

The Effect of Adding Some Pesticides in Inhibiting the Activity of the Urease Enzyme in Sandy Loam Soil Treated with Different Organic Sources

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Summary

Due to the increased use of agricultural pesticides for the purpose of reducing agricultural diseases and thus increasing production, this research was conducted to find out the effect of four types of agricultural fungicide (Difenoconazole 25% EC), insecticide (Demethoate 40% EC), Herbicide (Atlants) and Biocide (*Trichoderma harzianum*) with four different concentrations. (Zero, according to the recommendation, double of the recommendation, and four times the recommendation) in inhibiting the activity of the enzyme urease in soils treated with five types of organic sources separately (Cows' manure, poultry wastes mixed with sawdust, alfalfa grinded, molasses of yellow corn grinded and urea) for five incubation periods (7, 14, 21, 28 and 35) days. The results showed the highest percentage of inhibition of the enzyme at the incubation period of 14 and 21 days from the addition of the fungicide and insecticide and at 28 and 35 days of adding the biocide and herbicide compared with the rest of the incubation periods, and the treatments of alfalfa grinded showed the lowest percentage inhibition of enzyme activity in fungicide, herbicide and biocide treatments, while it showed the highest rate of inhibition in the insecticide treatments. The poultry waste mixed with sawdust showed the highest rate of inhibition in the fungicide, insecticide and biocide treatments, while it showed the lowest rate of inhibition with the herbicide. The percentage of enzyme inhibition was increased by increasing the concentration of the pesticide compared to the control treatment.

Key words

Insecticide, urease inhibition, Pesticides, Cows' manure, fungicide, Trichoderma

* Research extracted from the doctoral thesis of the first researcher

1. Introduction

Soil is a vital system containing many free enzymes, microbial intracellular enzymes and immobilized extracellular enzymes (Vineela Deborah *et al.* 2013). The microbes present in the soil are a major source in the production of various enzymes (Zhang *et al.*, 2009). Many chemical, biological and biochemical reactions take place in the soil which are catalyzed by the enzymes that are cofactors consisting of proteins with catalytic properties that increase the speed of the reaction without changing the properties

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of the enzyme after the end of the reaction (Tabatabai, 1994). The mineralization of soil organic matter and the conversion of nitrogen, phosphorous and bound sulfur into a non-bound form ready for plants (Kumari and Rao, 2017). Soil enzymes play a major role in energy transfer through the decomposition of soil organic matter and nutrient cycling and thus play an important role in the plants growth (Srinivasa *et al.* 2017). The urease enzyme is one of the enzymes widely spread in nature, as it is produced by different types of bacteria, fungi, plants, algae and invertebrates, and it is found in the soil in the form of external enzymes that represent most of the soil enzymes (Krajewska, 2009). The addition of organic matter to the soil increases soil carbon stocks and its ability to retain water that increases plant production (Cabrera *et al.*, 2009; Powlson *et al.*, 2012) and maintains the readiness of nutrients by chelating them with the elements (Sparks 1995). Soil organic matter is a rich source of nutrients necessary for the plants, especially nitrogen, phosphorous, potassium and sulfur, and it plays a major role in creating favorable conditions for plant growth by improving the soil's physical, chemical and biological properties, as it increases the Cation Exchangeable Capacity (CEC) of soil and reduces the loss of elements. The nutrients in soil are also a source for preparing the carbon and energy needed for microorganisms activities (Al-Naimi, 1999). Zhang *et al.* (2020) found a great relationship between organic carbon in soil, invertase enzyme activity, urease, and amylase, with a slight effect on catalase activity. Al-Ansari *et al.* (2019) found that the activity of urease enzyme for soils treated with organic wastes was higher than in untreated soils at all temperatures degrees.

Pesticides are part of modern agriculture to maintain crop production and provide food for a large number of people. The use of pesticides leads to environmental and human health problems, as the widespread use of pesticides affects the pests in the soil (Misra *et al.* 2014; Wanga *et al.* 2020), pollinators (Bartlewicz *et al.*, 2016), and rhizosphere microbial groups (Singh *et al.*, 2015). Kumari *et al.* (2018) studied the effect of six types of herbicides, showed that used of atrazine decreased the enzyme activity at zero day, then the enzyme activity was inhibited and maximum activity was reached after 60 days. Also, found that tembotrione herbicide showed higher activity compared to topamezone. Bielinska and Pranagal (2007) found that the addition of the triazine herbicide significantly inhibited enzyme activity in the soil.

The aim of the present study is to know the effect of some chemical insecticides, fungicides, and herbicides in inhibiting the activity of the urease enzyme in soils containing cow's manure and poultry waste mixed with sawdust, alfalfa grinded, Yellow corn molasses and urea fertilizer, and comparing it with the effect of the biocide (*Tricoderma harzianum*).

Materials and methods

Study soil

A sandy loam soil collected from the Al-Barjisiya area - Al-Zubair district - Basra governorate. The samples were collected from the surface layer (0-30 cm) and transferred to the laboratory. Soil were air dried, grind and sieved with 2 mm diameter. The physical, chemical and biological properties of soil were measured according to the standard methods (Table 1).

Table 1. Some soil physical ,chemical and biological properties.

Soil texture g.Kg ⁻¹			urease activities ($\mu\text{gNH}_4^+ \cdot \text{g}^{-1}$ soil. 2 hour ⁻¹)	EC (dS. m ⁻¹)	pH	C/N	total nitrogen (g. Kg ⁻¹ soil)
sand	clay	salt					
681.4	77.6	241.0	14.3	8.4	7.8	6.45	1.15

Pesticides:

four types of pesticides were used in the present study.

1: fungicide

Difenoconazole 25% EC usage rate according to recommendation 50 ml per 100 liters of water.

2: Insecticide

Of organic phosphorous compounds Demethoate 40% EC rate of application according to recommendation 100 ml per 100 liters of water.

3: Herbicide

The herbicide Atlants is a systemic pesticide developed in the form of fine granules soluble in water, a mixture of two active substances:

1-Mesosulfuron –methyl 30 g .Kg⁻¹ chemical structure

Methyl 2- (3- (4,6-dimethoxy-pyrimidin-2-yl) ureidosulfonyl) -4-methanesulfonamidomethyl -benzoate

2- Iodosulfuron-methyl-sodium 6g. Kg⁻¹ Its chemical composition

Methyl 4-iodo-2 [3- (4-methoxy-6-methyl-1,3,5-triazin-2-yl) ureidosulfonyl] benzoate, sodium salt

in addition to Mefenpyr-diethyl 90 g. Kg⁻¹ as a safety agent and the rest Carrier materials.

4: Biocide

Tricoderma, a biocide containing more than 19×10^7 spores per gram of *Trichoderma harzianum*. This pesticide is used according to the recommendation of 1 g. 40 ml⁻¹ water.

Each pesticide was added in four concentrations, including zero (comparison), field recommendation, double the field recommendation, and four times the field

recommendation . Each pesticide treatments incubated for 7, 14, 21, 28 and 35 days to estimate the urease activity in the soil.

Sources of organic nitrogen

Five sources of organic nitrogen (Cows' manure , poultry wastes mixed with sawdust, ground alfalfa , molasses of yellow corn ground and urea) were added at a rate of 5% on the basis of the dry weight of the soil, and urea fertilizer was added at a level of 125 mg N .kg⁻¹ soil.(Table 2)

Cows' manure

The waste was brought from one of the cowshed fields in the Al-Madinah district, it was air dried, milled, and passed through a 1 mm sieve.

Poultry waste mixture + sawdust

The waste was brought from a poultry field in the Al-Madinah district, it was air-dried, milled, and passed through a 1 mm sieve.

alfalfa grinded

The alfalfa plant was brought from the farms of the Al-Madinah district, air dried, milled and passed from a 1 mm sieve.

molasses of yellow corn ground

It was brought from the Al- Dear district, air dried, milled, and passed through a 1 mm sieve.

Urea fertilizer (46% N)

Table (2): Some of the chemical properties of the organic residues used in the study.

Chemical Properties	Yellow corn molasses grinded	alfalfa grinded	Poultry waste + sawdust	cows' manure	measuring unit
pH(5:1)	8.2	8.2	9.8	8.5	-----
E.C.(5:1)	4.23	11.41	7.94	8.04	dS. m ⁻¹
Total nitrogen	9.80	51.0	19.9	18.9	gm. Kg ⁻¹
Organic carbon	345.0	310.5	207.0	224.2	gm. Kg ⁻¹
Organic matter	594.7	535.0	356.0	386.6	gm. Kg ⁻¹
C:N Ratio	35.2	6.08	10.4	11.86	-----

Measurement of urease enzyme activity

Forty grams of dry soil was placed and mixed with 5% organic matter in 50 ml plastic boxes and wetted with distilled water at 60% of the saturation capacity and left for 15 days, then the above-mentioned pesticides were added separately at concentrations

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according to the recommendation, double the recommendation, and four times recommendation . Soils treated with the above-mentioned organic sources were used without pesticides as comparison treatments. Enzyme activity was measured according to the Tabatabai and Bremner method (1972) after 7, 14, 21, 28 and 35 days of pesticide addition and incubation at 30 ° C by incubating 5 g of soil with 0.2 ml toluene and 9 ml of buffer solution. Tris (hydroxymethyl) (THAM) aminomethan at pH = 9 and add 1 ml of 0.2 M urea solution as a substrate at 37 ° C for two hours, then add 35 ml of 2.5 M KCl solution containing 100 ppm Ag₂SO₄ as an inhibitor of enzyme activity, complete the volume to 50 ml with the same solution, then the ammonium ion resulting from the enzyme activity was estimated using the Kjeldahl apparatus according to Bremner and Edwards (1965) using Heavy MgO, receiving the ammonia with 2% boric acid , then correcting with hydrochloric acid at a concentration of 0.005 M. The inhibition ratio was calculated compare with comparison treatments.

The percentage of inhibition the urease enzyme activity was calculated using the following equation and as reported in (Bremner *et al.*, 1991).

$$\frac{C - T}{C} \times 100$$

whereas:-

T: the amount of ammonium released in the soil treated with the pesticide.

C: The amount of ammonium released in the soil not treated with pesticide (comparison treatment).

statistical analysis

All experiments were carried out with three replications as a factorial experiment. With a Complete Randomized Design (CRD), the studied traits were analyzed using an analysis of variance using SPSS-19 software. The averages of the coefficients were compared using the Reversed Least Significant differences (RLSD) at a probability level of 0.05, according to Al-Rawi and Khalaf Allah (1980).

Results and discussion

The effect of fungicide on the activity of urease enzyme in the soil:

Table (3) showed the percentage of inhibition of urease activity in the case of adding the fungicide to soil treated with different organic sources as a source of organic nitrogen. The highest rate of inhibition of urease enzyme activity appeared when adding the fungicide in the treatment of poultry waste mixed with sawdust, the rate of inhibition reached 54.18%, while soil treated with alfalfa grinded was the lowest inhibition rate 28.66%. As for the treatments of cows' manure, yellow corn and urea, the rate of inhibition was 49.05%, 52.39% and 37.22%, respectively. The activity of the urease enzyme is affected by the type of fungicide, its concentration, method of addition, and incubation time (Jastrzębska and Kucharski, 2007). The results are in agreement with Sukul (2006) who indicated that the addition of the fungicide metallaxyl inhibited urease

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activity. Jastrzębska and Kucharski (2007) also noted, when adding ciprodinil and a mixture of demoxystropin + epoxiconazole, a negative effects of these pesticides on urease activity. Increase the rate of inhibition of enzyme activity in the soil

Table (3) The effect of adding different concentrations of the fungicide Difenconazole 25% EC on inhibiting the activity of the urease enzyme in the soil under different incubation periods.

Incubation time (days)	The concentration of the pesticide	Organic source type					Incubation time * pesticide concentration
		Urea	Yellow corn molasses	alfalfa grinded	Poultry waste + sawdust	cows' manure	
7	recommendation	19.0	33.3	17.3	44.6	15.7	25.98
	2 * recommendation	25.7	48.8	21.7	58.4	27.1	36.34
	4 * recommendation	34.2	55.5	68.4	69.2	52.8	56.02
14	recommendation	8.0	58.8	7.4	40.4	48.0	32.52
	2 * recommendation	37.3	67.6	14.1	71.4	70.0	52.08
	4 * recommendation	53.3	70.5	32.0	80.9	76.0	62.54
21	recommendation	32.8	51.9	4.5	48.6	50.0	37.56
	2 * recommendation	43.2	55.7	12.4	62.1	64.2	47.52
	4 * recommendation	55.2	65.3	27.4	75.6	76.1	59.92
28	recommendation	35.1	38.7	20.2	40.5	38.4	34.58
	2 * recommendation	55.5	44.8	32.1	48.6	56.4	47.48
	4 * recommendation	64.8	59.1	38.0	62.1	66.6	58.12
35	recommendation	19.4	14.8	38.1	21.0	15.7	21.80
	2 * recommendation	30.5	53.1	42.4	34.2	31.5	38.34
	4 * recommendation	44.4	68.0	53.9	55.2	47.3	53.76
Type of organic source * pesticide concentration * incubation time		RLSD 0.01=1.918 0.05=1.41					0.01=0.993 0.05=0.715
The effect rate of the type of organic source		37.22	52.39	28.66	54.18	49.05	Effect rate of cuddling time
		RLSD 0.01=0.702 0.05=0.474					
Incubation time * Organic source type	7	26.30	45.86	35.80	57.40	31.86	39.44
	14	32.86	65.63	17.83	64.23	64.66	49.04
	21	43.73	57.63	14.76	62.10	63.43	48.33
	28	51.80	47.53	30.10	50.40	53.80	46.72
	35	31.43	45.30	44.80	36.80	31.50	37.96
		RLSD 0.01=1.159 0.05=0.862					0.01=0.702 0.05=0.474
							The effect rate of the pesticide concentration
Pesticide concentration * type of organic source	recommendation	22.86	39.5	17.5	39.02	33.56	30.488
	2 * recommendation	38.44	54	24.54	54.94	49.84	44.352
	4 * recommendation	50.38	63.68	43.94	68.6	63.76	58.072
		RLSD 0.01=1.033 0.05=0.737					0.01=0.543 0.05=0.367

Recommendation to add pesticide 0.5 ml. liter⁻¹

by increasing the incubation period from 7 to 14 days, as the rate of inhibition increased from 39.44% at the 7-day incubation period to 49.04% at the 14-day incubation period, then the inhibition rate decreased to 48.33%, 46.72% and 37.96% for periods 21, 28 and 35 days respectively. The results agree with the findings of Micuti *et al.* (2018). The urease activity decreased by 73% and 35% at the 21-day incubation period from the addition of the fungicide Rpdomal Gold MZ68WG and Bravo 500 SC, respectively. The increase in the concentration of the fungicide increased the rate of inhibition of urease enzyme activity among the treatments, as the rate of inhibition rate reached 30.48%, 44.35% and 58.07% for the recommended concentrations, recommendation *2 and Recommendation* 4, respectively. These results are in agreement with those of Bacmaga *et al* (2015). The addition of a high concentration of the fungicide azoxystrobin inhibited urease activity. Fungicides negatively affect enzymatic activities (Riah *et al.*, 2014). The increased concentration of pesticide increased the inhibition of urease enzyme activity in soil, as the rate of inhibition increased when increasing the concentration from the recommendation to four times the recommendation from 25.98% to 56.02% at the 7-day incubation period and from 32.52% to 62.54% at the 14-day incubation period and from 37.56% to 59.92% at the 21-day incubation period, and from 34.58% to 58.12% at the 28-day incubation period, and from 21.80% to 53.76% at the 35-day incubation period. Singh and Kumar, (2008) indicated a decrease in urease activity in soils to which acetamiprid was added by up to 35% over a period of 43 days. The addition of the chemical fungicide dazomet (dazomet DZ) negatively affected the regeneration of the soil microorganisms in the rhizosphere and also led to a significant decrease in the activity of urease enzyme in the soil compared to the soil untreated with the pesticide (Chen *et al.* 2001). The treatments of ground alfalfa showed the lowest rate of inhibition of enzyme activity during most of the incubation periods ranged between 35.80% at the 7-day and 44.80% at the 35-day incubation period, and poultry residues mixed with sawdust showed the highest inhibition rate, which ranged between 57.40% at a period 7-days and 36.80% at the 35- days incubation period. The results also indicate that the effect of the incubation period on the inhibition of enzyme activity differed according to the type of the organic source, as the results show that the incubation period increased from 7 to 14 days, which led to an increase in the rate of inhibition of the enzyme activity in the treatment of cows' manure, poultry wastes and yellow corns, and then a decrease in the rate of inhibition with increase the incubation period from 21 days to 35 days. As for the urea treatments, the rate of inhibition increased with the increase of the incubation period from 7 days to 28 days, then the rate of inhibition decreased at the 35-day incubation period. As for the treatment of ground alfalfa, the lowest rate of inhibition was at the incubation period of 21 day, then the rate of inhibition increased for subsequent periods.

The treatment of poultry waste mixed with sawdust showed the highest rate of inhibition of enzyme activity in the soil. It ranged between 39.02% when adding the pesticide at a

concentration according to the recommendation and 68.60% when adding the pesticide at a concentration of four times the recommendation. The treatment of ground alfalfa showed the lowest rate of inhibition of the activity, which ranged between 17.50% at the recommendation and 43.94% at four times the recommendation. The results are consistent with Rasool & Reshi (2010) who indicated a significant decrease in the activity of the urease enzyme after adding high concentrations of the fungicide. Liliana and Rao (2015) indicated a decrease in the activity of urease enzyme when various pesticides were added to organic soils.

The results of the triple interactions (Table 3) among incubation period, pesticide concentration and organic source type indicate the highest inhibition of enzyme activity, which was 80.9% in poultry waste mixed with sawdust at a concentration of four times the recommendation and the incubation period of 14 days. The lowest percentage of inhibition of enzyme activity was 4.5% in ground alfalfa soil at concentration of recommendation and incubation period of 21 days.

The effect of adding the insecticide (Demethoate 40% EC).

Table (4) showed, the highest inhibition of urease enzyme activity after adding the insecticide in ground alfalfa treatments, as it was 42.41%, which did not differ significantly from the treatment of poultry waste mixed with sawdust, which reached 42.08%, while soil treatment with urea showed the lowest rate of inhibition 30.99%. As for the treatment of cows' manure and yellow corn molasses, the inhibition rate of urease activity was 33.59% and 33.15%, respectively. The use of insecticides decreased urease activity in the soil and reduced urea hydrolysis (Antonious, 2003).

The results indicated an increase in the inhibition of enzyme activity with increasing the incubation period from 7 to 21 days, which was 35.41% at the 7-day and 44.63% at the 21-day incubation period, then it decreased to 33.00% and 30.12% at 28 and 35 days, respectively. The results are consistent with the findings of Micuti *et al.* (2018) who found that urease activity decreased after 14 and 21 days of the addition the insecticide Mospilan20SG and Vertimec 1.8% RC, respectively. Rasool & Reshi (2010) reported that increasing the incubation period decreases the enzyme urease activity. Increasing the concentration of insecticide led to an increase the inhibition rate of urease enzyme activity, as the rate of inhibition ratios reached 20.09%, 37.30%, 51.94% for the recommended concentrations, recommendation *2 and recommendation* 4, respectively. These results are in agreement with Rasool & Reshi (2010) who noticed a decrease in the activity of the urea enzyme when adding the pesticide mancozeb compared to the comparison treatment, as adding the pesticide with a concentration of N₁₀₀ (adding the pesticide at a concentration of 100 times from the recommendation

Table (4) The effect of adding different concentrations of the insecticide Demethoate 40% EC on the activity of the urease enzyme under different incubation periods.

Incubation time (day)	The concentration of the pesticide	Organic source type					Incubation time * pesticide concentration
		Urea	Yellow corn molasses	alfalfa grinded	Poultry waste + sawdust	cows' manure	
7	recommendation	16.1	23.0	32.6	31.8	25.7	25.84
	2 * recommendation	28.5	32.9	37.5	40.9	31.4	34.24
	4 * recommendation	52.3	45.0	46.6	46.9	40.0	46.16
14	recommendation	10.5	13.0	13.5	30.9	21.5	17.88
	2 * recommendation	34.2	44.9	34.5	52.3	41.1	41.40
	4 * recommendation	40.7	79.7	50.0	64.2	54.9	57.90
21	recommendation	10.4	17.3	17.6	35.1	42.8	24.64
	2 * recommendation	43.2	40.3	39.8	56.7	57.1	47.42
	4 * recommendation	61.9	57.6	60.7	64.8	64.2	61.84
28	recommendation	16.3	10.2	21.8	31.5	15.3	19.02
	2 * recommendation	25.4	22.4	59.0	44.7	20.5	34.40
	4 * recommendation	30.9	36.7	77.0	52.6	30.7	45.58
35	recommendation	18.9	6.3	16.9	15.7	7.6	13.08
	2 * recommendation	32.4	21.2	50.0	26.3	15.3	29.04
	4 * recommendation	43.2	46.8	78.7	36.8	35.8	48.26
Type of organic source * pesticide concentration * incubation time		RLSD 0.01=1.918 0.05=1.412					0.01=0.993 0.05=0.715
The effect rate of the type of organic source		30.99	33.15	42.41	42.08	33.59	Effect rate of cuddling time
		RLSD 0.01=0.702 0.05=0.474					
Incubation time * Organic source type	7	32.30	33.63	38.90	39.86	32.36	35.41
	14	28.46	45.86	32.66	49.13	39.16	39.06
	21	38.50	38.40	39.36	52.20	54.70	44.63
	28	24.20	23.10	52.60	42.93	22.16	33.00
	35	31.50	24.76	48.53	26.26	19.56	30.12
		RLSD 0.01=1.159 0.05=0.862					0.01=0.720 0.05=0.474
							The effect rate of the pesticide concentration
Pesticide concentration * type of organic source	recommendation	14.44	13.96	20.48	29.00	22.58	20.09
	2 * recommendation	32.74	32.34	44.16	44.18	33.08	37.30
	4 * recommendation						
		45.80	53.16	62.60	53.06	45.12	51.94
		RLSD 0.01=0.993 0.05=0.715					0.01=0.543 0.05=0.367

the recommendation 1 ml liter⁻¹

of 60 kg. ha⁻¹) inhibited enzyme activity by 52% . Table (4) showed the interaction between the incubation period and the concentration of the pesticide in inhibiting the enzyme activity, increasing the concentration of the added pesticide increased the inhibition of urease enzyme activity in the soil, as the rate of inhibition increased when

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increasing the concentration from the recommendation to four times the recommendation from 25.84% to 46.16% at the 7-day and from 17.88% to 57.90% at the 14-day and from 24.64% to 61.84% at the 21-day, and from 19.02% to 45.58% at the 28-day, and from 13.08% to 48.26% at the 35-day incubation period. The results indicate that the effect of the incubation period on inhibiting the enzyme activity differed according to the type of organic source, as the results show an increase in the incubation period from 7 to 21 days, which led to increase the rate of inhibition of enzyme activity in the treatment of cattle manure, poultry manure and urea, then the rate of inhibition decreased with increasing the incubation period from 21 days to 35 days.. As for the treatment of corn citrus, the rate of inhibition increased at the incubation period of 7 to 14 days, then the inhibition rate decreased for subsequent periods. This is in agreement with Nasreen *et al.* (2012) who found a decrease in urease activity after 24 hours of incubation that persisted up to 20 days as well as confirmed by Anuradha *et al.* (2015) The urease activity decreased gradually with an increase in the incubation period from 30-40 days.

The treatment of poultry waste mixed with sawdust when adding the pesticide with the concentration of the recommendation and double of the recommendation showed the highest rate of inhibition of enzyme activity in the soil, it ranged from 29.00% when pesticide added at a concentration according to the recommendation to 44.18% when adding the pesticide at a concentration double of the recommendation. The treatment of urea showed the lowest rate of inhibition for all pesticide concentrations, it ranged from 14.44% when adding the pesticide with a concentration according to the recommendation to 45.80% when adding the pesticide at a concentration of four times the recommendation. Lethbridge and Burns (1976) obtained inhibition of the enzyme urease in sandy clay loam soil by 40-50% and 14-23% in silt loam soil, when different concentrations of pesticides (thimet, malathion, accothion) were added to these two soils. The results of the triple interaction (Table 4) between the incubation period, the pesticide concentration and the type of organic source indicate the highest inhibition of enzyme activity 78.7% in the treatment of alfalfa grinded after 35 days of adding the pesticide at a concentration of four times the recommendation. while the lowest inhibition rate of enzyme activity is 6.3% in the yellow corn molasses treatment after 35 days of adding the pesticide at the concentration of the recommendation.

The effect of adding the herbicide Atlants

The highest rate of inhibition of the urease enzyme activity appeared in the treatment of cows' manure, which reached 59.40% (Table 5), while the lowest rate of inhibition, 25.98%, when treating poultry waste mixed with sawdust. Inhibition of urease activity by 30.21%, 50.37% and 29.20%, respectively. This is in agreement with Bielinska and Pranagal (2007) who found the addition of the herbicide triazine led to a significant inhibition of enzyme activity in the soil. The inhibition rate increased from 33.32% at a 7-day to 37.95%, 39.28%, 40.35% and 44.24% for 14, 21, 28 and 35 days incubation

periods, respectively .The results are consistent with the findings of Kucharski *et al.* (2009) who found increasing of inhibition with increasing incubation time for the herbicide Mocarz 75 WG.

Table (5) showed an increase in the concentration of the herbicide led to an increase in the inhibition rate of urease enzyme activity among the treatments, as the rate of inhibition reached 25.91%, 36.92% and 54.26% for the recommended concentrations, 2 * recommendation and 4 * recommendation, respectively. These results are in agreement with Bharathi *et al.* (2011) who found that addition of the herbicide at a high concentration reduced the activity of urease because the microorganisms were affected by the osmotic stress caused by the herbicides and the soil, they stated that the enzyme activity is affected by the soil properties and the type of herbicide.

The increase in the concentration of pesticide and the incubation period increased the inhibition of urease enzyme activity in the soil, as the rate of inhibition increased when increasing the concentration from the recommendation to four times the recommendation from 23.90% to 45.09% at the 7-day and from 22.79% to 54.27% at the 14 days incubation period, from 26.26% to 52.50% at the 21 days incubation period, and from 26.15% to 57.95% at the 28 days incubation period, and from 30.43% to 61.50% at the 35 day incubation period. The results are consistent with Kumari *et al.* (2018) who found that effect of pesticides varies with the herbicide type , concentration, and period of incubation. Rasool & Reshi (2010) also indicated that there was a significant decrease in the activity of the enzyme urease after adding high concentrations of the pesticide.

The results indicate that the effect of the incubation period on the inhibition of the enzyme activity differed according to the type of the organic source, as the results show an increase in the incubation period from 7 to 14 days, which led to an increase in the rate of inhibition of the enzyme activity in the treatment of cows' manure , then a decrease in the rate of inhibition with an increase in the incubation period from 21 days. As for the treatment of poultry wastes mixed with sawdust, the rate of inhibition increased with the increase of the incubation period from 7 days to 28 days and the increase of the incubation period to 35 days did not significantly affect the inhibition rates. As for the treatment of ground alfalfa, the inhibition values ranged between 38.31% at a period of 7day and 35.97% at 35 days incubation period. Increasing the incubation period from 7 days to 35 days led to an increasein the rate of inhibition of the

Table (5) The effect of adding different concentrations of the herbicide Atlants on the activity of the urease enzyme under different incubation periods.

Incubation time (day)	The concentration of the pesticide	Organic source type					Incubation time * pesticide concentration
		Urea	Yellow corn molasses	alfalfa grinded	Poultry waste + sawdust	cows' manure	
7	recommendation	1.9	38.8	34.4	0.0	44.2	23.90
	2 * recommendation	3.8	46.6	34.9	16.6	52.8	30.99
	4 * recommendation	21.9	53.3	45.5	31.8	72.8	45.09

14	recommendation	5.3	35.2	7.4	7.1	58.8	22.79
	2 * recommendation	20.0	79.7	38.2	9.5	76.4	36.79
	4 * recommendation	28.0	47.0	48.1	61.9	86.2	54.27
21	recommendation	28.3	38.4	2.6	0.0	61.9	26.26
	2 * recommendation	43.2	48.0	21.5	13.5	69.0	39.09
	4 * recommendation	52.2	53.8	15.0	67.5	73.8	52.50
28	recommendation	20.0	51.0	7.1	7.8	44.7	26.15
	2 * recommendation	30.9	55.1	25.1	23.6	50.0	36.96
	4 * recommendation	36.3	67.3	65.0	63.1	57.8	57.95
35	recommendation	32.4	55.3	17.0	7.8	39.4	30.43
	2 * recommendation	45.9	53.1	39.0	18.4	47.3	40.79
	4 * recommendation	67.5	72.3	51.8	60.5	55.2	61.50
Type of organic source * pesticide concentration * incubation time		RLSD 0.01=1.918 0.05=1.412					0.01=0.993 0.05=0.715
The effect rate of the type of organic source		29.20	50.37	30.21	25.98	59.40	Effect rate of cuddling time
		RLSD 0.01=0.702 0.05=0.474					
Incubation time * Organic source type	7	9.20	46.29	38.31	16.16	56.67	33.32
	14	17.77	40.68	31.27	26.18	73.85	37.95
	21	41.29	46.79	13.07	27.02	68.25	39.28
	28	29.09	57.82	32.42	31.57	50.87	40.35
	35	48.65	60.28	35.97	28.94	47.36	44.24
		RLSD 0.01=1.159 0.05=0.862					0.01=0.720 0.05=0.474
							The effect rate of the pesticide concentration
Pesticide concentration * type of organic source	recommendation	17.60	43.79	13.73	4.58	49.84	
	2 * recommendation	28.79	48.55	31.78	16.36	59.15	36.92
	4 * recommendation	41.21	58.78	45.11	56.99	69.21	54.26
		RLSD 0.01=0.993 0.05=0.715					0.01=0.543 0.05=0.367

The herbicide (Atlants) was used in the research. Concentration 0.8 g+1.2ml.liter⁻¹

enzyme activity when treating yellow corn molasses and urea as the values increased from 46.29% to 60.28% and from 9.2% to 48.65%, respectively. . The addition of the triazine herbicide leads to a significant inhibition of enzyme activity in the soil (Bielinska and Pranagal, 2007).

The treatment of cows' manure and for all the added concentrations of the pesticide showed the highest rate of inhibition of enzyme activity in the soil. It ranged between 49.84% when adding the pesticide at a concentration according to the recommendation and 69.21% when adding the pesticide with a concentration of four times the recommendation and the treatment of poultry waste mixed with sawdust showed the lowest rate of inhibition of activity at the concentration of the recommendation and double of the recommendation. It ranged between 4.58% when adding the pesticide at a concentration according to the recommendation and 16.36% when adding the pesticide at a double of the recommendation. The increased concentration of the added pesticide

increased the rate of inhibition of enzyme activity in all treatments. Abbas *et al.* (2015) mentioned the addition of the herbicide bromoxinil to a decrease in urease activity by 30%, dehydrogenase activity by 36% and phosphatase activity by 34%, and the reason was attributed to a decrease in the number of microorganisms. The herbs inhibited the activity of the urea enzyme, especially when using urea fertilizer. The results of the triple interactions (Table 5) among the incubation period, the concentration of the pesticide and the type of organic source, the highest rate of inhibition of the enzyme activity was 86.2% when adding the pesticide at a concentration of four times the recommendation and the incubation period of 14 days for the treatment of cows' manure no inhibition of enzyme activity was shown when treating poultry waste mixed with sawdust by adding the recommendation and an incubation period of 7 days.

The effect of adding the biocide (*Trichoderma*)

The highest rate of inhibition of urease enzyme activity when adding the biocide was in the treatment of poultry waste mixed with sawdust (Table 6), as the rate of inhibition reached 52.80%, while the soil treated with ground alfalfa showed the lowest rate of inhibition, which amounted to 24.81%. Yellow corn molasses and urea, the rate of inhibition of urease enzyme activity was 49.32%, 48.62% and 41.36%, respectively. The results are consistent with that of Naseby *et al.* (2000) The urease activity decreased with the addition of trichoderma compared with the control treatment. On the other hand, Halifu *et al.*, (2019) indicated that the addition of trichoderma to the soil increased the activity of urease enzyme by 38.02% in the rhizosphere as compared to the comparison treatment.

Table (6) shows an increase in the inhibition of enzyme activity in the soil by increasing the incubation period from 7 to 28 days, as the inhibition rate increased from 31.84% at the 7-day to 56.29% at the 28-day incubation period, then the inhibition rate decreased to 50.83% at the 35-day incubation period. The increase in the concentration of the biocide added to the soil increased the rate of inhibition of urease enzyme activity between the treatments as the

Table (6) The effect of adding different concentrations of the biocide Tricoderma on the activity of the enzyme urease in the soil under different incubation periods

Recommendation concentration 1 g .40 ml⁻¹

Incubation time (day)	The concentration of the pesticide	Organic source type					Incubation time * pesticide concentration
		Urea	Yellow corn molasses	alfalfa grinded	Poultry waste + sawdust	cows' manure	
7	recommendation	20.0	16.6	8.6	0.0	25.7	14.20
	2 * recommendation	38.1	24.4	32.6	36.3	42.8	34.89
	4 * recommendation	52.3	42.2	43.7	40.9	52.8	46.42
14	recommendation	15.7	14.4	4.9	11.9	23.5	14.13
	2 * recommendation	32.8	44.9	23.4	28.5	33.3	32.63
	4 * recommendation	50.0	60.8	48.1	54.7	49.0	52.56
21	recommendation	34.3	32.6	8.5	45.9	23.8	29.05
	2 * recommendation	43.2	55.7	18.3	67.5	47.6	46.50
	4 * recommendation	52.2	68.2	34.6	78.3	59.5	58.61
28	recommendation	40.9	51.0	13.6	64.8	61.5	46.39
	2 * recommendation	47.2	59.1	24.0	72.9	71.7	55.05
	4 * recommendation	61.8	67.3	42.0	86.4	79.4	67.44
35	recommendation	30.1	54.8	12.1	56.5	44.1	39.56
	2 * recommendation	43.8	66.6	24.2	69.7	53.2	51.54
	4 * recommendation	57.5	69.8	33.0	75.0	71.4	61.37
		RLSD 0.01=1.967 0.05=1.486					0.01=0.993 0.05=0.715
The effect rate of the type of organic source		41.36	48.62	24.81	52.66	49.32	Effect rate of cuddling time
		RLSD 0.01=0.702 0.05=0.474					
Incubation time * Organic source type	7	36.82	27.77	28.36	25.75	40.47	31.84
	14	32.89	40.09	25.51	31.74	35.29	33.10
	21	43.28	52.24	20.48	63.96	43.65	44.72
	28	50.00	59.18	26.59	74.77	70.94	56.29
	35	43.83	63.80	23.13	67.10	56.28	50.83
		RLSD 0.01=1.159 0.05=0.862					0.01=0.720 0.05=0.474
							The effect rate of the pesticide concentration
Pesticide concentration * type of organic source	recommendation	28.23	33.94	9.57	35.85	35.75	28.67
	2 * recommendation	41.07	50.19	24.54	55.04	49.77	44.12
	4 * recommendation						
		54.79	61.72	40.33	67.10	62.46	57.28
		RLSD 0.01=1.033 0.05=0.737					0.01=0.543 0.05=0.367

inhibition ratio 28.67%, 44.12% and 57.28% for the recommended rate of concentrations, Recommendation *2 and Recommendation *4 respectively. The increase in the concentration of the pesticide increased the inhibition of urease enzyme

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activity in the soil at all incubation periods, as the rate of inhibition increased when increasing the concentration from the recommendation to four times the recommendation from 14.20% to 46.42% at the 7-day incubation period and from 14.13% to 52.56% at the 14 days incubation period, from 29.05% to 58.61% when the incubation period is 21 days, from 46.39% to 67.44% when the incubation period is 28 days, and from 39.56% to 61.37% at the 35 day incubation period.

The treatment of ground alfalfa showed the lowest rate of inhibition of enzyme activity at most of the incubation periods and ranged between 28.36% at the 7-day incubation period and 23.13% at the 35-day incubation period, and the treatment of cows' manure showed the highest rate of inhibition of most of the treatments and ranged between 40.47% at 7-day to 56.28% at 35-day incubation period. Increasing the incubation period from 7 days to 28 days led to an increase in the rates of inhibition of the activity of the enzyme urease in all organic sources (except for the treatment of yellow corn molasses), followed by a decrease in the inhibition of the enzyme activity.

The treatment of poultry waste mixed with sawdust and for all the added concentrations of the pesticide showed the highest rate of inhibition of enzyme activity in the soil. It ranged between 35.85% when adding the pesticide at a concentration according to the recommendation and 67.10% when adding the pesticide at a concentration of four times the recommendation, between 9.57% when adding the pesticide at a concentration according to the recommendation and 40.33% when adding the pesticide at a concentration of four times the recommendation.

The results of the triple interaction (Table 6) among the incubation period, the pesticide concentration and the type of organic source indicate the highest percentage of inhibition of enzyme activity in the treatment of poultry waste mixed with sawdust and it reached 86.4% when adding the pesticide at a concentration of four times the recommendation and the incubation period of 28 days did not show inhibition of the enzyme activity at pesticide at the recommendation concentration and incubation period of 7 days

Conclusions

1. Insecticide have the highest inhibition rate of urease activity than the fungicides, herbicides, and biocides.
2. The treatments of alfalfa grinded showed the lowest rate of inhibition of enzyme activity in the fungicide, herbicide and biological treatments.
3. Poultry waste mixed with sawdust showed the highest rate of inhibition of fungal, insecticidal and biological treatments, while it showed the lowest rate of inhibition with herbicide.
4. The highest rate of enzyme inhibition was at the 14 and 21 days incubation period after adding the fungicide and insecticide and at 28 and 35 days after adding the biocide and herbicide.

5. The inhibition percentage of enzyme was increased with increasing the concentration of the pesticides .

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