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Research article

STUDIES ON THE IMPACT OF PESTICIDES ON SOIL MICROBIAL RESPIRATION

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ABSTRACT: The present study aims to evaluate the effect of cypermethrin and chlorpyrifos on soil micro-organisms. The impact of these insecticides on soil microbial respiration was studied in the laboratory through replicated trials using cypermethrin and chlorpyrifos at 0,5,10,15,20,50, and100 ppm levels of fortification against the heterotrophic activities of soil micro-organisms which were measured in terms of the Carbon dioxide (CO_2) evolved in the incubation chamber. The CO_2that evolved was determined after an incubation period spanning 5, 20, 35, and50 days. The Carbon dioxide evolved from the soil was related with the microbial population and their activities. The results indicated the trend of a gradual increase in CO_2 that evolved (0.73 to 0.96 mg/g) against the fortification range of 5 to100 ppm with cypermethrin after50 days of incubation as compared to control. Though there was a gradual decrease in CO_2 that evolved (1.73 to 1.53 mg/g) with chlorpyrifos after 50 days of incubation as compared to the control, but there was no adverse effect on the soil's micro-flora after treatment with chlorpyrifos. Thus, the effect of cypermethrin and chlorpyrifos on soil micro-flora in terms of CO_2evolution revealed no adverse effect on soil microbial activity.

Keywords: soil micro-flora, pesticide fortification, CO_2 evolution.

INTRODUCTION

Recent studies reveal that agricultural practices today use increased amounts of pesticides which has become a matter of concern in the context of the environment in general, and eco-toxicology, in particular. [1]The insecticides that are used frequently and which eventually reach the soil from the crop plants usually accumulate in the top layer of the soil where there is maximum microbial activity.[2] Some pesticides in the soil affect the non-target and beneficial micro-organisms[3,4,5] and their activities that are essential for maintaining soil fertility. [6] One of the main activities of these soil micro-organisms is the decomposition of organic matter in the soil. Through this process, most of the carbon content of the organic matter in the soil is released in the form of CO_2.Therefore; the amount of CO_2 liberated is dependent on both, the nature of organic matter and the microbial community of the soil [7] along with the conditions of decomposition. Soil respiration which measures the amount of CO_2 liberated, therefore, indicates the activity of these microorganisms in the soil. Thus, soil respiration is good index of the activity of these micro-organisms involved in the decomposition of the organic matter [8] (Komal et al. 1999). Soil respiration index measurement has been used frequently to evaluate the side effects of toxic chemicals, such as, insecticides in the soil. [9, 10] Ananyeva has reported that changes in soil respiration have been used as criteria for insecticide toxicity. The impact of pesticides on various soil microbial parameters, including soil respiration, has been previously reported by various authors. [11,12,13] There are many reports regarding the favourable effects of insecticides on the growth and activities of micro-organisms in the soil.[14] On the other hand, there are some insecticides which can adversely affect the growth of soil microorganisms[15] but this effect on soil microbial activities is temporary. However, the effect of different insecticides on the growth and activities of micro-organisms in the soil, since different groups of insecticides exhibit manifold variations in toxicity. [16, 17]

Therefore, the present study has been undertaken to investigate the impact of select insecticides, namely cypermethrin and chlorpyrifos under controlled laboratory conditions on soil from the agricultural experimental farm in Nagpur, India.

MATERIALS AND METHODS

Soil sample Preparation

The soil sample used in the present study was collected from the experimental farm (at Mahurzari, Nagpur, India).The soil sample was sieved to remove plant materials, stones etc. After the sieving, the soil was homogenized. The soil was pre-incubated at room temperature (30^0C ± 1) for 10 days in the laboratory which allowed the soil's microbial population to stabilize also minimizing the effect of soil handling and preparation. [18] After the pre-incubation, the soil was used for the experiment.

Experiment Design and Treatments-:

The treatment involved mixing two insecticides i.e. cypermethrin 25ECand chlorpyrifos 20EC–separately with the soil. Their effect on the soil micro-flora was evaluated by the fortification of the soil at 0,5,10,15,20,50, and100 ppm using three replicated sets. The observations were recorded at intervals of 5, 20, 35, and 50 days in terms of the CO_2 that evolved in the incubation chamber.

Preparation of the incubation chamber:

The incubation chamber was prepared using a conical flask (500ml) with a tightly fitted rubber stopper. In each flask, we took 50gm soil that had been mixed with an appropriate quantity of water in order to maintain field conditions and then, this had been mixed with10 ml of insecticide suspension prepared in water of different concentrations i.e.0, 5, 10, 15, 20, 50, and 100ppm. A glass vial (25ml) filled with 10mlof 0.5N NaOH was kept suspended in each conical flask (incubation chamber) with the help of a thread hooked from the cork inside the chamber. The rubber stopper was fitted tightly as shown in Figure1.

Method of taking reading after completion of the incubation period:

[The readings were taken separately for cypermethrin and chlorpyrifos after 5days, 20days, 35days, and50days in different sets prepared as shown in Figure 1.]

Reading after 5 days

●The vials were taken out of each conical flask (incubation chamber) after 5 days from the commencement of the experiment. The **content of the vials were titrated with 0.7N HCL using 1N $BaCl_2$ solution and a phenolphthalein indicator.** (Endpoint- pink → colourless).

The amount of CO_2 evolved from the soil was determined after considering a blank reading of 10 ml 0.5N NaOH on titration with 0.7NHCl, and then, calculating the following formulae

$$\textbf{CO}_2\textbf{ evolved (mg/g soil)} = \frac{(B-A)\times 2.2}{0.1\times N/50}$$

Where,

A= ml of 0.7NHCl required to neutralize NaOH in the vial from incubation chamber.

B= ml of 0.7NHCl required for blank reading.

N= Normality of acid used [(1mlof 0.1NHCl is equivalent to 2.2mg CO_2)]

The same procedure was repeated for taking readings of CO_2that evolved after 20 days, 35days, and and50 days and, from the readings, we calculated the CO_2that evolved.

The calculated quantity of CO_2per gram soil from observations for the incubation period of 5days, 20 days, 35days, and 50 days were considered together and used for comparison.

The data collected of the evolved CO_2as against various fortification levels of cypermethrin (25EC) and chlorpyrifos (20EC) for different incubation periods derived from the three replications (Table numbers 1 to 8) was used then, to evaluate the effect on the micro-flora present in the soil.

RESULTS

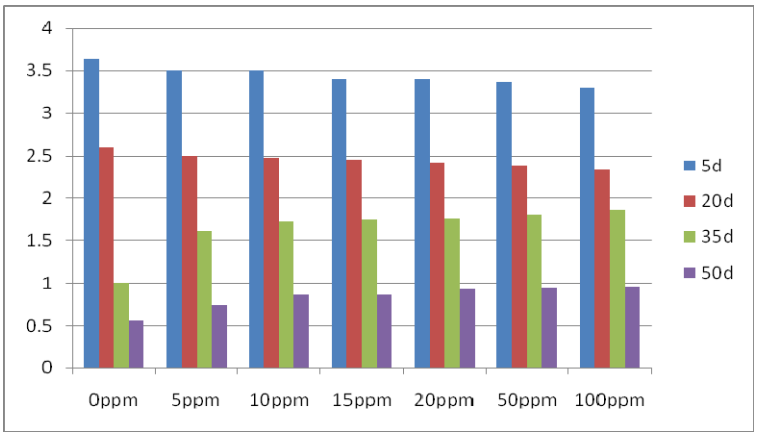
The observations were recorded for the Carbon dioxide that evolved during the incubation period from5daysto50 days against various fortification levels of cypermethrin (25EC) and chlorpyrifos (20EC).

Influence of Cypermethrin (25EC) on CO_2evolution from soil was studied from observations recorded in Tables1 to 4. These observations had been recorded during the incubation period from5daysto 50days.

The mean data (Tables1 to 4) on CO_2evolution against various fortification levels of cypermethrin indicated the trend of a gradual decrease of CO_2evolutionas against the increasing levels of fortification of the soil with pesticides as seen in Figure 2.

Similarly, the influence of Chlorpyrifos (20EC) on CO_2evolution from soil was studied from the observation Tables 5 to8 which recorded data during the incubation period from5days to50days.
The mean data (Tables1 to 4) on CO_2evolution against various fortification levels of chlorpyrifos indicated the trend of a gradual decrease of CO_2evolution initially up to20 days and then, a very gradual increase of CO_2evolution as against the increasing levels of fortification of the soilafter20 days as seen in Figure 3.

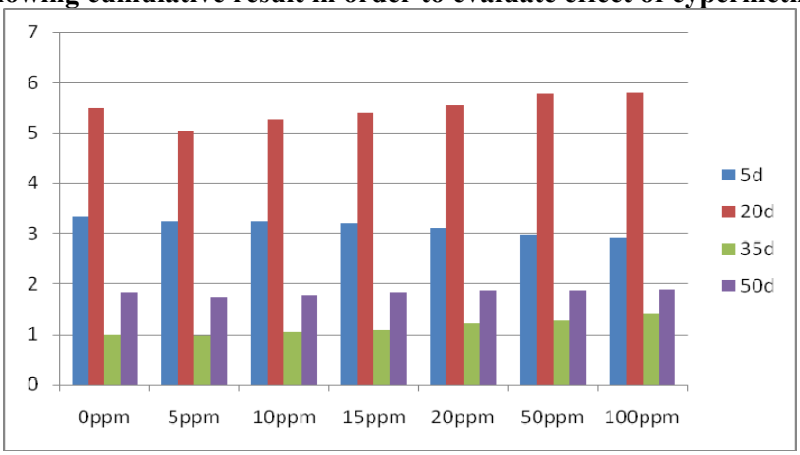
Figure 1.Pictures showing incubation chambers prepared for taking readings



Level of cypermethrin fortification (ppm) →

Figure 2.Evolution of CO_2in mg/g soil at different fortification levels of cypermethrin

Figure 2. Bar graph showing cumulative result in order to evaluate effect of cypermethrin on soil respiration



Level of chlorpyrifos fortification (ppm) →

Figure 3. Evolution of CO2in mg/g soil at different fortification levels of chlorpyrifos

Bar graph showing cumulative result in order to evaluate effect of chlorpyrifos on soil respiration

DISCUSSION

The effects of cypermethrin (25EC) and chlorpyrifos (20EC) were studied through a pot experiment in the laboratory. In the experiment, the soil collected from the experimental farm was treated separately with selected insecticides in the replicated trials using 0,5,10,15,20,50, and100ppm levels of fortification against the activities of the soil's micro-organisms.

The influence of the insecticide was evaluated by considering the CO_2that evolved which was measured after an incubation period spanning 5, 20, 35, and50 days.

When the soil was treated with cypermethrin, the CO_2 that evolved in the incubation chamber indicated that there was a gradual decrease in microbial activity when compared from5days to 50days with increasing concentrations as seen inTables1 to 4. However, when compared with untreated soil, the treated soil, irrespective of the concentrations of the cypermethrin mixed, did not show any adverse effect on the micro-flora. Thus, our result supported the work of [19] who reported that cypermethrin had no adverse effect on soil microbes.

Table 1.Effectof cypermethrin on micro-flora present in soil in terms of carbon dioxide evolved after5days: [Blank Reading: 4.2ml]

Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	RI	RII	RIII	Mean (±S.D)
0	3.7	3.6	3.6	3.63 (± 0.16)
5	3.5	3.6	3.4	3.5(±0.08)
10	3.4	3.5	3.6	3.5 (± 0.08)
15	3.4	3.4	3.4	3.4 (± 0.00)
20	3.5	3.4	3.3	3.4(±0.086)
50	3.4	3.4	3.3	3.36 (± 0.216)
100	3.2	3.4	3.3	3.3 (± 0.816)

Table 2.Effectof cypermethrin on micro-flora present in soil in terms of carbon dioxide evolved after20days: [Blank Reading: 4.3ml]

Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	RI	RII	RIII	Mean (±S.D)
0	2.5	2.7	2.6	2.6(± 0.0836)
5	2.4	2.6	2.5	2.5 (± 0.816)
10	2.4	2.5	2.5	2.47(±0.0471)
15	2.35	2.55	2.45	2.45(±0.08167)
20	2.4	2.45	2.4	2.42(±0.02449)
50	2.4	2.3	2.45	2.38(±0.1405)
100	2.4	2.3	2.3	2.33(±0.133)

Table 3.Effectof cypermethrin on micro-flora present in soil in terms of carbon dioxide evolved after35days: [Blank Reading: 4.2ml]

Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	RI	RII	RIII	Mean(±S.D)
0	1.1	1.0	1.1	1.0(± 0.416)
5	1.65	1.6	1.7	1.6(± 0.405)
10	1.7	1.8	1.7	1.73(± 0.1173
15	1.75	1.8	1.7	1.75(± 0.040)
20	1.45	1.65	2.2	1.76(± 0.3522)
50	1.8	1.7	1.9	1.8(± 0.0816)
100	1.9	1.8	1.9	1.86(± 0.1645)

Table 4.Effectof cypermethrin on micro-flora present in soil in terms of carbon dioxide evolved after50days: Blank Reading: 4.0ml

Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	RI	RII	RIII	Mean (±S.D)
0	0.6	0.6	0.5	0.56(± 0.098)
5	0.8	0.75	0.65	0.73(± 0.0936)
10	0.8	0.8	1.0	0.86(± 0.1428)
15	0.6	1.1	0.9	0.86(± 0.2318)
20	0.9	0.9	1.0	0.93(± 0.0918)
50	1.0	1.0	0.95	0.95(± 0.0408)
100	1.1	1.1	0.9	0.96(± 0.1474)

Similarly, when soil was treated with chlorpyrifos, the CO_2 that evolved in the incubation chamber showed different behavioural patterns for chlorpyrifos. The readings of the evolved CO_2were compared with data collated from the period between 5 days and50 days. This showed that initially (for the range5-20 days) there was a gradual decrease in the CO_2that evolved. This is evident in Tables 5 and6.Thus, the insecticide treatment can be said to display a short-term inhibitory effect on microbial activity. But this microbial activity seems to significantly increase in the period post35 days up to 50 days as seen inTables7 and8. [20].

Table 5.Effectof chlorpyrifos on micro-flora present in soil in terms of carbon dioxide evolved after 5days: [Blank Reading: 4.0ml]

Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	RI	RII	RIII	Mean (±S.D)
0	3.2	3.4	3.4	3.33(± 0.176)
5	3.4	3.1	3.2	3.23(± 0.1926)
10	3.1	3.3	3.3	3.23(± 0.1744)
15	3.4	3.3	3.2	3.2(± 0.8103)
20	3.2	3.0	3.1	3.1(± 0.0816)
50	3.1	2.7	3.1	2.96(± 0.2739)
100	3.0	3.2	3.2	2.9(± 1.1902

Table 6.Effectof chlorpyrifos on micro-flora present in soil in terms of carbon dioxide evolved after20days: Blank Reading: 7.9ml

Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	RI	RII	RIII	Mean (±S.D)
0	5.4	5.7	5.4	5.5(± 0.1414)
5	5.0	5.0	5.1	5.033(± 0.0746)
10	4.9	5.4	5.5	5.266(± 0.2755)
15	5.8	5.4	5.0	5.4(± 0.3265)
20	5.6	5.5	5.6	5.566(± 0.0982)
50	5.8	5.7	5.8	5.766(± 0.0995)
100	5.9	5.7	5.8	5.8(± 0.0816)

At the same time, when microbial activity was compared between the untreated soil and the treated soil, there was a small but significant inhibition of soil microbial activity that was observed during the first three weeks, [21] as noted by Pandey and Singh (2004). Thereafter, soil microbial activity recovered within 35 days [22] as seen in Tables 3 and 4 with no adverse effects (on microbial activity)attributed to the insecticide present in the soil. It is in that regard that our result did not support the work. [23]

Table 7.Effectof chlorpyrifos on micro flora present in soil in terms of carbon dioxide Evolved after35 days: [Blank Reading: 3.9ml]

Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	RI	RII	RIII	Mean (±S.D)
0	0.7	1.3	1.0	1.0(± 0.2449)
5	0.8	0.9	1.2	0.966(± 0.1737)
10	1.2	0.8	1.1	1.03(± 0.1891)
15	1.1	1.0	1.1	1.066(± 0.06036)
20	1.1	1.3	1.2	1.2(± 0.08164)
50	1.3	1.4	1.1	1.266(± 0.1313)
100	1.6	1.2	1.4	1.4(± 0.6376)

Table 8.Effect of chlorpyrifos on micro-flora present in soil in terms of carbon dioxide evolved after 50 days: [Blank Reading: 3.5ml]

Level of fortification in (ppm)	CO2 evolved in mg/g soil			
	RI	RII	RIII	Mean(±S.D)
0	2.0	1.7	1.8	1.83(± 0.1666)
5	1.8	1.6	1.8	1.73(± 0.1429)
10	1.7	1.7	1.9	1.766(± 0.106)
15	1.8	1.8	1.9	1.83(± 0.1666)
20	1.9	1.9	1.8	1.866(± 0.1343)
50	2.0	1.7	1.9	1.866(± 0.1343)
100	1.9	2.0	1.8	1.9(± 0.0816)

CONCLUSION

The present study reveals that when the soil is treated with cypermethrin and chlorpyrifos, the adverse effect on microbial activity is seen to be minimal. Cypermethrin was observed to have a consistently stimulatory effect, whereas chlorpyrifos was less simulative. However, both did not show adverse effects on microbial activity as compared with their effects on untreated soils or the control.

Therefore, it may be concluded that cypermethrin and chlorpyrifos to protect the plant can be safely used such that their effects will not be drastic but minor in nature.

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