sousa et al., 2004; Xiuguo et al., 2009) and with other pesticides (Monkiedje et al., 2002; Bendig et al., 2007; Scelza et al., 2008; Cycon et al., 2010).

In general the values of the dehydrogenase activity were greater in silty loam soil than in silty soil so, we can say that the enzyme is also affected by different soil features such as soil type (Cycon et al., 2010).

Effects on rhodanese activity

This enzyme belongs to the family of transferases. Data of Tables (9, 10) showed the effects of the interaction of three doses of three type of pesticides and incubation time reached to 120 days on the activity of this enzyme. It was clear that all the interaction treatments oscillated

between the increase and decrease at intervals of experiment. As a collective mean of the effect of the three factors studied (Tables 1, 2), the fungicide carbendazim was the least effective pesticide in the inhibition of rhodanese activity. To the best of our knowledge, there is severe shortage of recent research associated with the impact of pesticides on the enzyme activity of rhodanese in soil.

Effect of tested pesticides on soil respiration

The soil respiration is commonly used parameter reflecting microbial activity, which allows assessing the potential perturbations of the carbon transformation processes in soil treated with pesticides and other xenobiotics (Cycon et al., 2010; Ramezani et al., 2010). The effects of soil treatments and incubation times on microbial activities according to soil respiration are summarized in table (11, 12). Soil respiration was significantly and strongly affected by each factor considered and also by each of their interactions. The release of CO₂ reached a maximum, ranging from 3. 600 to 5.445 mCO₂ g⁻¹ dry soil h⁻¹ for silty soil samples and from 5.085 to 6.750 mCO₂ g⁻¹ dry soil h⁻¹ for silty loam soil samples after 45 days of incubation and then decreased with time. In the study of (Crouzet et al., 2010) soil respiration responses peaked at 42 days post-exposure. Respiration was highest in the silty soil than in the silty loam soils. This result was in agreement with Crouzet et al., (2010) and Ramezani et al., (2010) which reported that soil respiration differ from soil type to another one. The general pattern was that the magnitude of soil respiration response was dependent on exposure time, pesticide type and dose, Crouzet et al., 2010, (Tables 1, 2). All the tested

pesticides increased soil respiration either in silty soil or silty loam and the fungicide Carbendazim ranked first. Our results in this point agreed with the results of Devare et al., (2007), Eisenhauer et al., (2009) and Rahmansyah et al., (2009). As indicated by many studies, the changes in microbial respiration in soils treated with pesticides have been related closely to the dosages of pesticides used (Chen et al., 2001; Cernohlávková et al., 2009; Crouzet et al., 2010 Cycon et al., 2006, 2010). The three used doses significantly increased soil respiration especially the dose of 1 mg kg⁻¹ dry soil.

In general, our collective results as shown in Figures 1, 2, and 3 appeared that Metribuzin, Carbendazim and Imidacloprid had contrasting effects on the soil enzyme and respiration and

Metribuzin had the most inhibitory effect on the selected microbiological processes of the two types of soil, this attributed with the finding of Sabra et al. (1997) and carbendazim had the least effect. Certainly, the inhibitory effect of these pesticides increased with the concentration, so avoiding overdose is useful (Riah et al., 2014). Although the experiments presented herein are limited by the controlled laboratory conditions adopted (i.e. absence of soil fauna and leaching), they may be suitable for providing information on the intensity and nature of the response of soil to an applied pesticides. Furthermore, such investigations may be helpful for further studies aimed at validating and extrapolating the data to natural situations.

Table (2): Effect of main tested factors on soil enzymes and respiration of silty soil.

Tested Factors		Ph.ase μg/g/h	Ur.ase mmole/g/h	Dehy.ase ηmole/g/h	Rho.ase mmole/g/h	Respiration mg/g/h
Time Days	0	74.326c	28.770e	0.0900a	51.778cd	3.355d
	1	92.553e	27.963b	0.1610b	49.745c	1.544a
	2	54.202b	28.609de	0.3026c	46.545b	2.678c
	7	113.939f	28.606de	0.0833a	46.316b	1.792b
	14	147.661h	28.241bc	0.0408a	36.648a	1.637a
	21	144.209g	27.689a	0.0849a	50.140cd	1.784b
	45	46.650a	28.222bc	0.0275a	50.839cd	4.339e
	60	54.578b	28.356cd	0.0341a	48.946bc	2.725c
	120	82.647d	27.590a	0.0798a	53.189d	3.299d
	L.S.D _{0.05}	0.777	0.272	0.0438	2.372	0.094
Pesticides	M	87.049a	28.341b	0.0871a	47.519a	2.306a
	C	92.812c	28.191ab	0.0865a	50.453b	2.769c
	I	90.394b	28.150a	0.1284b	47.743a	2.643b
	L.S.D _{0.05}	0.4485	0.157	0.0253	1.369	0.055
Conc. Ppm	0	97.924d	28.145a	0.1488b	48.967a	2.458a
	1	88.294b	28.282a	0.0766a	48.960a	2.602b
	10	79.619a	28.316a	0.0876a	47.598a	2.621b
	100	94.503c	28.165a	0.0897a	47.431a	2.608b
	L.S.D _{0.05}	0.51796	n.s	0.0292	n.s	0.063

M: Metribuzin, C: Carbendazim, I: Imidacloprid. Means of periods, pesticides and doses followed by the same letter are not significantly different according to LSD (P < 0.05) three way ANOVA (SNK) test.

Table (3): Effect of main tested factors on soil enzymes and respiration of silty loam soil.

Tested Factors		Ph.ase μg/g/h	Ur.ase mmole/g/h	Dehy.ase ηmole/g/h	Rho.ese mmole/g/h	Respiration mg/g/h
Time Days	0	99.614d	28.033f	0.2798f	54.027d	8.921h
	1	92.717b	27.723b	0.1954d	61.634i	1.884b
	2	114.597f	29.399i	0.3999g	48.194d	3.457e
	7	167.725g	28.391g	0.1087c	34.807a	2.188c
	14	213.956i	27.833c	0.1186c	38.935b	1.750a
	21	187.189h	27.863d	0.2388e	40.498c	2.260c
	45	65.898a	28.512h	0.0564a	55.805f	5.456g
	60	96.287c	27.948e	0.0691b	58.994h	2.713d
	120	103.261e	27.154a	0.0761b	57.647g	3.723f
	L.S.D _{0.05}	1.715	0.0287	0.0116	0.978	0.0987
Pesticides	M	125.589a	28.103b	0.1614a	49.953b	3.755b
	C	126.697b	28.077a	0.1665a	51.677c	3.911c
	I	128.123c	28.105b	0.1863b	48.551a	3.118a
	L.S.D _{0.05}	0.099	0.0166	0.0067	0.565	0.0569
Conc. ppm	0	135.817d	28.194c	0.2062c	53.686d	2.625a
	1	123.235b	28.122b	0.1709b	51.201c	4.172d
	10	126.822c	28.040a	0.1394a	48.854b	3.901c
	100	121.337a	28.024a	0.1692b	46.450a	3.681b
	L.S.D _{0.05}	1.143	0.0191	0.0077	0.652	0.0658

M: Metribuzin, C: Carbendazim, I: Imidacloprid. Means of periods, pesticides and doses followed by the same letter are not significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test.

Table (4): Effect of Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme alkaline phosphatase (μg Pi/g dry soil/ h.) in silty soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		52.55	141.23	49.23	113.43	160.06	203.37	47.34	56.22	57.93
Metribuzin	1	96.93	70.93	59.90	93.17	215.66	149.21	44.66	67.34	95.50
	10	81.69	69.76	58.55	86.44	155.49	124.46	42.41	59.00	92.09
	100	73.89	59.81	56.58	55.15	81.69	94.06	36.67	48.87	82.50
Carbendazim	1	70.48	85.27	58.28	127.96	153.51	148.40	56.13	56.40	97.47
	10	91.37	97.11	54.79	130.83	115.58	86.71	47.44	42.23	92.87
	100	79.45	74.69	96.85	118.27	149.12	160.42	58.55	40.44	78.37
Imidacloprid	1	70.48	85.27	58.29	127.96	153.51	148.40	56.13	56.40	97.47
	10	91.37	97.11	54.79	130.83	115.58	86.71	47.44	42.23	92.87
	100	79.45	74.69	96.84	118.27	149.12	160.42	58.55	40.44	78.37

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD_{0.05} for interaction (time*pesticides*doses) = 2.69.

Table (5): Effect of Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme alkaline phosphatase ($\mu\text{g Pi/g dry soil/ h.}$) in silty loam soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		105.27	99.26	136.21	178.80	220.49	212.50	72.72	90.57	106.50
Metribuzin	1	94.96	63.56	91.46	168.76	188.66	252.00	75.68	106.70	92.45
	10	106.08	67.25	86.17	188.30	233.94	137.70	65.55	105.50	122.40
	100	76.58	20.89	163.02	118.54	255.47	143.80	70.39	102.80	100.20
Carbendazim	1	105.63	8.967	108.77	178.53	271.25	186.70	74.87	114.00	106.50
	10	101.24	13.81	102.13	178.35	215.20	184.40	66.89	82.41	106.80
	100	81.06	81.06	146.16	108.68	98.82	175.00	33.63	89.13	104.70
Imidacloprid	1	95.68	123.11	84.29	154.68	172.52	106.40	50.39	85.63	84.47
	10	117.73	107.06	81.51	178.35	190.99	192.60	61.33	97.74	99.53
	100	100.61	124.10	103.03	202.11	269.90	230.00	73.89	99.89	102.50

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD0.05 for interaction (time*pesticides*doses) = 5.93.

Table (7): Effect of Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme Urease (mmole urea /g dry soil /h.) in silty soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		28.47	27.42	29.60	28.56	28.41	27.60	28.17	27.80	27.24
Metribuzin	1	30.69	27.91	27.90	27.55	28.22	27.80	28.22	30.41	27.97
	10	30.86	28.57	27.27	28.73	27.90	28.00	28.33	27.95	28.07
	100	30.08	28.05	28.05	28.91	28.01	27.30	28.12	27.98	28.12
Carbendazim	1	27.91	27.90	28.56	28.92	28.06	27.90	28.37	28.02	27.67
	10	28.46	27.74	27.87	28.67	28.25	27.70	28.12	30.02	27.85
	100	28.47	28.39	29.86	28.90	28.39	27.70	28.26	29.39	27.63
Imidacloprid	1	28.56	27.76	29.82	28.70	28.22	27.70	28.33	27.45	27.24
	10	28.12	28.64	29.39	28.59	28.23	27.70	28.25	27.81	27.45
	100	26.68	28.30	29.45	28.75	28.39	27.50	28.17	27.84	27.36

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD0.05 for interaction (time*pesticides*doses) = 0.94.

Table (8): Effect of Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme Urease (mmole urea /g dry soil /h.) in silty loam soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		28.02	27.90	29.19	28.29	28.01	28.80	28.33	28.14	27.05
Metribuzin	1	28.25	28.02	29.47	28.57	26.40	27.50	28.18	28.02	27.27
	10	28.15	27.89	29.41	28.51	28.13	27.40	28.12	27.89	27.28
	100	27.41	27.09	29.65	28.24	27.96	27.70	30.60	27.53	27.40
Carbendazim	1	27.68	28.07	29.60	28.45	28.14	27.60	28.28	27.81	27.27
	10	27.91	27.75	29.67	28.54	27.74	27.50	27.98	27.59	26.96
	100	28.36	27.30	29.91	28.34	27.87	27.30	27.98	28.56	26.84
Imidacloprid	1	28.40	27.92	29.29	28.14	27.92	27.70	30.26	27.94	27.20
	10	28.33	27.74	29.24	28.70	28.07	27.40	28.00	27.90	27.22
	100	27.84	27.19	28.97	28.34	27.73	27.80	27.75	27.72	27.27

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD0.05 for interaction (time*pesticides*doses) = 0.099

Table (9): Effect of the Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme Dehydrogenase (η mole TPF/g dry soil/ h.) in silty soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		0.117	0.323	0.527	0.080	0.049	0.107	0.028	0.028	0.081
Metribuzin	1	0.052	0.078	0.475	0.096	0.040	0.069	0.030	0.033	0.099
	10	0.048	0.055	0.141	0.018	0.017	0.067	0.028	0.040	0.031
	100	0.053	0.042	0.105	0.017	0.017	0.067	0.022	0.017	0.041
Carbendazim	1	0.095	0.216	0.087	0.018	0.033	0.099	0.017	0.018	0.037
	10	0.111	0.105	0.140	0.052	0.027	0.078	0.017	0.030	0.053
	100	0.054	0.051	0.140	0.040	0.017	0.034	0.033	0.061	0.066
Imidacloprid	1	0.081	0.039	0.108	0.017	0.017	0.071	0.017	0.041	0.088
	10	0.146	0.234	0.269	0.312	0.094	0.096	0.017	0.040	0.070
	100	0.090	0.146	0.585	0.217	0.081	0.116	0.017	0.047	0.249

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$). three way ANOVA (SNK) test. LSD0.05 for interaction (time*pesticides*doses) = 0.152.

Table (10): Effect of the Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme Dehydrogenase (η mole TPF/g dry soil/ h.) in silty loam soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		0.282	0.149	0.633	0.113	0.183	0.308	0.040	0.080	0.059
Metribuzin	1	0.343	0.151	0.216	0.189	0.090	0.214	0.030	0.080	0.098
	10	0.371	0.220	0.171	0.125	0.059	0.140	0.024	0.067	0.067
	100	0.296	0.225	0.218	0.067	0.064	0.177	0.053	0.111	0.098
Carbendazim	1	0.314	0.075	0.609	0.017	0.090	0.202	0.092	0.017	0.106
	10	0.035	0.084	0.346	0.089	0.092	0.222	0.031	0.043	0.118
	100	0.416	0.348	0.210	0.061	0.151	0.217	0.017	0.017	0.101
Imidacloprid	1	0.346	0.090	0.464	0.148	0.112	0.241	0.193	0.046	0.042
	10	0.224	0.229	0.360	0.131	0.083	0.198	0.098	0.095	0.042
	100	0.167	0.475	1.305	0.136	0.134	0.306	0.017	0.114	0.064

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD0.05 for interaction (time*pesticides*doses) = 0.040.

Table (11): Effect of the Pesticides Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme Rhodanese (mmole SCN/g dry soil / h.) in silty soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		59.604	58.994	47.256	40.549	35.213	38.567	62.805	47.866	49.848
Metribuzin	1	52.591	48.018	48.018	58.384	39.939	54.116	43.750	51.524	51.524
	10	62.805	40.396	48.018	47.409	41.006	48.933	53.506	52.744	51.982
	100	41.463	40.396	45.274	42.988	30.030	52.896	41.159	35.366	53.811
Carbendazim	1	39.482	49.543	42.835	53.963	40.854	82.470	36.128	73.018	56.860
	10	54.726	61.738	53.963	47.561	31.707	51.677	51.067	47.866	48.018
	100	46.646	35.671	45.274	52.134	41.311	68.293	55.488	46.037	64.939
Imidacloprid	1	45.732	50.457	46.494	40.701	36.890	41.006	42.988	39.787	55.183
	10	40.549	38.720	40.701	48.933	35.823	48.018	34.756	49.543	56.250
	100	58.537	58.689	46.189	42.073	36.585	38.567	62.805	47.866	50.152

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD0.05 for interaction (time*pesticides*doses) = 8.203.

Table (12): Effect of the Pesticides Metribuzin, Carbendazim and Imidacloprid on the activity of enzyme Rhodanese (mmole SCN/g dry soil / h.) in silty loam soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		44.055	109.76	47.561	35.671	37.805	32.317	58.841	62.957	54.268
Metribuzin	1	72.561	50.915	50.000	32.774	42.835	38.720	54.878	55.793	55.640
	10	61.585	53.963	49.238	45.274	39.177	34.909	45.274	54.878	75.915
	100	43.750	35.976	51.067	31.250	37.957	41.921	41.159	52.439	65.549
Carbendazim	1	64.939	67.530	46.494	30.030	38.872	43.902	70.122	58.232	57.165
	10	50.762	46.494	57.165	36.433	32.927	50.000	54.878	68.445	53.049
	100	46.037	40.091	53.049	41.311	48.933	48.171	59.146	62.043	50.915
Imidacloprid	1	69.817	55.335	40.854	36.890	39.482	41.311	65.244	51.372	51.067
	10	49.543	39.329	45.884	29.421	41.921	49.848	46.951	48.171	57.622
	100	57.165	20.884	42.226	27.287	31.707	40.244	55.488	67.683	62.043

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD_{0.05} for interaction (time*pesticides*doses) = 3.384

Table (13): Effect of the Pesticides Metribuzin, Carbendazim and Imidacloprid on the respiration (mCO₂ /g dry soil / h.) in silty soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		1.525	1.700	2.590	1.570	1.615	2.180	5.445	2.555	2.945
Metribuzin	1	1.830	1.520	2.200	1.895	1.580	1.660	3.600	2.035	2.425
	10	1.830	1.570	2.590	1.635	1.615	1.130	4.455	2.555	2.620
	100	1.830	1.310	2.330	1.635	1.525	0.960	4.725	3.735	3.995
Carbendazim	1	2.745	1.435	3.240	2.090	1.700	1.090	3.780	2.750	3.345
	10	2.405	1.570	3.115	1.765	1.785	2.270	4.275	3.345	3.865
	100	3.150	1.570	1.685	2.025	1.655	1.920	3.015	2.490	3.475
Imidacloprid	1	1.235	1.830	2.590	1.895	1.660	1.830	4.005	2.685	4.520
	10	1.830	1.440	3.370	2.090	1.660	1.830	4.050	2.620	3.475
	100	1.830	1.180	3.240	1.765	1.615	2.180	3.825	2.815	2.945

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD_{0.05} for interaction (time*pesticides*doses) = 0.326.

Table (14): Effect of the Pesticides Metribuzin, Carbendazim and Imidacloprid on the respiration (mCO₂ /g dry soil / h.) in silty loam soil at different time intervals.

Treatments	Dose mg/kg	Days after treatments								
		0	1	2	7	14	21	45	60	120
Control		1.830	1.700	2.855	2.025	1.700	2.270	5.085	2.945	3.210
Metribuzin	1	2.065	1.700	3.500	2.025	1.740	2.180	6.075	1.765	3.865
	10	3.640	2.745	3.760	2.220	1.830	1.830	6.750	2.685	4.520
	100	3.725	2.480	3.890	2.220	1.700	2.180	5.175	2.685	3.605
Carbendazim	1	15.555	2.090	3.890	2.285	1.875	2.270	6.075	3.210	4.255
	10	15.555	1.440	3.875	2.545	1.830	2.610	4.950	1.970	4.255
	100	13.725	1.435	3.630	2.220	1.785	2.440	4.995	2.555	3.865
Imidacloprid	1	14.640	1.960	3.115	2.220	1.655	2.350	6.255	2.295	3.735
	10	1.830	2.220	3.370	2.025	1.740	2.180	5.130	3.475	3.345
	100	1.830	1.440	3.890	2.415	1.745	2.270	4.815	3.075	3.605

All values are means of three replicates samples, significantly different according to LSD ($P < 0.05$) three way ANOVA (SNK) test. LSD_{0.05} for interaction (time*pesticides*doses) = 0.341

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الآثار الجانبية لثلاثة مبيدات تطبق على التربة على نشاط انزيمات وتنفس التربة تحت ظروف متحكم بها

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الملخص العربي

أجريت التجربة في المختبر للتحقيق من تأثير ثلاثة من المبيدات التي تطبق على التربة والتي تنتمي إلى ثلاثة مجموعات متغايرة، مبيد الأعشاب الميتروبيوزين (Metribuzin)، مبيد فطري الكربندازيم (Carbendazim) ومبيد حشري إيميداكلوبريد (imidacloprid) على نشاط الإنزيمات في التربة، الفوسفاتيز القاعدي، اليورياز، الديهيدروجينيز، الرهودينيز وكذلك على عملية تنفس التربة في نوعين من التربة، السلتية والصلتية اللومية. تم تطبيق المبيدات بتركيز ٠، ٠.١، ١٠، ١٠٠ ملجم/كيلوجرام تربة جافة. وقدّر نشاط هذه العمليات الميكروبيولوجية في التربة بعد ٠، ١، ٢، ٧، ١٤، ٢١، ٤٥، ٦٠، ١٢٠ يوما من تطبيق المبيدات. وأظهرت الدراسة أن التأثير على نشاط الانزيمات وعملية تنفس التربة يتوقف على الزمن الذي تم عليه التقييم بعد المعاملة بالمبيد. وكان واضحا أنه بعد استعمال المبيدات يزيد معدل النشاط لمدة ٢٤ ساعة ومن ثم ينخفض إلى أدنى مستوى بعد ٤٨ ساعة. وسجلت أعلى قيمة لنشاط العمليات بعد ١٤ يوما من معاملة التربة بالمبيدات. تستعيد هذه العمليات الحيوية نشاطها مره أخرى بعد ٤٥ يوما والعودة إلى النشاط العادي مع مرور الوقت بعد ١٢٠ يوما. بالنسبة لمبيد الأعشاب (metribuzin) كان أعلى مثبط لنشاط الانزيمات وتنفس التربة، يليه المبيد الحشري إيميداكلوبريد وأقل تأثير حدث من قبل المبيد الفطري carbendazim اعتمادا على تركيز المبيدات. وقد سجلت النتائج أن انزيم الفوسفاتيز كان أكثر الانزيمات المقدرة حساسية لجميع المبيدات وأقل الانزيمات حساسية هو انزيم اليورياز في كلا نوعي التربة. وكانت العمليات البيولوجية المقدرة أكثر تأثرا في التربة السلتية عن السلتية لوم.