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Effect of Some Organophosphorus Insecticides on Soil Microorganisms Populations under Lab Condition

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Abstract Three insecticides malathion, diazinon and dimethoate were used separately to the soil at 50, 100 and 250 ppm. Their effects were examined at 24, 48 and 72 hrs on the population of bacteria, fungi and actinomycetes under lab conditions. The microbial communities were investigated using the standard dilution plate technique. All soil microbes affected with insecticides, the inhibition style similar in all microorganism which is depending on concentration and time of exposure. All results were significant ($p \leq 0.05, 0.01$).

Keywords Microbial Population, Insecticides, Soil Microorganisms, Malathion, Diazinon, Dimethoate

1. Introduction

Microbial ecology studies the relationship between microorganisms and their environment. A major aim of it is to study the abundance, localization and activities of microorganisms *in situ* in order to understand their eco-physiological roles in natural ecosystems[1]. Understanding microbial ecology is extremely important, because the relationships between microorganisms and their environme nts have a crucial role in environmental restoration, food production and bioengineering of useful products such as antibiotics, food supplements and chemicals. Using microbes to study ecology helps to understand complex ecosystems because bacteria are small but with very large populations and can be studied thanks to their speed of reproduction, which is rapid when compared to larger organisms. Biological and biochemically mediated processes in soil and water are significant for ecosystem functions[2]. Soil representing a good environment of great species of bacteria, fungi, viruses, algae and protozoa because it is complex, heterogeneous and dynamic part of environment. Microorganisms are found in large numbers in soil with bacteria and fungi being the most prevalent. It is stated that usually more than 10^9 microorganisms are present per gram of soil representing 4000 to 7000 different genomes and biomass of 300 to 3000 kg per ha[3]. Soil microbes communities effected by many conditions one of these insecticides used, their effects are variable according to types, doses and field conditions. Malathion, diazinon and dimethoate are anon-systemic, wide-spectrum organophosp hate insecticides effects on soil diversity microbes, Dimethoate (0.2%), phorate at 300 fg/g and malathion at 100-300 fg / g had specifically toxic effect on one type of microorganisms but stimulated the growth of another type[4, 5, 6, 7].

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2. Materials and Methods

2.1. Site Description and Soil Samples Collection

This study was conducted at the Environmental research center / university of technology, Baghdad, Iraq, from April-November 2010. The annual temperature averages 31.2°C and the annual rainfall averages 67.5 mm while annual average of relative humidity 42. Experiments were arranged based on randomized complete block design with three replications, soil samples were collected from university of technology gardens belong to (Typic Torrifluvent) at a depth 10-20 cm by sterile spatula, putted in polyethylene clear bags (capacity 1 and 2) Kg and transported to the lab within one hour, soil was dried by air, pulverized and sieved by 2mm mesh. Some chemical and physical properties of soil were determined according to[8, 9].

2.2. Pesticide Solutions Prepare and Experimental Design

Three commercial 40% insecticide solutions (Malathion, Diazinon and Dimethoate) (Agro-china Co., China) were used to study their effects on soil microbes communities. 1000 ppm stock solution of each insecticide were prepared and kept in dark cold conditions until use. 10 gm of dry sieved soil putted in screw cap universal tube, three concentrations of each insecticide were added separately (50, 100, 250) ppm for (24, 48 and 72)hrs with three replications of each treatment, tubes without added considered as control. Total microorganisms and yeast-mold were determined using the dilution method[10].

1. **Total viable count of soil bacteria** was determined by adding 1ml of soil suspension at appropriate dilution (10^{-4}, 10^{-5} and 10^{-6}) on solidified nutrient agar (Biomark Labolatories, India) and incubated for 48 hrs at 28±2ºC.

2. **Total soil fungi** was determined on potato dextrose agar(Biomark Labolatories, India) supplemented by 100µg/ml *chloramphenicol,* 1 ml of appropriate dilution (10^{-4}, 10^{-5} and 10^{-6}) was added and separated thoroughly, all petri dishes incubated for 72 hrs at 28±2ºC.

3. **Total count of Actinomycetes** was determined as shown above by using starch ammonium agar (Biomark Labolatories, India) and all petri dishes incubated for 10 days at 28±2ºC.

2.3. Statistical Analyses

Complete randomized designed was followed in this study, all data was analyzed by using SPSS software program version 16 by using ANOVAI test and multiple comparisons.

3. Result and Discussions

3.1. Chemical and Physical Properties of Soil

Table 1. Soil chemical and physical properties

Property	Value
pH	7.23
Electronic conductivity	3.15 mS/m
Organic matter	21.3 gm/kg
Cation exchange capacity	26.5 cmol+/kg
Potassium K^+	0.1 mg/kg
Phosphor Ph	4.5 mg/kg
Humidity	27.6%
Volumetric distribution of soil particles %	Clay 35%
	Silt 44%
	Sand 21%
Total microbes	Total bacteria 4.2×10^7 cell/gm
	Total fungi 2.3×10^3cell/gm

Table 1. shows some chemical and physical properties of 10 samples of cultivated soil composite, electronic conductivity was 3.15 mS/m which dependent on percent of humidity 27.6 % and percent of clay 35%, from the table 1 we can see reduction of humidity rate because of sedimentary nature of the soil that makes them lose of water easily and make ions concentrated, beside loses of nutrients, their microbial communities are poor as compare with another soils that have high content of organic matter and high humidity rate[11].

3.2. The Effects of Insecticides on Soil Bacteria

The effects of insecticides on the number of bacteria cells per gram of dry soil are presented in table 2. From the results we can see reduction and decreasing in the total bacterial count with increasing in the insecticide concentrations and incubation time, as a compare with control group. When we calculate the inhibition percent at 250 ppm of three insecticides at 24, 48 and 72 hours the readings were 16%, 24% and 40% for malathion, 16%, 44% and 48% for diazinon 24%, 32% and 40% for dimethoate respectively the most potential impact was for diazianon with significant deference at P≤0.05, 0.01 the results of current study didn't agree with many studies used insecticides on soil microbial communities[12, 7] who notice that the presence of pesticides led to an increase in the total number of soil microbes, nevertheless, the present study shows that the presence of insecticides led to inhibition in the growth rate of soil bacteria.

This may be due to unexposed previously of these bacteria to such insecticides and not developed a resistant mechanisms for these toxic substances or the exposed time un sufficient for Consumption these materials by bacteria as a source of nitrogen and carbon or the vegetation cells of bacteria are affected negatively by insecticides while the spores stayed alive, AL. Rasslany and her collogues, 2010 observed that microbial population suffered significant decreasing after 7 days of cypermethrin exposed in loamy sand soil, while Suhayl and Al-Samaraay 2010 found that a mixture of herbicide at recommended concentrations have a potential impact on 12 strains of local *Azotobacter sp.* on the third day, while Ahmed and Ahmed 2006 found that some insecticides cause significantly reduction in bacteria number in the lab scale while filed didn't show any effects of these materials.

Table 2. Effects of insecticides on total bacterial count $\times10^6$ cell/gm

concentration ppm / Insecticides	Malathion,			Diazinon			Dimethoate		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control	25±0.23	25±0.43	25±0.65	25±0.23	25±0.43	25±0.65	25±0.23	25±0.43	25±0.65
50	23 ±0.32*	22 ±0.18*	20 ±0.44*	24±0.63*	23±0.17*	22±0.54*	21±0.44*	20±0.18*	19±0.56*
100	23 ±0.16*	21 ±0.45*	17 ±0.36*	22±0.27*	21±0.18*	21±0.13*	21±0.76*	18±0.13*	17±0.45*
250	21±0.23*	19 ±0.23*	15 ±0.29*	21±0.34*	14±0.45*	13±0.67*	19±0.65*	17±0.45*	15±0.18*

- *Significant at P≤0.05, 0.01
- Each number refer M±SD of three replicate

3.3. The Effects of Insecticides on Soil Fungi

The fungi less prevalent than bacteria in the soil but their important role as a decomposers not less than other soil microbes, impact of three insecticides on fungi was shown in table 3. the influence on fungi is similar that in bacteria, the inhibition style was dependent on concentration level beside of incubation time the most inhibition rate was seen in 250 ppm at 72 hrs reduction percent at 250 ppm 44%, 47% and 58% for malathion 33%, 35% and 41% for diazinon 44%, 52% and 59 % for dimethoate.

The spores forming ability of fungi enable them to resist hard conditions like high temperature, alkalinity, and acidity…. Ect, But in the current study the presence of these substances affect negatively on total number of fungi. In spite of hydrolyses susceptible of insecticides but their impacts was obvious on viability of fungi these result absolutely disagree with[7] and be through with other[16].

Table 3. Effects of insecticides on total fungal count $\times 10^3$ cell/gm

concentration ppm / Insecticides	Malathion,			Diazinon			Dimethoate		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control	18±0.11	17±0.45	17±0.65	18±0.11	17±045	17±0.65	18±0.11	17±045	17±0.65
50	16±0.21*	15±0.32*	12±0.67*	16±0.65*	15±0.67*	13±0.21*	16±0.21*	13±0.34*	12±0.65*
100	12±0.45*	11±0.51*	9±0.58*	14±0.21*	12±0.87*	12±0.66*	13±0.66*	11±0.23*	10±0.24*
250	10±0.65*	9±0.17*	7±0.65*	12±0.43*	11±0.25*	10±0.53*	10±0.41*	8±0.76*	7±0.65*

- *Significant at $P \leq 0.05$, 0.01
- Each number refer M±SD of three replicate

3.4. The Effects of Insecticides on Soil Actinomycetes

Table 4. Effects of insecticides on total number of Actinomycetes $\times 10^2$ cell/gm

concentration ppm / Insecticides	Malathion,			Diazinon			Dimethoate		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control	55±0.31	57±0.21	61±0.13	55±0.31	57±0.21	61±0.13	55±0.31	57±0.21	61±0.13
50	46±0.61*	43±0.62*	41±0.98*	48±0.45*	45±0.63*	43±0.51*	45±0.91*	44±0.14*	39±0.15*
100	35±0.45*	31±0.65*	31±0.87*	41±0.61*	36±0.31*	32±0.64*	38±0.44*	37±0.54*	28±054*
250	21±0.65*	19±0.29*	17±0.55*	28±0.13*	30±0.25*	28±0.65*	26±0.71*	25±0.56*	21±0.15*

- *Significant at $P \leq 0.05$, 0.01
- Each number refer M±SD of three replicate

The results of the effect of different rates of insecticides at different times on actinomycetes population are shown in table 4. All treatment significantly ($P \leq 0.05$, 0.01) reduced in actinomycetes number in the soil. It is clear that the impact associated with the concentration and incubation time. The highest influence can be seen in 250 ppm at 72 incubation periods with inhibition rate 72.13% when treated with malathion, 54% with diazinon and 65.6% with Dimethoate. These results correspond with[17] and his colleagues 2007 when he tests two insecticides karate and thiodan on four different soil microorganisms. The three insecticides malathion, diazinon and Dimethoate have the same effects on three types of soil microorganisms.

REFERENCES

[1] Grenni P. 2011. Effects of Pesticides and Pharmaceuticals on soil and water bacterial communities, PhD thesis in Environmental Science University of Milano-Bicocca .

[2] Zabaloy MC, Garland JL, Gomez MA, 2008. An integrated approach to evaluate the impacts of the herbicides glyphosate, 2, 4-D, and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina. Appl. Soil Ecol. 40:1-12.

[3] Ranjard L and Richaume, AS 2001. Quantitative and qualitative microscale distribution of bacteria in soil. Research in Microbiology 152: 707-716.

[4] Martinez-Toldo, MV; Salmeron V; Rodelas B; Pozo C and Gonzalez-Lopez J. 1993. Studies on the effects of a chlorinated hydrocarbon insecticide, lindane, on soil microorganisms. Chemosphere, 27:(11), 2261-2270.

[5] Gonzalez-Lopez J; Martinez-Toledo MV; Rodelas B and Salmeron V 1993. Studies on the effects of the insecticide, phorate and malathion, on soil microorganisms. Env. Toxic. Chem. 12 (7): 1209-1214.

[6] Mandic L; Dukic D; Govedarica M and Stamenkovic S 1997. The effect of some insecticides on the number of amylolytic microorganism and azotobacters in apple nursery soil. Jug. Voc., 31 (2): 177-184

[7] Digrak M and Kazanici F 2001. Effect of some organophosphorus insecticides on soil microorganisms. Turk. J. Biol., 25: 51-58.

[8] Page AL; Miller RH and Keeney DR 1982. Methods of soil analysis. Part (2).2nd ed. Wisconsin. USA.

[9] Black CA. 1965. Method of Soil Analysis . Part (1). Physical properties Am. Soc. Agron. Inc. puplisher Madison. Wisconsin, USA.

[10] Collins CH; Lyne RM; Grange JM; Collins and LyneÕs. 1989. Microbiological Methods. Sixth Edition. London, Butterworths Co., Ltd., 410.

[11] Talati NR; Attri SC and.Mathur SK. 1975. Moisture studies and their relationship with some of the soil charcteristic. J.Indian.S.S.32:91-588.

[12] Stanlake GJ and Clark JB. 1975 Effects of a Commercial Malathion Preparation on Selected Soil Bacteria. Applied Microbiology, Vol 30, (2) p. 335-336.

[13] AL. Rasslany IA; Kadhum SJ and Faker SJ 2010. Effect of insecticide Cypermethrin on the biological community in loamy sand soil, J Thi Qar Science Vol 2, P 81-90.

[14] Suhayl FM and Al-Samaraay EK 2010. Effects of deferent levels of a herbicides mixture on *Azotobacter sp* at deferent incubation time. Diyala Journal for Pure Science Vol: 6 (4) P 192-201.

[15] Ahmed S and Ahmad MS 2006. Effects of insecticides on the number of soil bacteria under laboratory and field conditions Pak. Entomol. Vol. 28(2) P 63-68.

[16] Dalshad RA 2012. Effect of some pesticides on soil microorganisms in Kirkuk province under field conditions, Journal of Al-Rafidain Agr.Vol: 40. (1).

[17] Adeba ayo TA; Ojo OA and Olaniran OA 2007. Effect of two insecticides karate and thiodan on population dynamics of four different soil microorganisms. Research Journal of Biological Sciences Vol: 2 (5), P557-560.