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Effect of Chlorpyrifos on Soil Microbial Health

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Abstract:
The experiment was conducted to record the effect of different concentrations of chlorpyrifos (50, 100, 150, 200, 500 and 1000 ppm) on total viable bacterial, fungal and actinomycetes count in soil. Interaction of treatment and sampling days recorded maximum viable bacterial count (20.93 CFU/g), fungal count (13.30 CFU/g) and actinomycetes count (20.60 CFU/g) at 50 ppm treated chlorpyrifos soil on 10th day (for bacteria) and 5th day (for fungi and actinomycetes) of incubation which was significantly higher than all other treatments and sampling days. Further microbial count decreased with increase in chlorpyrifos concentration and sampling days. Minimum viable count of bacteria (2.02 CFU/g), fungi (1.37 CFU/g) and actinomycetes (1.52 CFU/g) recorded at 1000 ppm was significantly lower. So it was concluded that with increase in concentration of chlorpyrifos (50 to 1000 ppm), there was decrease in soil microbial count.

Keywords: *Actinomycetes, Bacterial, Chlorpyrifos, Concentration, Fungal.*

1. Introduction

Organophosphorous (OP) insecticides such as parathion, methamidophos and chlorpyrifos are a group of highly toxic chemicals widely used in plant protection. As these insecticides cause extensive damage to non-target organisms, studies regarding their degradation have received considerable attention from soil microbiologists. Several reports suggest that contamination of soil by these insecticides as a result of their bulk handling at the farm yard or following application in the field or accidental release lead to contamination of a wide range of water and terrestrial ecosystems (Singh *et al* 2004). Insecticides in soil and water can be degraded by biotic and abiotic pathways however biodegradation by microorganisms is the primary mechanism of insecticide break down and detoxification in many soils. Chlorpyrifos, [O,O–diethylO–(3,5,6-trichloro-2-pyridyl) phosphorothioate] is one of the widely used organo-phosphate insecticides, effective against a broad spectrum of agricultural and house hold pests and characterized by P-O-C linkages. It is effective in controlling insects, termites on grains, cotton, vegetable crops, lawns and ornamental plants. Its half life in soil varies from 10 to 120 days, with 3, 5, 6-trichloro-2-pyridinol (TCP) as the major degradation product. The accumulation of TCP which has anti-microbial properties prevents the proliferation of chlorpyrifos degrading microorganisms, thus enhanced microbial degradation of chlorpyrifos is prevented (Singh and Walker 2006). Chlorpyrifos undergo transformation in the soil by the abiotic hydrolysis and microbial degradation. Since pesticides are toxic by design, they have the potential to adversely affect the ecosystem health. Thus pesticides when used in the field to increase crop production besides combating insect pests, also affect the population and activity of beneficial microbial communities (Pandey and Singh 2004). Microbial communities of soil also interact with plant roots and soil constituents at the root-soil interface (Khan *et al* 2009, Attitalla *et al* 2011). The addition of pesticides may disturb the equilibrium and thus fertility of the soil. The present study was therefore primarily focused to evaluate effect of chlorpyrifos on soil microorganisms.

2. Materials and Methods

Chemicals: A commercial preparation of chlorpyrifos i.e. Classic*20 (20% EC) obtained from Cheminova India Ltd, Panoli, Distt. Bharuch was used as a substrate for the present study. Other chemicals and media for biochemical tests were of AR grade and were procured from Hi-Media Laboratories Pvt. Ltd., Mumbai.

Soil was collected from unsprayed field of PAU at the depth of 10-15cm from five randomly selected spots. The soil samples were pooled and air dried for further processing. Each air dried soil sample was mixed aseptically and separately with different concentrations (50, 100, 150, 200, 500 and 1000 ppm) of commercially available chlorpyrifos, Classic 20% EC and a sample of unsprayed soil was run along with as a control. Sample was aseptically transferred to one liter sterilized flask and incubated at 30±1°C for two months and then recorded for viable microbial count. One gram of soil was added to nine ml sterilized 0.85 per cent saline solution, which was mixed on a shaker for ten minutes and then serially diluted upto 10^{-6} for taking viable counts of fungi,

actinomycetes and bacteria, respectively. One ml of each dilution was mixed with twenty ml of sterilized molten medium (Soil extract medium, Rose Bengal agar medium, Kenknight's agar medium) separately for isolation of viable bacteria, fungi and actinomycetes respectively in separate petri-plates which were incubated at 25°C for fungi and at 28°C for bacteria and actinomycetes. The experiment was conducted in triplicate. The viable bacterial, fungal and actinomycetes counts were recorded on 0, 5, 10, 15, 30, 45 and 60 days of incubation. The results were evaluated by analysis of variance (ANOVA) and the statistical significance (P=0.05) of difference between means within factors (insecticides and incubation time) was evaluated using Fisher's protected LSD method (Petersen, 1994).

3. Results and Discussion

Soil samples were collected from unsprayed fields of honey bee at Entomological research farm PAU Ludhiana. These samples were processed in the laboratory and fortified with six different concentrations of commercial grade chlorpyrifos to study its effect on total viable soil microbial count. The viable bacterial, fungal and actinomycetes count was recorded at regular intervals of incubation.

3.1. Total Viable Bacterial Count

The results presented in Table 1 revealed that with increase in concentration of chlorpyrifos, there was a decrease in bacterial population. Minimum count (2.02) was recorded at 1000 ppm which was significantly lower than all other treatments. When sampling days were compared the maximum bacterial count (12.51) was recorded on 10^{th} day of incubation, which reduced with further increase in sampling days and it was 9.65, 5.99, 3.40 and 2.08 on 15^{th}, 30^{th}, 45^{th} and 60^{th} day of incubation respectively. When interaction of treatment and sampling days were compared maximum bacterial count (20.93) was recorded at 50 ppm treated chlorpyrifos soil on 10^{th} day of incubation which was significantly higher than all other treatments.

Conc. (ppm)	Bacterial count on different sampling days (CFU×10^6/g)							
	0	5	10	15	30	45	60	Mean
50	11.53	12.53	20.93	16.80	10.00	6.70	2.53	11.57
100	10.10	10.23	18.36	13.66	8.66	3.53	1.73	9.47
150	8.10	9.23	15.36	11.20	6.53	1.80	1.23	7.64
200	3.26	3.96	6.00	3.53	2.73	1.30	0.96	3.11
500	2.93	3.20	4.70	2.46	2.00	1.03	0.93	2.46
1000	2.03	2.83	3.73	2.10	1.73	0.93	0.83	2.02
0 (Control)	18.23	18.80	18.50	17.80	10.27	8.53	6.37	14.07
Mean	8.03	8.68	12.51	9.65	5.99	3.40	2.08	

Table 1: Effect of different concentrations of chlorpyrifos on viable bacterial population in soil.

Values are Mean

LSD at 0.05

Treatment (T) = 0.093

Sampling date (SD) = 0.093

Interaction (T×SD) = 0.25

3.2. Total Viable Fungal Count

The results presented in Table 2 revealed that with increase in concentration of chlorpyrifos, there was a decrease in fungal population. Minimum count (1.37) was recorded at 1000 ppm which was significantly lower than all other treatments. When sampling days were compared the maximum fungal count (9.35) was recorded on the 5^{th} day of incubation, which reduced further with increase in sampling days. When interaction of treatment and sampling days were compared maximum bacterial count (13.30) was recorded at 50 ppm treated chlorpyrifos soil on the 5^{th} day of incubation.

Conc. (ppm)	Fungal count on different sampling days (CFU×10^3 /g)							
	0	5	10	15	30	45	60	Mean
50	11.00	13.30	11.30	5.60	2.60	1.60	1.30	6.67
100	9.60	12.00	8.60	4.00	2.00	1.66	1.00	5.55
150	9.30	9.66	6.60	2.30	2.00	1.66	1.00	4.65
200	8.00	8.60	3.66	2.40	1.30	1.00	0.67	3.67
500	6.00	6.60	2.60	1.70	1.30	1.00	0.33	2.79
1000	3.60	4.30	1.66	0.00	0.00	0.00	0.00	1.37
0 (Control)	11.30	11.00	10.60	9.60	8.50	5.00	3.67	8.52
Mean	8.40	9.35	6.57	3.66	2.53	1.70	1.14	

Table 2: Effect of different concentrations of chlorpyrifos on viable fungal population in soil.

Values are Mean
LSD at 0.05
Treatment (T) = 0.99
Sampling date (SD) = 0.99
Interaction (T×SD) = 2.61

3.3. Total Viable Actinomycetes Count

The results presented in Table 3 revealed that that mean actinomycetes count decreased with increase in chlorpyrifos concentration and the maximum mean count was observed on the 5th day of incubation. At higher concentration (1000 ppm) no actinomycetes count was recorded after the 5th day of incubation under laboratory conditions.

Conc. (ppm)	Actinomycetes count on different sampling days (CFU×10^4 /g)							
	0	5	10	15	30	45	60	Mean
50	16.00	20.60	18.60	15.60	9.60	4.00	0.00	12.05
100	11.30	15.00	12.30	10.0	8.60	2.60	0.00	8.54
150	10.00	13.60	8.30	4.60	1.33	1.00	0.00	5.55
200	8.00	12.00	7.30	3.66	1.00	1.00	0.00	4.71
500	6.00	9.30	5.60	2.60	1.00	1.00	0.00	3.64
1000	4.60	6.00	0.00	0.00	0.00	0.00	0.00	1.52
0 (Control)	18.60	17.60	17.30	15.30	12.30	10.30	4.60	13.71
Mean	10.64	13.44	9.91	7.39	4.83	2.84	0.66	

Table 3: Effect of different concentrations of chlorpyrifos on viable actinomycetes population in soil.

Values are Mean
LSD at 0.05
Treatment (T) = 0.75
Sampling date (SD) =0.75
Interaction (T×SD) =1.98

Our results are in corroboration with Ahmed and Ahmad (2006) who evaluated the effect of chlorpyrifos on soil bacterial population and reported that addition of chlorpyrifos in plates brought a reduction in bacterial population at different concentrations, which were

significantly different from control and further reported that minimum count was found at 1000 ppm. Similarly Rani *et al.* (2008) reported high viable bacterial counts at 200 mg/L of chlorpyrifos, whereas at higher concentrations of chlorpyrifos (400 - 700 mg/L) the count was low as compared to the control. Further Nazia Sultan *et al.* (2010) who conducted an experiment to study the toxic effects of organophosphorus insecticides on soil bacterial population reported that treatment of soil with chlorpyrifos (100, 1000, 2000 and 10,000 ppm) brought about a reduction in bacterial population at almost all concentrations. They further reported that chlorpyrifos had maximum deleterious effect on soil bacterial population among all the four insecticides i.e. endosulfan, phorate, chlorpyrifos and polytrin, tested for their effect on soil bacterial population.

It was observed that after a small lag phase of twenty four hours bacterial count started increasing and gave maximum count on 10^{th} day of incubation and then it decreased and gave minimum count on 60^{th} day. Rani *et al* (2008) discussed that an initial rise in microbial counts in soil treatments could be due to the fact that microorganisms need an adaptation period to produce the necessary degradative enzymes which may account for the lag phase at different concentrations of chlorpyrifos and subsequent decline later on must have been due to the fact that microbial populations that were tolerant of the treated insecticide were susceptible to the products of soil insecticide interactions, which could have possibly been anti-microbial.

Das and Mukherjee (2000) studied the effect of different insecticides on soil fungal population and reported that fungi respond differently to various insecticides. They found that application of insecticides like HCH, carbofuran and fenvalerate did not significantly effect Aspergillus population, however, Fusarium, Trichoderma and Rhizopus were increased by these insecticides. On the other hand, insecticides had no effect on Penicillium population. They also studied the effect of different insecticides on soil actinomycetes population and reported that actinomycetes respond differently to insecticides treatment like Streptomyces population increased due to the incorporation of insecticides while those of Nocardia and Micromonospora population was decreased.

So it was concluded that for all treatments the bacterial count increased up to 10^{th} day of incubation and then after it decreased. For both fungi and actinomycetes count increased up to 5^{th} day and then decreased thereafter. These observationss support earlier workers (Rache and Coats 1988, Das *et al* 1995) who reported that numbers of soil microoganisms were stimulated through the utilization of chlorinated hydrocarbon, organophosphate and synthetic pyrethroid insecticides. It was also observed that soil bacterial, fungal and actinomycetes population decreased with increase in chloryrifos concentration. The decrease in the total population of soil bacteria, fungi and actinomycetes with increase in Chlorpyrifos concentration from 2 to 10 mg/kg was reported by Shan *et al* (2006). They also recorded a gradual decrease in bacterial, fungal and actinomycete count in control soil samples with increase in sampling days.

Thus, chlorpyrifos when used in the field to increase crop production besides combating insect pests also affect the population and activity of beneficial microbial communities. So its application has to be judicious as it causes a significant decrease in microbial population, which is directly related to the soil health.

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