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Effect of Malathion on Microbial Population, Acid and Alkaline Phosphatase Activity of Soil

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Abstract: ***In this study the response of microbial populations, acid and alkaline phosphatase activities of microorganisms in garden soil after incorporation of the pesticide Malathion [S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate] were examined. The effect of malathion was observed over a period of ten days in three different concentrations each viz., 1%, 0.5% and 0.2%. Total microbial count was highly affected in presence of malathion and persisted up to 7 days when the population of soil microbes was found to be highest in untreated soil sample ($7.55x10^6$ cells per gram of soil) followed by samples having 0.2% ($6.2x10^6$ cells per gram), 0.5% ($1.4x10^6$ cells per gram) and undiluted concentrations ($0.6x10^6$ cells per gram) of the pesticide. Phosphatase activity in soil was unaffected initially (up to 3 days) and then sharply decreased on day 7 with 10.359µg/g/hr, 15.263µg/g/hr, 14.44µg/g/hr and 10.144µg/g/hr of p-nitrophenol in control, 1%, 0.5% and 0.2% concentrations of malathion treated soil respectively.***

Keywords: Malathion, organophosphate pesticide, garden soil, microbial population, acid and alkaline phosphatase

1. Introduction

Soil is the unconsolidated top layer of the earth's crust, formed by mineral particles, organic matter, water, air and living organisms. It is an extremely complex and dynamic medium containing many free enzymes, immobilized extracellular enzymes and enzymes within microbial cells which forms the substratum for the growth of a wide range of microflora and microfauna. Microorganisms, including fungi and bacteria, affect chemical exchanges between roots and soil and act as a reserve of nutrients. They also secrete different chemicals which affect the soil in different ways. A wide variety of synthetically produced chemicals including insecticides, fungicides, herbicides and other pesticides are utilized in modern agriculture which on injudicious application greatly affect the soil microorganisms and soil enzymes. These functions are worthy of study because of their socio-economic as well as environmental importance. Enzymes are the organic catalyst in biological reactions without themselves being altered. Soil enzymes indicate the soil quality and play an important role in organic matter decomposition and nutrient cycling. One such enzyme, Phosphatase, is a hydrolysing enzyme that removes a phosphate group from its substrate by hydrolysing phosphoric acid mono esters into a phosphate ion and a molecule with a free hydroxyl group. A common phosphatase in many organisms is alkaline phosphatase. Alkaline phosphatase is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids, called dephosphorylation. Acid phosphatase is a widely found soil enzyme, used to free attached phosphate groups from other molecules. Phosphatase enzymes are also used by soil microorganisms to access organically bound phosphate nutrients. An assay on the rates of activity of these enzymes may be used to ascertain biological demand for phosphates in the soil.

Malathion is a pesticide of relatively low human toxicity and the most commonly used organophosphate insecticide in the US [1]. It is a cholinesterase inhibitor, acts as non-systemic pesticide and acaricide with contact, stomach and respiratory action, widely used in agriculture, residential landscaping, public recreation areas, and in public health, pest control programs such as mosquito eradication, to control many insect pests in a wide range of crops [2]. When a synthetic pesticide is released into the environment, only a small fraction of it reaches the target organism, while the remaining interferes with local metabolism or enzymatic activities, and also affects human health by entering into the food chain, which is a matter of public concern. Thus, it is required to estimate soil biological responses to the pesticides in terms of soil enzyme activities.

2. Materials and Methods

The soil samples were collected from the college garden on September 3, 2015. Separate plots that received no application of pesticides were used as controls. Around 20 kg of the soil sample was collected to a depth of 10-15 cm with no history of pesticide treatment.

In the laboratory, plant material and soil macrofauna were removed, and soil samples sieved and mixed and then in semi dry condition divided and put in 1kg polythene bags. 10 bags of soil were kept untreated (to be used as control), the rest were treated with the pesticide of different concentrations, 3 bags each for recommended dose of the pesticide, 2 times and 5 times dilution of the recommended dose respectively. Controls, with soil only, were included within all tests.

2.1 Dosage

For recommended dose (T1= 0.002 ml per ml of water)
0.6 ml of Malathion (25%) in 299.4 ml of distilled water = 300 ml of 1% Malathion
For 2x diluted dose (T2=0.0016 ml per ml of water)
150 ml of T1 in 150 ml of distilled water = 300 ml of 0.5% Malathion
For 5x diluted dose (T3=0.0006 ml per ml of water)
60 ml of T1 in 240 ml of distilled water = 300 ml of 0.2% Malathion

Where T1 the dose recommended for use by the farmers and T2 and T3 are dilutions made from the recommended dose of the pesticide.

The biological activity of the soil microbes and the physico-chemical properties of the soil were studied and represented in the form of tables and graphs. Soil microbes from treated and control soils were grown on nutrient agar (Peptone- 10g, NaCl- 5g, Agar- 2%, Distilled water-1000ml, pH- 7.5) media and their population count was made by dilution plate technique [3]. Phosphatase activity was measured colorimetrically by using PNPP (paranitrophenyl phosphate). Aliquots of soil sample (5gm each) were mixed with 20 ml of paranitrophenyl phosphate (10µg/ml) in culture tubes, incubated for two hours, except for the blank sample, and then centrifuged. The blank sample was mixed with PNPP and immediately centrifuged. Now from each centrifuged supernatant, 1ml is taken in labelled test tubes and 2ml of 0.1N NaOH is added. The absorbance of each sample is then estimated at 420nm by using a UV-visible spectrophotometer. The standard curve was prepared by taking 2, 4, 6, 8 and 10µg/ml solutions of p-nitrophenyl phosphate in tris buffer (acidic and alkaline) and the total amount of p-nitrophenol is expressed as µg/g of soil per hour.

3. Result and Discussion

The changes in the total microbial population, alkaline and acid phosphatase activity recorded on day 0, day 3, day 7 and day 10 have been tabulated as follows:

On day 0, microbial population remained almost equal in treated as well as untreated soil with $7.45x10^6$ cells per gram of soil in control soil sample, $7.2x10^6$ cells, $7.35x10^6$ cells and $7.15x10^6$ cells per gram of soil in soil sample treated with recommended, 2 times diluted and 5 times diluted dosage of malathion respectively. From day 3 onwards, microbial population was highest in control soil sample with $7.35x10^6$ cells, $7.55x10^6$ cells and $7.65x10^6$ cells per gram of soil on day3, day 7 and day 10 respectively, whereas it was lowest in the soil sample treated with recommended dose of malathion showing $1.5x10^6$ cells, $0.6x10^6$ cells and $1.5x10^6$ cells per gram of soil on day 3, day 7 and day 10 respectively. With increase in the time interval of application of the pesticide, the microbial population decreases from day 0 to 10th day in case of treated soil. These results can be supported by several works on the effect of malathion which have been published previously by various workers. Malathion at 100-300mg/g had specifically toxic effect on certain type of microorganisms but stimulated the growth of another types [4]-[6]. Earlier works show the inhibition percent of soil bacteria by malathion at 250ppm after 24, 48 and 72 hours to be 16%, 24% and 40% respectively and that of soil fungi to be 44%, 47% and 58% respectively [7].

Effect of malathion on alkaline and acid phosphatase activity showed similar results. On day 0, malathion did not affect the phosphatase activity very much and the phosphatase activity was found to be same at all concentrations of the pesticide. On 3rd day also, the pesticide was not much effective on the enzyme activity. But on 7th day the activity of alkaline and acid phosphatase was found to decrease with increase in concentration of the pesticide. Finally on 10th day it was found that the activity of alkaline phosphatase increased slowly but the acid phosphatase activity of the microbes showed a further decreasing effect towards the action of malathion. This shows that malathion is slow in action, moderately persistent in soil and its effect on the enzyme activity gradually decreases after the 10th day of its application [8]-[11]. Also, the effect of different concentrations of the pesticide was not same against the acid phosphatase activity of soil microbes as they show variation during different periods of time. This is because Malathions are less effective on soil microbes and their activities [12]. In addition to that, population of phosphate solubilizing bacteria depends on different soil properties (physical and chemical properties, organic matter, and Phosphorus content) and cultural activities [13].

It was observed that the effect of the pesticides on the soil microbial population was not uniform in all cases and their effect diminished gradually after a few days. This is because several microorganisms play a major role in the breakdown or degradation of pesticides in soil. Most of the pesticides applied in the soil suppress the microbial activity in soil but are soon lost from the soil, resulting in the microbial population to rebound. Some investigations resulted in the identification of microbial isolates which are apparently responsible for the accelerated degradation of individual pesticides [14]-[16]. Most of the organophosphate and carbamate pesticides like malathion and parathion disappear more quickly than organochlorines and other pesticides [17]. The most influential bacterial strains on the acceleration of malathion degradation rate include *Bacillus licheniformis*, *B. pseudomycoides* and *Pseudomonas aeruginosa* [18].

3.1 Dilution plate result for Malathion treated soil

Table 1: Effect of different concentrations of malathion on microbial population per gram of control and treated soil

DOSE→ TIME↓		CONTROL (number x 10^6)	RECOMMENDED (number x 10^6)	2x DILUTED (number x 10^6)	5x DILUTED (number x 10^6)
0hr	Number of cells/gm of soil	7.2	7.1	7.2	7.5
		7.7	7.3	7.5	6.8
	Mean	7.45	7.2	7.35	7.15
	SD	0.17	0.07	0.1	0.24
	Remarks	Creamy, white, small, round colonies	Creamy, white, small, round colonies	Large, white, mycelia like colonies	Large, white, mycelia like colonies
Day 3	Number of cells/gm of soil	7	1.7	2.3	4.8
		7.7	1.3	3.5	5.2
	Mean	7.35	1.5	2.9	5
	SD	0.24	0.14	0.42	0.14
	Remarks	Creamy, white, small, round colonies	Creamy, white, small, round colonies	Large, white, mycelia like colonies	Large, white, mycelia like colonies
Day 7	Number of cells/gm of soil	7.2	0.7	1.1	5.5
		7.9	0.5	1.7	6.9
	Mean	7.55	0.6	1.4	6.2
	SD	7.2	0.7	1.1	5.5
	Remarks	Creamy, white, small, round colonies	Creamy, white, small, round colonies	Large, white, mycelia like colonies	Large, white, mycelia like colonies
Day 10	Number of cells/gm of soil	7.4	1.9	2.5	4.8
		7.9	1.1	2.7	3.3
	Mean	7.65	1.5	2.6	4.05
	SD	0.17	0.28	0.07	0.53
	Remarks	Creamy, white, small, round colonies	Creamy, white, small, round colonies	Large, white, mycelia like colonies	Large, white, mycelia like colonies

3.2 Alkaline phosphatase activity of Malathion treated soil

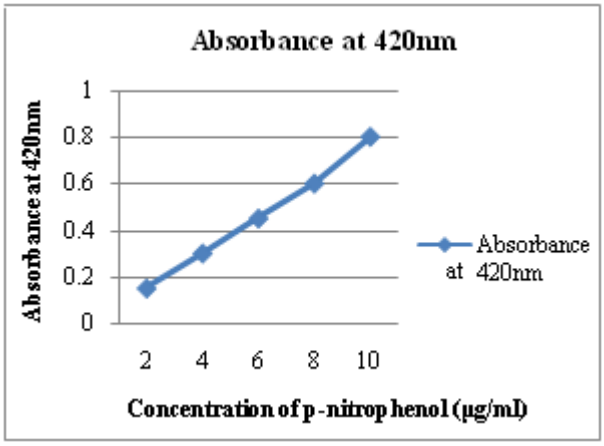

Figure 1: Standard curve of P-nitrophenol in alkaline medium at 420 nm

3.3 Acid phosphatase activity of malathion treated soil

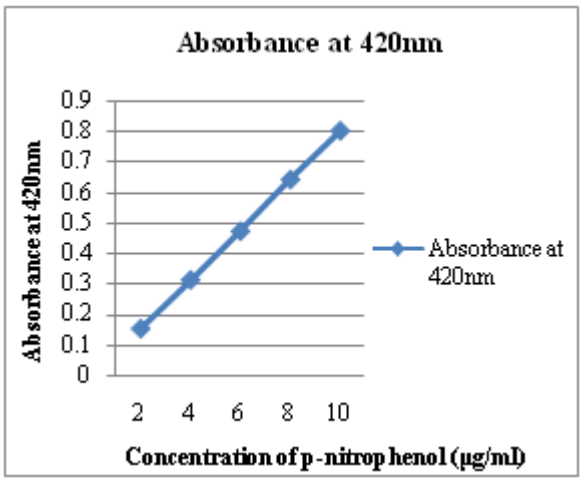

Figure 2: Standard curve of P-nitrophenol in acidic medium at 420 nm

Table 2: Effect of different concentrations of malathion on alkaline phosphatase activity of soil microbes during different periods of time

	dose of the pesticide			
days ↓ Amt.→	control (µg/g/hr)	Recommended (µg/g/hr)	2times diluted (µg/g/hr)	5times diluted (µg/g/hr)
0hr	6.899	7.374	6.498	5.044
S.D.	0.113	0.044	0.029	0.034
3rd day	7.695	10.383	9.999	9.704
S.D.	0.119	0.004	0.010	0.004
7th day	0.025	0.072	0.141	0.129
S.D.	0.008	0.024	0.047	0.143
10th day	0.319	0.569	0.202	0.397
S.D.	0.059	0.223	0.048	0.008

Amt. = amount of p-nitrophenol (µg/g/hr)
S.D. = standard deviation of amount of p-nitrophenol (µg/g/hr)

Table 3: Effect of different concentrations of malathion on acid phosphatase activity of soil microbes during different periods of time

	dose of the pesticide			
days ↓ Amt.→	control (µg/g/hr)	Recommended (µg/g/hr)	2times diluted (µg/g/hr)	5times diluted (µg/g/hr)
0hr	80.878	94.571	78.46	109.352
S.D.	0.268	0.313	0.222	0.911
3rd day	121.174	195.251	181.897	189.897
S.D.	0.251	0.956	0.332	39.433
7th day	10.359	15.263	14.441	10.144
S.D.	0.055	0.246	0.173	4.741
10th day	2.329	7.693	7.373	5.974
S.D.	0.246	0.019	0.112	0.304

Amt. = amount of p-nitrophenol (µg/g/hr)

S.D. = standard deviation of amount of p-nitrophenol (µg/g/hr)

4. Conclusion

The widespread agricultural use of pesticides resulted in these chemicals entering soil and water ecosystems [19]. Pesticides applied to soil at planting persist during the development of plant roots. Therefore, a portion of the pesticide likely interacts with microorganisms in soil and rhizosphere [20].

The use of pesticides in minimum effective doses may be beneficial but due to unjustifiable use their harmful effects have become manifold and far reaching. Sadly, chemical pesticides still continue to be used in large proportions in many parts of the world which interfere with the soil-microbe and soil-enzyme interactions, ultimately leading to environmental degradation. As such, it is hoped that this work and its results will be beneficial to the agricultural communities and thereby encourage them to switch to less toxic or biological alternatives for our own wellbeing.

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