# The Influence of Pesticide Application Rates on the Activity of Some Soil Enzymes

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## ABSTRACT

The activity of four soil enzymes (cellulase, dehydrogenase, phosphatase and urease) were investigated after using different rates of herbicides (penoxalin, fluometuron) and insecticides (carbofuran and aldicarb) at different times of application.

Increasing the application rates of the tested compounds generally increased soil cellulase and urease activity, while dehydrogenase activity was decreased comparable with control treatment. Penoxalin herbicide decreased phosphatase activity, although the other compounds increased its activity. However, there were some exceptions at certain rates and times. Fluometuron decreased cellulase, phosphatase and urease activity at 1 and 38 days, and at the late periods respectively. It increased dehydrogenase activity at 1 and 56 days. Penoxalin decreased urease activity at 1 and 56 days, and increased dehydrogenase activity at the beginning of the test. Phosphatase activity was increased up to the 17th day from application. Carbofuran and aldicarb insecticides decreased both urease and phosphatase activity at 1,3 and 56 days and at 35 and 56 days respectively. They increased dehydrogenase activity at 5 days.

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## INTRODUCTION

Considerable interest in the effect of pesticides on non-target organisms has been recently developed. Since large quantities of pesticides are used in modern agriculture, the effect of many pesticides on altering the activity and microbial population, as related to soil fertility, as been investigated[1-5].

Different herbicides and some of their mixtures in amounts comparable to those which are used in agriculture did not affect the overall microbial activity of the soil microflora and soil dehydrogenase activity[6]. Triazine herbicides had no effect on soil micro-organisms, or on the biochemical processes they mediated in the soil[7]. Asulam treatments significantly reduced the cellulose decomposition[8] and had very little effect on phosphatase or dehydrogenase activity. Urease activity slightly increased when added at 16 and 160 ppm[9]. Atrazine, simazine and prometryne did not cause a significant change in the cellulose decomposing activity of the soil, even when used over long periods; while monolinuron, ametryne and 2,4-D strongly reduced this activity[10]. Carbofurna had a singificant, but temporary, effect on microbial populations and their activities[11,12].

The aim of the present work is to investigate the effect of adding different concentrations of herbicides and insecticides on dehydrogenase, cellulase, phosphatase, and urease activities in soil.

## MATERIALS AND METHODS

### Compounds used and their rates of application:

#### Herbicides

1. Penoxalin (33% EC) - (1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzineamine was used at three rates: 2,5, 5.0 and 10.0 L/feddan (which was 0.025mg, 0.05mg and 0.1mg/100g soil).
2. Fluometuron (80% wp) - [1,1-dimethyl-3-($\alpha$,$\alpha$,$\alpha$-trifluoro-m-tolyl) urea] was used at 1.25, 2.5 and 5.00 Kg/feddan (equal to 0.0125 mg, 0.025 mg and 0.05 mg/100 g soil).

#### Insecticides

1. Aldicarb (10% G) - [2-methyl-2-(methyl-thio) propionaldehyde O-(methyl carbamoyl) oxime] at 10.0, 20.0 and 40 Kg/feddan (equal to 0.1 mg, 0.2 mg and 0.4 mg/100 g soil).
2. Carbofuran (10% G) - 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate at 10.0, 20.0 and 40.0 Kg/feddan (equal to 0.1 mg, 0.2 mg and 0.4 mg/100 g soil).

### Soil Treatment

A sandy-clay-loam soil previously cultivated with beans for 30 days was collected from El-Nobaria Research Station area, air dried and seived. Portions of soil (200 g) were placed in plastic pots and treated with the mentioned doses of the pesticides. Soil moisture was adjusted at its half field capacity at all times of the test. Each treatment was replicated four times in a complete randomized design. All pots were incubated at room temperature (18 ± 2°C).

### Determination of Soil Enzymes Activity

Cellulase Activity - The method of Pancholy and Rice (1973)[13] was used. It depends on determining the amount of reducing sugar formed after incubating the soil with carboxy methyl cellulose (CMC).

Dehydrogenase Activity - The method is based upon the determination of triphenyl formazan (TPF) formed after the reduction of 2:3:5-triphenyl tetrazolium cholride (TTC), according to the procedure described by several workers[13,14,15].

Phosphatase Activity - Soil-phosphatase activity was determined by using p-nitro-phenyl di-sodium orthophosphate as a substrate with the determination of p-nitrophynol released colourimetrically, according to Tabatabi and Bremner (1969)[16].

Urease Activity - The method is based upon determination of urea residue after incubation of soil sample with urea as a substrate, according to Zantira and Bremner (1975)[17].

## Results and Discussion

The effect of certain herbicides and insecticides at different concentrations on soil dehydrogenase, cellulase, phosphatase and urease activity as a percentage ratio of the control treatment were recorded in Tables 1,2,3, and 4. The results of analysis of variance showed that the differences between concentrations of the compounds were generally significant in the case of phosphatase and dehydrogenase activity, and were not significant in urease activity. Concentrations of fluometuron and carbofuran were only significant on the cellulase activity. On the other hand, the interaction between concentration of

compound and time of application were significant. This means that the comparisons between concentrations of the compound must be undertaken at the same period of its application.

Generally aldicarb and carbofuran insecticides decreased the dehydrogenase activity at all tested time,except the activity at 5 and 56 days, when it increased (Table 1) compared with control treatment. The inhibitive effect at the beginning could be attributed to the parent compound, while the second one could be attributed to the insecticide by-products. Penoxalin herbicide increased dehydrogenase activity at 1 and 5 days, and decreased the activity at other times. Fluometuron increased the activity at 1 and 56 days and decreased the activity at other times. The decreased dehydrogenase activity recorded indicated the inhibitive effect of the compound by-products. Another stimulatory effect appeared at 56 days from application. It could be attributed to the compound decomposition.

Data in Table 2 showed that the used compounds increased the activity of cellulase enzyme at 1, 3, 10 and 56 days and decreased the activity at 5 and 38 days from application, compared with control treatment. The two herbicides concentrations increased the activity of cellulase enzyme at 1 day from application, and this increase was parallel with increasing of concentrations, while at 3 days the trends of increasing activity were reversed. This means that the primary by-products of the two herbicides decreased the activity of cellulase compared with the parent molecule. The increasing of activity at 10 days reffered to the effect of other by-products. The increase in activity at 3 days means that the micro-organism takes 3 days to cover its lage period, and the insecticidal effect becomes similar to the herbicides effect after that.

Data in Table 3 showed that the two herbicides increased the activity of phosphatase enzyme at 1,3,10 and 17 days but decreased its activity at 35 and 56 days from application, compared with control treatments. Also, the difference between the activity of penoxalin, its high and low concentrations, was not significant at all times. While there were no significant differences between the concentration of fluometuron at any time, except at 1 and 35 days, the highest concentration gave the highest activity. This data indicated that the micro-organisms which excreted phosphatase enzyme are capable of utilizing the herbicides or their by-products[18]. The decreasing of activity at the late periods means the inhibitive effect of another by-product of the compounds. Carbofuran insecticide increased phosphatase activity until 35 days and then decreased to the end of the test. Aldicarb decreased the activity the first day, and then increased at 3 days. Generally the enzyme activity decreased slightly up to the end of the experiment.

Data in Table 4 indicated that the differences between penoxalin concentration on urease activity at 10, 13 and 35 days; at 35 days for fluometuron; at 10 and 35 days for carbofuran; and at 5 and 35 days for aldicarb were significant. Also, the recorded data indicated that all compounds had an inhibitive affect on urease activity (% activity < 100%) at 1 and 56 days, but the activity increased from 10 until 35 days from application compared with control (% activity >100%). This indicated that the compounds had a toxic effect on the micro-organisms which excreted urease enzyme at the beginning and that after that these micro-organisms adapted themselves to the compounds or their by-product. The slight decrease of the activity at the

Table 1

Percentage means of soil dehydrogenase activity for the tested compounds at different rates and different time intervals.

| Compound | Rate/fad | % activity after different intervals (days) 1 | 3 | 5 | 10 | 13 | 21 | 31 | 56 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| Penoxalin | 2.5 L | 149.89 | 64.10 | 118.47 | 96.29 | 97.43 | 41.35 | 54.54 | 90.47 | 89.07 |
| | 5 L | 174.87 | 72.64 | 94.25 | 93.82 | 64.10 | 47.62 | 69.7 | 97.62 | 79.33 |
| | 10 L | 189.15 | 72.65 | 124.62 | 106.17 | 71.79 | 55.13 | 75.76 | 90.47 | 101.97 |
| | | $LSD_{0.05}$ for interaction = 25.21 | | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 8.91 | | | | | | | | |
| Fluometuron | 1.25 Kg | 164.17 | 65.52 | 74.29 | 69.13 | 69.23 | 85.21 | 75.76 | 104.76 | 88.51 |
| | 2.5 KG | 178.44 | 71.22 | 98.39 | 75.30 | 56.41 | 85.21 | 75.76 | 109.52 | 93.78 |
| | 5 Kg | 182.01 | 74.07 | 68.27 | 83.95 | 53.84 | 75.19 | 96.97 | 114.28 | 93.57 |
| | | $LSD_{0.5}$ for interaction = 12.70 | | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 4.49 | | | | | | | | |
| Carbofuran | 10 Kg | 132.04 | 74.21 | 94.38 | 76.54 | 46.15 | 95.23 | 90.91 | 119.04 | 91.06 |
| | 20 Kg | 78.51 | 78.34 | 119.14 | 54.32 | 75.43 | 92.73 | 84.85 | 132.39 | 113.46 |
| | 40 Kg | 78.51 | 68.38 | 100.40 | 85.18 | 79.48 | 82.70 | 118.18 | 123.80 | 92.08 |
| | | $LSD_{0.05}$ for interaction = 20.35 | | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 7.19 | | | | | | | | |
| Aldicarb | 10 Kg | 82.08 | 78.34 | 132.53 | 69.13 | 82.05 | 62.65 | 109.09 | 122.38 | 96.03 |
| | 20 Kg | 84.24 | 82.61 | 100.40 | 69.14 | 74.10 | 77.74 | 93.94 | 114.28 | 85.80 |
| | 40 Kg | 92.79 | 88.31 | 132.53 | 75.30 | 100. 00 | 72.68 | 121.21 | 90.47 | 96.66 |
| | | $LSD_{0.05}$ for interaction = 17.52 | | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 6.19 | | | | | | | | |

Table 2

Percentage means of soil cellulase activity for the tested compounds at different rates and different time intervals.

| Compound | Rate/fad. | % activity after different intervals (days) 1 | 3 | 5 | 10 | 38 | 59 | Mean |
|---|---|---|---|---|---|---|---|---|
| Penoxalin | 2.5 L | 148.56 | 168.44 | 100.08 | 138.60 | 87.823 | 104.16 | 124.61* |
| | 5 L | 150.86 | 135.93 | 93.103 | 145.83 | 102.116 | 104.16 | 122.00 |
| | 10 L | 162.35 | 144.51 | 102.41 | 138.60 | 89.86 | 107.64 | 124.23 |
| | | $LSD_{0.05}$ for interaction = 12.26 | | | | | | |
| Fluometuron | 1.25Kg | 148.58 | 146.84 | 102.41 | 128.47 | 87.82 | 106.10 | 120.03 |
| | 2.5 Kg | 166.66 | 124.58 | 97.76 | 145.83 | 91.90 | 121.52 | 124.71 |
| | 5 Kg | 166.29 | 126.62 | 107.07 | 125.00 | 93.95 | 135.41 | 125.72 |
| | | $LSD_{0.05}$ for interaction = 8.79 | | | | | | |
| | | $LSD_{0.05}$ for concentration = 3.11 | | | | | | |
| Carbofuran | 10 Kg | 123.56 | 126.63 | 97.76 | 135.41 | 91.90 | 128.47 | 117.29 |
| | 20 Kg | 122.12 | 126.62 | 95.43 | 131.94 | 112.74 | 107.77 | 116.10 |
| | 40 Kg | 120.68 | 124.58 | 100.08 | 114.91 | 108.24 | 104.57 | 112.18 |
| | | $LSD_{0.05}$ for interaction = 8.36 | | | | | | |
| | | $LSD_{0.05}$ for concentration = 2.95 | | | | | | |
| Aldicarb | 10 Kg | 107.70 | 122.54 | 95.43 | 139.02 | 100.07 | 125.00 | 114.96* |
| | 20 Kg | 109.18 | 134.79 | 104.74 | 138.88 | 114.37 | 104.16 | 117.69 |
| | 40 Kg | 110.62 | 118.45 | 116.38 | 135.24 | 95.24 | 121.52 | 116.24 |
| | | $LSD_{0.05}$ for interaction = 11.03 | | | | | | |

* Not significant

Table 3

Percentage means of soil phosphatase activity for the tested compounds at different rates and different time intervals.

| Compound | Rate/fad. | % activity after different intervals | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 10 | 17 | 35 | 56 | |
| Penoxalin | 2.5 L | 108.85 | 104.10 | 106.68 | 99.58 | 105.16 | 83.81 | 78.36 | 98.08 |
| | 5 L | 89.83 | 108.38 | 93.43 | 105.61 | 102.81 | 73.75 | 84.20 | 89.24 |
| | 10 L | 111.10 | 112.88 | 99.45 | 92.37 | 105.16 | 83.58 | 74.85 | 97.05 |
| | | $LSD_{0.05}$ for interaction = 8.46 | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 2.99 | | | | | | | |
| Fluometuron | 1.25 Kg | 102.10 | 130.25 | 98.85 | 103.95 | 103.75 | 62.25 | 90.44 | 98.80 |
| | 2.5 Kg | 109.60 | 130.25 | 95.23 | 102.31 | 104.69 | 78.54 | 91.61 | 101.75 |
| | 5 Kg | 114.43 | 127.20 | 98.25 | 104.74 | 110.78 | 103.92 | 93.17 | 107.52 |
| | | $LSD_{0.05}$ for interaction = 8.33 | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 2.94 | | | | | | | |
| Carbofuran | 10 Kg | 108.60 | 112.30 | 95.84 | 106.16 | 110.8 | 85.20 | 77.40 | 99.47 |
| | 20 Kg | 108.11 | 116.35 | 106.08 | 107.92 | 110.33 | 94.82 | 97.85 | 105.92 |
| | 40 Kg | 98.9 | 123.07 | 104.88 | 101.76 | 96.71 | 102.01 | 98.16 | 103.64 |
| | | $LSD_{0.05}$ for interaction = 9.64 | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 3.41 | | | | | | | |
| Aldicarb | 10 Kg | 91.39 | 150.71 | 94.63 | 103.96 | 100.94 | 71.83 | 98.16 | 101.66 |
| | 20 Kg | 96.42 | 147.69 | 107.89 | 98.46 | 102.81 | 90.03 | 92.78 | 105.15 |
| | 40 Kg | 92.36 | 139.87 | 105.48 | 100.99 | 107.04 | 95.30 | 98.22 | 105.61 |
| | | $LSD_{0.05}$ for interaction = 7.45 | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 2.63 | | | | | | | |

Table 4

Percentage means of soil urease activity for the tested compounds at different rate and different time intervals.

| Compound | Rate/fad. | % activity after different intervals | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 10 | 13 | 35 | 56 | |
| Penoxalin | 2.5 L | 95.26 | 128.20 | 115.41 | 177.33 | 150.03 | 122.79 | 97.46 | 126.64 |
| | 5 L | 92.89 | 107.69 | 107.40 | 185.33 | 129.37 | 174.83 | 95.79 | 127.61 |
| | 10 L | 90.53 | 107.69 | 106.94 | 97.33 | 98.93 | 202.72 | 98.31 | 114.63 |
| | | $LSD_{0.05}$ for interaction = 39.304 | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 12.98 | | | | | | | |
| Fluometuron | 12.5 Kg | 88.61 | 102.56 | 99.53 | 120.66 | 107.63 | 170.06 | 94.11 | 111.88 |
| | 2.5 Kg | 98.11 | 103.84 | 102.77 | 118.66 | 109.81 | 163.26 | 101.67 | 114.02 |
| | 5 Kg | 87.67 | 103.84 | 106.48 | 124.66 | 105.45 | 140.81 | 98.31 | 109.60 |
| | | $LSD_{0.05}$ for interaction = 10.722 | | | | | | | |
| | | $LSD_{0.05}$ for concentration = 3.79 | | | | | | | |
| Carbofuran | 10 Kg | 100.00 | 98.71 | 108.33 | 107.33 | 106.54 | 151.02 | 91.59 | 109.07* |
| | 20 Kg | 94.79 | 100.00 | 110.18 | 136.00 | 109.80 | 96.59 | 97.47 | 106.40 |
| | 40 Kg | 93.84 | 93.59 | 110.64 | 128.66 | 103.28 | 102.04 | 104.19 | 105.18 |
| | | $LSD_{0.05}$ for interaction = 14.97 | | | | | | | |
| Aldicarb | 10 Kg | 98.24 | 98.71 | 106.01 | 120.00 | 102.2 | 114.96 | 92.43 | 104.65* |
| | 20 Kg | 99.53 | 87.43 | 113.89 | 120.00 | 115.24 | 93.19 | 102.51 | 104.54 |
| | 40 Kg | 96.21 | 100.00 | 96.76 | 115.33 | 113.07 | 102.04 | 97.47 | 102.98 |
| | | $LSD_{0.05}$ for interaction = 13.57 | | | | | | | |

*Not significant

late periods (56 days) may be due to the absence of these by-products. The decreasing of the activity at 10 and 13 days with increasing penoxalin concentration indicated that this is an optimum concentration of penoxalin, while the increasing of enzyme activity at 35 days with increasing penoxalin concentration may be due to its metabolites The by-products of carbofuran had an opposit trend compared with penoxalin by-products.

Previous data showed that penoxalin increased the activity of urease, cellulase, phosphatase and dehydrogenase until 35, 10, 17, and 1 days from application, respectively compared with control.

Fluometuron inhibited the urease activity at the beginning of the test and had a slightly increasing effect along the experiment. In the case of cellulase, fluometuron increased the activity along the test except at 38 days, but the phosphatase activity increased up to 17 days. Its activity decreased in the late period. It increased the activity of dehydrogenase only at the first day.

Carbofuran increased urease activity from 5 to 35 days; cellulase activity along the experimental time; phosphatase activity until 35 days; but with dehydrogenase there was a remarkable inhibitive effect.

Aldicarb had no effect on urease activity at the beginning and started to increase urease activity up to 35 days, also with cellulase it had the same trend. It depressed the activity of phosphatase at the beginning of the experiment and increased its activity up to 17 days, while with dehydrogenase it decreased its activity up to 21 days, and slighly increasing at the end of the experiment.

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