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FIELD AND LABORATORY STUDIES FOR EVALUATING THE TOXICITY OF SOME PESTICIDES ON SOIL MICROORGANISMS.

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ABSTRACT

Incessant and indiscriminate use of some agrochemicals in agricultural production has elicited fears of changes in microbial populations and the activities of individual species of micro-organisms. Present study was carried out to study the effect of three pesticides (Chloropyrifos (1L/200L.Water), Lambada cyhalothrin(50g/100L.Water) and Emamectin benzoate(120g/400LWater)) at field application rate on total population count of microorganism in phasoulus vulgaris field . Also measured the sensitivity of some species, bacteria (*Rhizobium leguminosarum*. And *Bacillus thuringiensis*) , fungi (*Trichoderma harzianum and Trichoderma viridi*) and actinomycetes (*Streptomyces griseorubens and Streptomyces cavourensis* at field and double application rate to these pesticides .

Data indicated that, Chloropyrifos stimulated significantly the proliferation of all of the microorganism. Lambada-cyhalothrin decreased population of Bactria at 1 DAT (day after treatment) until 15 DAT and returned to increase at 21 DAT . For fungal population, the total count of the soil fungi was decreased after the addition of Lambada-cyhalothrin . Actinomycetes was also significantly inhibited during the period from 1 to 15 DAT by Lambada-cyhalothrin, and subsequently recovered to a similar level of control. Emamectin benzoate in general increased population of bacteria and actinomycetes and decreased population of fungi. Concerning the sensitivity study we found that , Chloropyrifos inhibited significantly the growth of the tested species at different rates with a positive correlation. Chloropyrifos was the most potent compound followed by Lambada-cyhalothrin while Emamectin benzoate did not happen any effect on tested species. For radical growth of *T.harzianum and T. viridi* Chloropyrifos , Lambada-cyhalothrin and Emamectin benzoate showed the same level in its impact on the bacteria and actinomycetes

Keywords: Lambada-cyhalothrin, Emamectin benzoate, Chloropyrifos, microbial populations, sensitivity of microorganism

INTRODUCTION

Pesticides are applied annually in modern agriculture to increase the production by controlling the harmful effects caused by the target organisms including insects, fungi, bacteria, viruses as well as grasses grown in between the economical crops (Liu and Xiong, 2001)

Organic substances of any kind cannot escape degradation by microorganisms. Insecticides are no exceptions. Many of them are utilized by microorganisms as energy and as nutritional sources (Venkateswarlu and Sethunathan 1984; Itoh 1991; Bhuyan *et al.,*. 1992). As a consequence, the population density of active microorganisms increases which favorably influence biological transformation of nutrient elements in soil.

Moorman (1989) found that total microbial populations in soils are unaffected or slightly affected by pesticide applications but populations and

activities of individual groups of microorganisms (e.g. cellulose decomposers) are largely affected. He also noted that pesticide concentrations greater than recommended doses cause interruptions in microbial activities. A decrease in the number of rhizobial survivors on the seeds of Bengal gram (Cicer arietinum) treated with Aldrin and lindane and inoculated with chickpea rhizobium (Suneja and Dogra 1984)

Early investigators, however, were of the opinion that not all pesticides have adverse effects on soil biota, Rosas and Carranza Destorani (1987) Reported that no effect of parathion have been showed on the growth and viability of Pseudomonas aeruginosa in soil. While, Schuster and Schroder (1990) found only slight and short-lived side effects on microbial activities which usually disappeared before further treatments.

There is no definite conclusion can be made on the effect of insecticides on microorganisms and their associated transformations of nutrients in soil, since different groups of insecticides exhibit manifold variations in toxicity (Matsumura and Boush, 1971; Simon-Sylvestre and Fournier, 1979).

Based on above reports, an experiments were conducted to investigate the effect of Chloropyrifos, lambada cyhalothrin and emamectin benzoate on population count of microorganism in soil, and the sensitivity of some strain (bacteria (Rhizobium sp. And *Bacillus thuringiensis)* , fungi (*Trichoderma harzianum and T. viridi*) and actinomycetes (*Streptomyces griseorubens* and *S. cavourensis*) to these pesticides.

MATERIALS AND METHODS

I – **Material**

1. pesticides

Three pesticides from different groups, which well known and commonly used in Egypt, were used as commercial formulation type in this working. All tested pesticides were obtained from Research Institute of Plant Protection

a. Chloropyrifos

Trade name : chlorzid 48 % EC

chemical name : *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate

b. Lambada-cyhalothrin

Trade name : lambada super 10% Wp

Chemical name: α- cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-imethyl cyclopropane carboxylate

c. Emamectin benzoate

Trade name : proclaim 5% S. G

Chemical name: (4"*R*)-5-*O*-demethyl-4"-deoxy-4"-(methylamino) avermectin A_{1a} + (4"*R*)-5-*O*-demethyl -25- de(1-methylpropyl) -4"- deoxy-4"-methylamino)- 25 -(1-methylethyl) avermectin A_{1a} (9:1)

2. Microorganisms

(Rhizobium *leguminosarum* And *Bacillus thuringiensis*) from genetic department , (*Trichoderma harzianum* and *T. viridi*) (*Streptomyces griseorubens* and *S. cavourensis)* from plant pathology department.

3. Media used

a. Media used for total population count

Nutrient agar medium (Society of American Bacteriologists, (1951) was used for enumerating of soil bacteria.

Viable counts for actinomycetes were performed using a starch casein agar (kuster&Williams 1966)

Streptomycin–rose Bengal medium (Martin, 1950) was used for estimation of fungal population.

b. Media used for pesticides sensitivity assessment

Yeast extract mannitol (YEM) media was used for *Rhizobium leguminosarum* (Vincent, 1970).).

Nutrient agar was used for *Bacillus thuringiensis* . Starch nitrite agar media was used for actinomycetes strains .

Potato Dextrose Agar (PDA) was used for fungi isolates .

II -Methods

1. Effect of tested pesticides on population count of microorganisms in phasoulus vulgaris field

a. **Field and soil samples**

Phasoulus vulgaris field were treated with the field application rate of the tested pesticides at Agriculture faculty farm, Mansura University, Egypt. The untreated field was without any pesticides treatment. Soil samples were taken at random at a depth of 15 cm from all over the field and mixed properly to make one composite sample. Sample were collected at intervals days of 24h, 7, 15, 21, 28 days after treatment in plastic bags for microbiological examinations.

Ten gm of soil was mixed with 90 ml of sterilized water and mixed thoroughly. 1 ml from the solution was then mixed in 9 ml sterilized water to make 10^{-2} dilution of this solution and in the same pattern dilutions up to 10^{-6} were prepared to determine the microbial count.

For chemical analysis the soil composite were dried in an electrical oven at 70ºc for 24 h. then mixed thoroughly and ground to pass through a 2 mm sieve.

The general characteristics of the soil were summarized in Table (I) .

Table (I): Some chemical and biological characteristics of the tested soil prior to chemical treatment

Mechanical analysis	Sand	3.7	**Chemical analysis**	E.C ds.m^{-1}	7.83	**Available nutrient (ppm)**	Total nitrogen	42.3	**Saturation S.P%**	42
	Silt	40.9		PH	1.72		Total phosphorus	4.1		
	Clay	31.5		Organic matter OM%	1.46		Total potassium	315		
	Total class	loamy		$CaCO_3$%	1.9					

b. **Analysis of microbial population**

Soil sample were analyses immediately after collection to enumerated the colony forming units (C.F.U) of bacterial , actinomycetes and fungi following the serial diluation technique and pour plate methods (Pramer and Schmidt 1965).

The nutrient medium in agar was prepared according to the choice of the microorganism to be cultured. It was sterilized in autoclave at pressure 1.05 kg cm2 and temperature 120 _C . It was cooled to 40–50 _C before use. After cooling the mouth of the flask containing medium was flamed and 20–25 ml of the medium was poured in sterilized petri-plates containing one ml of soil suspension. The medium was then allowed to solidify and incubated for 1–7 days at appropriate temperature. Incubation period and condition varied, depending on the nature of the organism to be enumerated. After the suitable incubation period, the plates were removed from the incubator and the colony forming units (CFU) were counted manually ranging between 30 and300.

CFU = mean No. of colony X inverted dilution

No. of microorganism / 1 g soil = CFU X 100/ (100 – humidity)

2. Pesticides sensitivity assessment

Sensitivity of bacteria (*Rhizobium leguminosarum* and *Bacillus thuringiensis*), fungi (*Trichoderma harzianum* and *T. viridi*) and actinomycetes (*Streptomyces griseorubens*(Preoobrazhenskaya, *et al.,* 1957) and *S. cavourensis* (Giolitti, 1961)) to tested pesticides at field and double field application rates(0.5, 1.00 ml/100 ml water for chloropyrifos; 0.05, 0.1g/100 ml water for Lambada-cyhalothrin, and 0.5, 1.00 ml/100ml water for Emamectin benzoate) was determined.

In media for bacteria and actinomysetes we made in center of Petri dishes wells by aseptically removing a strip of agar which were filled with the tested pesticides, Diameter of the growth inhibition was considered as a measure of tested pesticides sensitivity after incubation at 30 C° for 28 hr for bacteria and 7days for actinomycetes (Benimeli, *et al.,* 2003).

For fungi Sensitivity of fungi was tested on PDA amended with the tested pesticides. Isolates were first cultured on PDA media without any pesticides (pH 6.0) in the dark to provide conidia-free actively growing colonies. Disks (6 mm diameter) cut from the margin of these cultures were placed upside down in the centre of fungicide-amended and control (no fungicide) PDA dishes (9 cm diameter containing 10 ml of medium). Plates were incubated at 20°C in the dark and the diameter of actively growing colonies was recorded at suitable intervals, the last measure being taken 10 days after seeding. Three replicates were used for each concentration tested. Inhibition of radial growth was calculated as percentage of radial growth of the control (Figueras-Rota, *et al.,* 1996).

3 . Data analysis

The results of total population count were evaluated by tow- way analysis of variance (ANOVA) . one-way analysis of variance used for evaluating the results of microorganism sensitivity . The statistical significance (P<0.05) of difference between means within factors

(insecticides and time) was evaluated using costat version 6.303(Costat 1990)

RESULTS AND DISCUSSION

1- Effect of tested pesticides on total population count of microorganism in phasoulus vulgaris field

There are three possible effects on microbes: inhibition, no effect and stimulation (Dragun, 1998). The plate count results indicated that tested pesticides affect the total numbers of microorganism including bacteria, fungi and actinomycetes in treated soil in table (1). Results in table (1) show that, Chloropyrifos caused significant stimulated effect on bacterial population at indicated days compered with untreated check which recorded 119.34, 138.04, 171.70, 199.58, 223.38 (X 10^5 /gdw) at 1,7,15,21,and 28 days respectively . Chloropyrifos had effect on fungal population at one day after treatment , while at 7day after treatment Chloropyrifos caused a decrease on fungal population than untreated check which recorded 11.56(X10^3 gdw). But the following days Chloropyrifos achieved an increase in total population count of fungi but less than untreated, while, the total population count of actinomycetes was slightly increased at indicated days than untreated check which recorded 23.12, 31.96, 42.50, 57.46, 65.96 (X 10^4 /gdw)at 1,7, 15, 21, 28 respectively.

Our results revealed that Chloropyrifos caused an increase in total population count in tested microorganisms in soil. Increases in the numbers of bacteria and actinomycetes were observed after deltamethrin treatment, whereas no adverse effect was observed on fungal population (Zhang *et al.,* 1984) . Also Das *et al.,* (2003, 2005) stated that, application of the insecticides stimulated the population of bacteria, actinomycetes, and fungi in the rhizosphere soils. These results are in agreement with those obtained by (Tu, 1991; Pozo *et al.,*., 1995; Pandey and Singh, 2004; Shan *et al.,*., 2006 , El-Mongy and El-Ghany 2009),. Therefore, inconsistent trends or patterns of a pesticide are often observed. In contrast to the results here, significant inhibition of soil bacteria and fungi by chloropyrifos has also been reported by (Tu, 1970; Pandey and Singh, 2004; Shan *et al.,.,* 2006, Xiaoqiang, *et al.,* 2008).

On the other hand Lambada-cyhalothrin showed an inhibitory effect on soil bacterial, fungal, and actinomycetes populations (Table 2). Bacterial population was significantly reduced to 77.18 (X 10^5 /gdw) at one day after treatment with Lambada-cyhalothrin, compared with the untreated check. The inhibitory effect did not disappear until 15 DAT. At 21 and 28 DAT, it was returned to slight increase compared with the untreated check.

Population of soil fungi was significantly ($P < 0.05$) reduced to11.90(× 10^3/gdw) on day at the first (DAT) compared with that of the untreated check (Table 2). Despite the increase in total population count of fungi up to 28 of treatment. Its still lower than the untreated check which recorded 11.90, 16.16, 26.18, 25.59, 30.21 (× 10^5 /g dw) at 1, 7, 15, 21,28 DAT, respectively.

Lambada-cyhalothrin treatment had an inhibitory effect on total population count of actinomycetes during the period from 1 to 15 DAT. It was

recovered to a similar level of control at 21 day and slight increase was achieved at 28 d compared with untreated check recorded 66.26 CFU × 10^4 /g dw. This result agree with previous studies reduce fungal biomass or counts, but increase bacterial biomass or counts (Duah-Yentumi and Johnson, 1986; Monkiedje *et al.,* 2002) significant decrease in bacterial numbers with subsequent recovery is also reported (Tu, 1978) ,. In contrast to the results here, significant inhibition was observed in previous study (Das and Mukherjee 2000, Boucard *et al.,* 2008, Rache and Coats, 1988; Das *et al.,* 1995) who reported that, tested insecticides(HCH, phorate, carbofuran and fenvalerate at their recommended field rates did not have any deleterious effect on the growth and activities of the major soil microorganisms responsible for the transformations of C, N and P. and. No adverse effect on fungal population were observed by (Tu 1995), no effects have been reported for l-cyhalothrin (Cycon *et al.,* 2006) or deltamethrin (Germida *et al.,* 1987; Vig *et al.,* 2008 Lupwayi, *et al.,* 2009).

The effect of Emamectin benzoate on total population count of fungi in soil was almost similar to lambada-cyhalothrin. The bacterial population count was initially influenced by the pesticide and their residues in soil but recovered quickly (in about 7–28 DAT). The fungal population count was inhibited by Emamectin benzoate and its residues in soil during the periods of treatment scoring 17.00, 9.18, 13.47, 19.53, 24.82(CFU × 10^3 /g dw) at 1, 7, 15, 21, 28 DAT compared with the untreated check, in contrast the population count of actinomycetes was increased during the period from 1 to 15 DAT AND slight decrease in population count was observed up to 28 DAT.

Chukwudebe, *et al.,* 1997 and Krogh, *et al.,* 2009, reported that, ivermectin does not seem to have an adverse impact on the population of soil microorganisms. Furthermore, these levels are indicative of microbial active soils.

Generally, some pesticides stimulate the growth of microorganism, but other pesticides have depressive effects or no effects on microorganism.

The stimulation or inhibition of the microbial population in the experiment fields was observed the tested time intervals (1-28days) of treatment. This was most likely due to the presence of pesticides residues in the soil during that period. A long with the pesticides residues various other local climatic factors such as temperature, sun light, relative humidity , rain fall , pesticides concentration, soil type, and microbial composition in tested soil also affect abundance of soil microorganism

The effect of tested pesticides on given microorganism in this work can be arranged in descending order as follow: for Bacteria and Actinomycetes, Chloropyrifos had the most stimulated effect followed by Emamectin- benzoate. The Lambada-cyhalothrin was the least effective one. On the other hand, for Fungi, Emamectin benzoate had the most inhibitory effect followed by Chloropyrifos and the Lambada-cyhalothrin

Table (1): Effect of tested pesticides on total population count of microorganism in soil

bacteria (CFU × 10^5 /g dw)						
Treatment	Total population count at indicated days					**Mean of treatment**
	1	**7**	**15**	**21**	**28**	
Untreated check	87.55J ±5.15	111.18 I ±8.71	144.84F ±1.76	157.76E ±2.56	176.46D ±4.44	135.55c
Chloropyrifos	119.34H ±5.39	138.04FG ±5.13	171.7D ±2.12	199.58B ±5.97	223.38A ±3.67	170.40a
Emamectin benzoate	83.98JK ±5.03	135.32G ±2.56	163.28E ±2.91	178.77D ±3.64	200.9B ±6.20	152.45b
Lambada-cyhalothrin	77.18K ±5.23	87.87J ±1.74	124.78H ±2.56	160.25E ±3.24	189.86C ±4.64	127.98d
Mean of time	92.01*e*	118.10*d*	151.15*c*	174.09*b*	197.66*a*	
L.S.D	Treatment 3.13	Time 3.50	Interaction 7.01			
fungi (CFU × 10^3 /g dw)						
Treatment	Total population count at indicated days					**Mean of treatment**
	1	**7**	**15**	**21**	**28**	
Untreated check	18.88 HI ±1.5	21.08 GH ±1.7	28.56CD ±2.0	35.36 B ±1.5	38.76 A ±2.0	28.52a
Chloropyrifos	18.7 HI ±1.5	11.56 IM ±1.1	13.94KI ±1.1	22.44 FG **±**2.0	26.18 DE ±.58	18.56c
Emamectin benzoate	17.00 IJ ±1.5	9.18 M ±1.0	13.46I ±1.5	19.52 HI ±1.1	24.82 EF ±1.1	16.79d
Lambada-cyhalothrin	11.9 I ±2.1	16.16 JK ±2.0	26.18 DE ±0.5	25.58 E ±0.5	30.21 C ±1.1	22.00b
Mean of time	16.62*d*	14.49*e*	20.52*c*	25.72*b*	29.99*a*	
L.S.D	Treatment 1.07	Time 1.19	Interaction2.39			
actinomycetes (CFU × 10^4 /g dw)						
Treatment	Total population count at indicated days					Mean of treatment
	1	**7**	**15**	**21**	**28**	
Untreated check	18.88 L ±1.5	28.22G ±41	36.72D ±1.7	44.88D ±2.04	58.14B ±2.04	37.36c
Chloropyrifos	23.12H ±12.5	31.96F ±1.5	42.5D ±1.5	57.46B ±1.17	65.96A ±1.5	44.2a
Emamectin benzoate	26.18GH ±1.5	37.06E ±1.5	45.11D ±1.1	39.39E ±1.0	49.64C ±1.17	39.47b
Lambada-cyhalothrin	25.16H ±1.17	24.57H ±1.16	31.28F ±0.5	44.77D ±0.5	66.26A ±1.1	38.41bc
Mean of time	23.33*a*	30.45*b*	38.90*c*	46.62*d*	60.00*e*	
L.S.D0.05	Treatment 1.27	Time 1.42	Interaction2.84			

All values are means± SD of triplicate sample, means followed by the same capital letter in column (or row) (interaction) , same small letter in column (mean of treatment) and same italic letter in row (mean of time) are not significantly different at 0.05 level of probability (Duncan ,s Multiple Range Test)

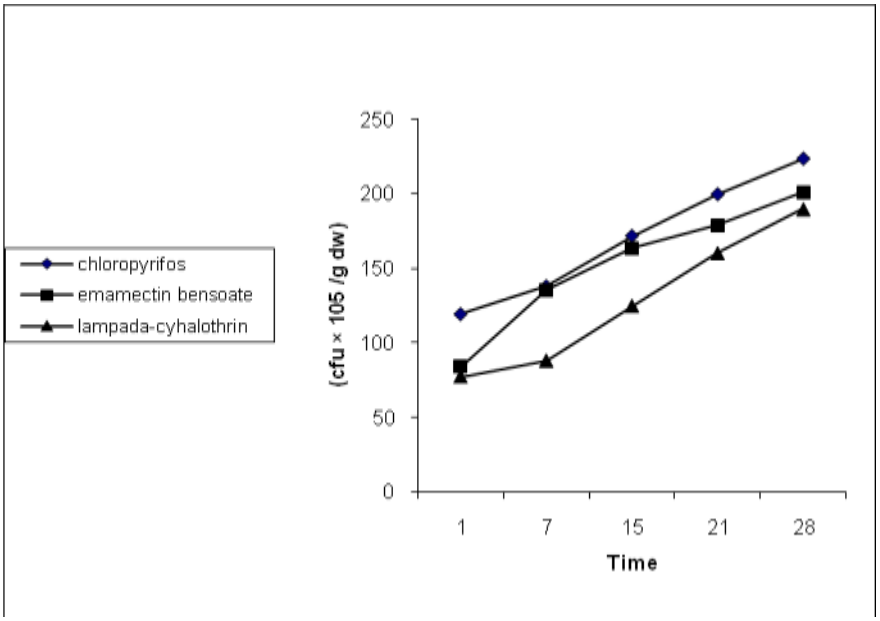

Fig. (1): Effect of tested pesticides on total population count of bacteria.

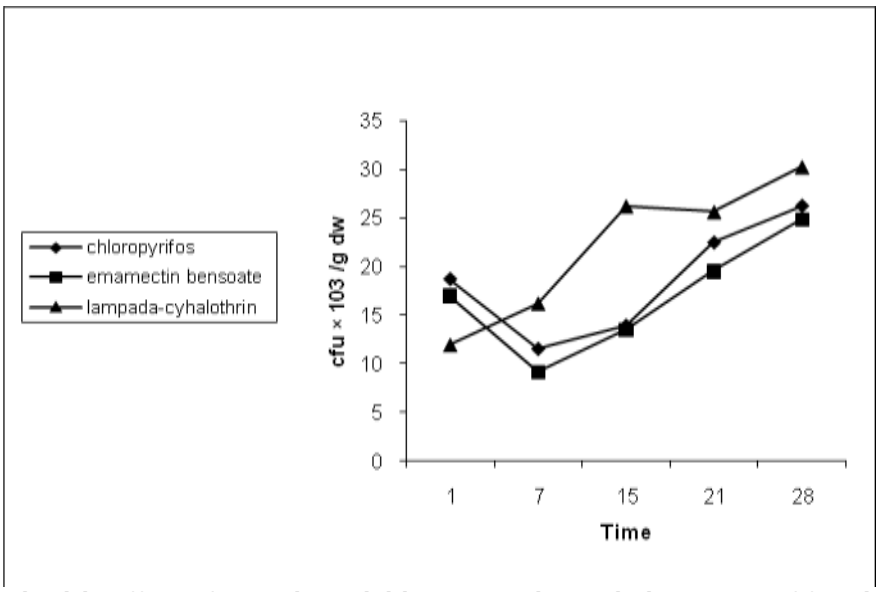

Fig. (2): Effect of tested pesticides on total population count of fungi.

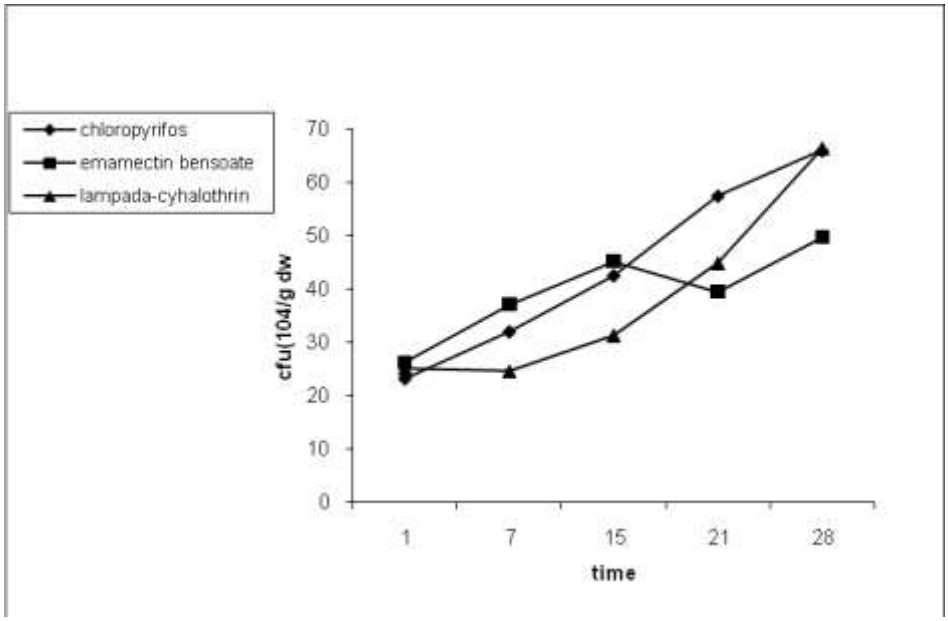

Fig. (3): Effect of tested pesticides on total population count of actinomysites.

2-Sensitivity of some micro-organism on tested pesticides in laboratory conditions.

Variable sensitivity of Bacteria, Actinomycetes and Fungi had been shown to the tested pesticides. Bacteria species showed higher sensitivity to Chloropyrifos and Lambada-cyhalothrin than Actinomycetes species at the two concentration levels. Which recorded (2.26, 4.33) and (1.93, 1.41) clear zone/cm for Bacteria and Actinomycetes, respectively at recommended rate of Chloropyrifos. Similar results were obtained for Lambada-cyhalothrin (table 2).

In contrast to the results here bacteria have shown resistance to O.P insecticides (guthion, methyl parathion and dimethoate) as reported by (Nazarian and Mousawi, 2005) .the resulting change in sensitivity or tolerance of microorganism to any given pesticides may be attributed to the varing metabolic bath ways which detoxify these compounds or break up or modify them into other forms that may further be more less deleterious.

Khan *et al.*(2009) found that chlorpyrifos decreased the number of chickpea *Rhizobium* in the rhizosphere by 90 % when applied as the commercial product Pyrifos 40 % EC (emulsifiable concentrate), while no effects were observed when it was applied as Lorsban 40 % EC. Lin, *et al.,* (1972) stated that, Disc inhibition tests of the rhizobia bacteria showed that*Rhizobium leguminosarum* and*Rhizobium trifolii* were most sensitive to the pesticides.

On the other hand, Fungi showed high tolerance level to Emamectin benzoate in which recorded 9.20 radial groeth/cm at recommended and double recommended rates similar that of untreated check, while Lambada-cyhalothrin slightly inhibited the growth of Fungi species. On contrary, Chloropyrifos had astrong inhibitory effect on the growth of Fungi, recording(0.75& 2.21)and (0.00& 0.36)radial growth at recommended and double recommended dose, receptivity.

Concerning, the pesticides used and then effect on the tested microorganism, data in table (2) showed that, Chloropyrifos significantly inhibited growth of all tested species at recommended and double recommended rates . Note inhibition increased with increasing the concentration of the pesticide and this effect signed by all the species being tested compared to the untreated check.

Lambada-cyhalothrin at recommended dose had slightly effect on growth of tested species but at double recommended dose it significantally inhibited growth in tested species. Similar results were obtained by (Maloney, *et al.,* 1992, Zhai, *et al.,* 2012) .

Emamectin benzoate had no any inhibitory effect on the growth of tested species except for *S.griseorubens* at double recommended dose had slightly inhibited growth scored 1.16 compared to the untreated check

CONCLUSION

The results in present investigation generated that, microorganisms in soil were able to use low rates of pesticides (field application rates) as a source of nutrients element and thus increase the total population count. In general Six different species of microorganism were able to grow in the presence of Emamectin benzoate and Lambada-cyhalothrin but could not able to grow in the presence of Chloropyrifos at recommended rates. We can say when we use pesticides at recommended rates in agriculture and therefore not excessive use of pesticides can be maintain the population of microbes in the soil and increase soil fertility**.** In future studies can be studied not only the impact of pesticides on the population of organisms, but also microbial activity under the influence of the pesticide**.**

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REFERENCES

Benimeli, C.S. ; Amoroso, M. J. ; Chaile, A. P. ; Castro, G. R. (2003) Isolation of four aquatic streptomycetes strains capable of growth on organochlorine pesticides. Bioresource Technol. 89 : 133–138

Bhuyan, S.; Sahu, S. K.; Adhya, T. K.; Sethunathan, N. (1992) Accelerated aerobic degradation ofy-hexachlorocyclohexane in suspensions of flooded and nonflooded soils pretreated with hexachlorocyclohexane. BioI. Fertil. Soils 12: 279 - 284.

Boldt, T.S.; Jacobsen, C.S. (1998) Different toxic effects of the sulphonylurea herbicides metsulfuron methyl, chlorsulfuron and thifensulfuron methyl on fluorescent pseudomonads isolated from an agricultural soil. FEMS Microbiol. Lett. 161:29–35.

Boucard, T. K. ; McNeill, C. ; Bardgett, R. D. ; Paynter, C. D. and Semple, K. T. (2008) The impact of synthetic pyrethroid and organophosphate sheep dip formulations on microbial activity in soil. Environ. Poll. 153, 1: 207–214

Chukwudebe, A.C., Atkins, R.H., Wislocki, P.G., (1997) Metabolic fate of emamectin benzoate in soil. J. Agric. Food Chem. 45: 4137–4146.

CoStat Software (1990). Microcomputer program analysis version 4.20,CotHort Software, Berkeley, CA.

Cycon, M., Piotrowska-Seget, Z., Kacynska, A.,(2006) Microbiological characteristics of a sandy loam soil exposed to tebuconazole and l-cyhalothrin under laboratory conditions. Ecotoxicology 15: 639–646.

Das, A.C. and Mukherjee, D. (2000) Soil application of insecticides influences microorganisms and plant nutrients. Appl. Soil Ecol. 14 : 55–62

Das, A.C., Chakravarty, A., Sen, G., Sukul, P., Mukherjee, D., (2005) A comparative study on the dissipation and microbial metabolism of organophosphate and carbamate insecticides in orchaqualf and fluvaquent soils of west Bengal. Chemosphere 58:579–584.

Das, A.C., Chakravarty, A., Sukul, P., Mukherjee, D., (1995). Insecticides: their effect on microorganisms and persistence in rice soil. Microbiol. Res 150: 187–194.

Das, A.C., Chakravarty, A., Sukul, P., Mukherjee, D., (2003) Influence and persistence of phorate and carbofuran insecticides on microorganisms in rice field. Chemosphere 53, 1033–1037.

Dragun, J., (1998) the soil chemistry of Hazarous Material, 2ed Edition. Amherst Scientific Puplishers , Massachusetts, 862.

Duah-Yentumi, S., Johnson, D.B., (1986) Changes in soil microflora in response to repeated applications of some pesticides. Soil Biol. Biochem. 18, 629–636.

El-Mongy, M. A. and El-Ghany, T. M. A. (2009)Field and laboratory studies for evaluating the toxicity of the insecticide Reldan on soil fungi. International Biodeterioration & Biodegradation 63 : 383–388

Figueras-Roca,; M. Cristani, C. and Vannacci, G. (1996) Sensitivity of Trichoderma isolates and selected resistant mutants to DMI fungicides. Crop Protection. 15. 615-620

Germida, J.J.; Onofriechuk, E.E.; Ewen, A.B. (1987) Effect of Nosema locustae canning microsporida and three chemical insecticides on microbial activity in soil. Can. J. Soil Sci. 67, 631–638.

Giolitti, G. (1961). *Streptomyces cavourensis*. Pp. 191. In: Waksman, S. A. The Actinomycetes. Vol. II, classification, identification and descriptions of genera and species. The Williams and Wilkins Co. Baltimore.

Itoh, K. (1991) Characteristics of microflora degrading insecticide alithion in soil. J. Pesticide Sci. 16: 77 - 83.

Juneja, S.; Dogra, R.C. (1978) Effect of aldrin on growth and oxidative metabolism of rhizobia. J. Appl. Microbiol. 44: 107–115.

Khan, H.; Zeb, A.; Ali, Z.; Shah, S.M. (2009) Impact of five insecticides on chickpea (*Cicer arietinum* L.) nodulation, yield and nitrogen fixing rhizospheric bacteria. Soil Environ., *28* : 56–59.

Krogh, A. k. ; Jensen, G. G. ; Schneider, K. N. ; Fenner, K.; Halling-S◌̊Ørensen, B.(2009) Analysis of the dissipation kinetics of ivermectin at different temperaturesand in four different soils. Chemosphere 75 : 1097–1104

Liu, Y.Y., Xiong, Y., (2001) Purification and characterization of a dimethoate-degrading enzyme of Aspergillus niger ZHY256 isolated from sewage. Appl. Environ. Microbiol. 67, 3746–3749.

Lupwayi, Z. N. ; Harker, N. K. ; Dosdall, M. L. : Turkington, K. T. ; b, Robert E. B.; John T. O'D.; Carcamo, A. H.; Otani, K. J. and Clayton, W. G.(2009) Changes in functional structure of soil bacterial communities due to fungicide and insecticide applications in canola. Agriculture, Ecosystems and Environment 130: 109–114

Maloney, S.E. ; Maule, A.; and Smith, A.R. (1992) Transformation of synthetic pyrethroid insecticides by athermophilic *Bacillus* sp, Arch. Microbiol. 158 : 282-286.

Martin, J.P., (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69: 215–232.

Matsumura, F., Boush, G.M.,(1971) Metabolism of insecticides by microorganisms. In: McLaren, microbial biodegradation. J. Agric. Food Chem. 36: 193–199.

Monkiedje, A.; Ilori, M.O.; Spiteller, M. (2002) Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl toand loam soil. Soil Biol. Biochem. 34: 939–948.

Moorman, T.B. (1989) A review of pesticide effects on microorganisms and microbial processes related to soil fertility. Journal of Crop Production 2: 14-23

Nazarian, A.; Mousawi, M., (2005) Study of bacterial resistance to organophosphorous pesticides in Iran. Iranian J. Environ. Health Sci. Eng. 2: 207–211.

Pandey S.; Singh D K, (2004). Total bacterial and fungal population after chlorpyrifos and quinalphos treatments in groundnut (*Arachis hypogaea* L.) soil. *Chemosphere*, 55: 197–205.

Pozo C, Martinez-Toledo M V, Salmeron S, Rodelas B, Gonzalez-Lopez J, (1995). Effect of chlorpyrifos on soil microbial activity. *Environ Toxicol Chem*, 14: 187–192.

Pramer, D.; Schmidt, E. L. (1965): Experimental soil microbiology. Burgers Publishing Co., Minneapolis 15, Minnesota, USA.

Preobrazhenskaya, T. P., Blinov, N. O. and Ryabova, I. D. (1957) Characteristics of antagonistic actinomycetes of the Griseus series (in Russian) p. 131-145. In Gauze, G. F., Preobrazhenskaya, T. P., Kudrina, E. S., Blinov, N. O., Ryabova, I. D. and Sveshnikova, M. A., Problems of classification of actinomycetes–antagonists. Government Publishing House of Medical Literature, Medgiz, Moscow.

Rache, K.D., Coats, J., (1988.) Comparative biodegradation of organophosphorus insecticides in soil. Specificity of enhanced microbial degradation. J. Agric. Food Chem., 36 :193–199

Rosas, S.B., Carranza Destorani, M.M., (1987) the action of pesticides on microorganisms. I. Parathion. Toxicity Assessment. 2: 293-303

Schuster, E., Schroder, D., (1990) Side effect of sequentially applied pesticides on non-target soil microorganisms—field experiment. Soil Biol. Biochem. 22: 367-373.

Shan, M.; Fang, H.; Wang, X.; Feng, B.; Chu, X. Q.; Yu, Y. L.z (2006). Effect of chlorpyrifos on soil microbial populations and enzyme activities. J. Environ. Sci., 18: 4–5.

Shin-Chsiang L, ; Funke, B. R. and Schulz, J. T.(1972) Effects of some organophosphate and carbamate insecticides on nitrification and legume growth. Plant and Soil. 37: 489-496

Simon-Sylvestre, G., Fournier, J.C.,(1979) Effect of pesticides on soil microflora. Adv. Agron. 31: 1–92.

Skujins, J. A.D. (Eds.), Soil Biochemistry, vol. 2. Marcel Dekker, New York, USA, 320–336.

Society of American Bacteriologists, (1951). Manual of methods for pure culture of bacteria. New York: McGraw-Hill.

Suneja, S.; Dogra, R.C. (1984) Effect of aldrin and lindane seed treatment on symbiosis of chickpea rhizobium with Bengal gram (Cicer arietinum). Tropical Agriculture 62: 110-114.

Tu, C. M. (1970). Effect of four organophosphorus insecticides on microbial activities in soil. Appl. Microbiol. 19: 479–484.

Tu, C. M. (1991). Effect of some technical and formulated insecticides on microbial activities in soil. J. Environ. Sci. Heal. B, 26: 557–573.

Tu, C.M. (1978) Effect of pesticides on acetylene reduction and microorganisms in a sandy loam. Soil Biol. Biochem. 10, 451–456.

Tu, C.M. (1995) Effect of five insecticides on microbial and enzymatic activities in sandy soil. J. Environ. Sci. Heal. B 30 : 289–306.

Venkateswarlu, K., Sethunathan, N. (1984) Degradation of carbofuran by *Azospirillum /ipoferum* and *Streptomyces* spp. isolated from flooded alluvial soil. Bull. Environ. Contam. Toxicol. 33: 556 - 560.

Vig, K., Singh, D.K., Agarwal, H.C., Dhawan, A.K., Dureja, P.,(2008) . Soil microorganisms in cotton fields sequentially treated with insecticides. Ecotoxicol. Environ. Safety 69: 263–276.

Vincent, J.M.,(1970) A Manual for the Practical Study of Root Nodule Bacteria, IBP Handbook No. 15. Blackwell Scientific Publications, Oxford, UK.

WHO (1990) Cyhalothrin, environmental health criteria, 99, Geneva,

Xiaoqiang,C. ; Hua, F.; Xuedong, P. ; Xiao,W.; Min, S. Bo, F. and Yunlong, Y (2008) Degradation of chlorpyrifos alone and in combination with chlorothalonil and their effects on soil microbial populations. J. .Environ. Sci. 20: 464–469

Zhai, Y.; Li, K.; Song, J.; Shi, Y. ; Yan, Y. (2012) Molecular cloning, purification and biochemical characterization of a novel pyrethroid-hydrolyzing carboxylesterase gene from *Ochrobactrum anthropi* YZ-1, *J. .Hazard. Mater.* (2010)

Zhang, L., Khan, S.U., Akhtar, M.H., Ivarson, K.C., (1984) Persistence, degradation and distributation of deltamethrin in an organic soil under laboratory conditions. J. Agric. Food Chem. 32: 1207–1211.

تأثير بعض المبيدات على تعداد الميكروبات فى التربة وحساسية بعض السلالات لهذه المبيدات.

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تم عمل تجربة لدراسة تأثير بعض المبيدات الحقلية (الكلوروبيريفوس ، الالمبادا سيهالوثرين و الايمامكتن بنزوات) بالتركيز الموصى به حقليا على التعداد الكلى للكائنات الحية فى حقل التربة و تمت الدراسة بمزرعة كلية الزراعة و اسفرت النتائج على التالى

احدث مبيد الكلوروبيريفوس زيادة فى تعداد الكائنات الحية فى التربة مقارنة بالتجربة الغير معاملة بينما احدث مبيد الالمبادا سيهالوثرين خفض فى تعداد البكتريا فى بداية المعاملة ثم بعد ذلك حدث زيادة تدريجية فى التعداد بينما حدث خفض فى تعداد الفطريات خلال فترة التجربة و حدث حفض فى تعداد الاكتينوميسيتس فى بداية المعاملة ثم تلاه زيادة عى التعداد .فى حين احدث مبيد الايمامكتن زيادة فى تعداد البكتريا و الاكتينوميسيتس و خفض فى تعداد البكتريا.

كما تم دراسة تأثير المبيدات المختبرة على بعض السلالات فى المعمل و هى (*Rhizobium leguminosarum,* Streptomyces griseorubens، *Bacillus thuringiensis* ,Streptomyces cavourensis ، *Trichoderma harzianum* ، *Trichoderma. viridi*) عند التركيز الموصى به و ضعف التركيز الموصى به و اوضحت النتائج ان مبيد الكلوروبيريفوس و الالمبادا سيهالوثرين ادى لتثبيط نمو الكائنات المختبرة عند التركيز الموصى به و حدث زيادة فى التثبيط عند زيادة مضاعفة التركيز بينما لم يحدث مبيد الايمامكتن تأثير على السلالات المختبرة عند التركيز الموصى به و عند مضاعفة التركيز ادث المبيد تثبيط طفيف فى نمو بعض السلالات .

قام بتحكيم البحث

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Table (2):Effect of tested pesticides on growth of some microorganisms considering different parameters

Treatment	Concentration	Bacteria (clear zone /cm)		Fungi (radical growth/cm)		Actinomycites(clear zone /cm)	
		B.thuringiensis)	*Rh.leguminosarum*	*T.harzianum*	*T. viridi*	*S.griseorubens*	*S.cavourensis*
Untreated check		0.00d ±0.00	0.00d ±0.00	9.200a ±0.00	9.200a ±0.00	0.00d ±0.00	0.00c ±0.00
Chloropyrifos	Recommended rate	2.26b ±0.12	4.333bc ±0.57	0.750c ±0.25	2.217c ±0.11	1.933b ±0.32	1.417b ±0.76
	Double recommended rate	2.91a ±0.38	7.417a ±0.38	0.000d ±0.00	0.700d ±0.36	2.700a ±0.69	2.383a ±0.25
Lambada-cyhalothrin	Recommended rate	0.00d ±0.00	3.783c ±0.55	8.900a ±0.36	8.850b ±0.62	0.00d ±0.00	1.17b ±0.13
	Double recommended rate	1.783c ±0.30	5.050b ±0.67	2.050b ±0.18	2.250c ±0.5	2.167b ±0.76	2.083a ±0.57
Emamectin benzoate	Recommended rate	0.00d ±0.00	0.00d ±0.00	9.200a ±0.00	9.200a ±0.00	0.00d ±0.00	0.00c ±0.00
	Double recommended rate	0.00d ±0.00	0.00d ±0.00	9.200a ±0.00	9.200a ±0.00	1.167c ±0.57	0.00c ±0.00
L.S.D0.05		0.332	0.736	0.313	0.292	0.405	0.330

All values are means± SD of triplicate sample ,means followed by same letter with in column are not significantly different according to LSD's Multiple Range test (P< 0.05)