EFFECT OF INSECTICIDES ON THE TOTAL NUMBER OF SOIL BACTERIA UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT

The laboratory and field studies were conducted to determine the effects of chlorpyrifos 40EC, imidacloprid 200SL, cypermethrin 10EC, endosulfan 35EC, carbofuran 20EC, and cypermethrin 10EC bifenthrin 10EC, at four concentrations (125, 250, 500, and 1000 ppm), and at field application rates, respectively on total number of soil bacteria's population. Nutrient Agar medium was used for the count of bacterial populations. Soil samples at 15-20cm depths were taken from agricultural fields, largely under sugarcane cultivation. Results obtained from both studies revealed that chlorpyrifos caused significant reduction in number of soil bacteria, however, in the filed experiment, effect disappeared at 21 day after application. Bifenthrin on the other hand increased the number of bacterial population at 250 (50.97) and 500 (41.69) ppm in the laboratory studies over the control (15.52) while in the field trail, bacteria population was unharmed, with increase (13.97) at day 7 after application over the pre-treatment number (11.30).

Key words: chlorpyrifos, bifenthrin, imidacloprid, carbofuran, endosulfan, cypermethrin, soil, bacteria, population

INTRODUCTION

In modern agriculture, pesticides are frequently used in the field to increase crop production. Besides combating insect pests, insecticides also affect the population and activity of beneficial microbial communities in soil (Pandey and Singh, 2004), especially in the case where these insecticides are applied as termiticides.

The effect of insecticides on soil microbial communities was variable with insecticide types, their doses and field conditions. Low concentration of lannate stimulated the growth, the toxic effect (high concentration) lasted only a few days and as such chemical did not represent a danger to the soil microorganisms (Bakalivanov, 1990; Cernakova, 1993). Population of microorganisms in the soil containing cis-isomer and trans-isomers of cypermethrin at different time durations had no adverse effects on soil microbes (Binner *et al.*, 1999). Stimulatory effect has been reported for carbofuran at all doses under laboratory condition (Dordevic *et al.*, 1998*;* Das and Mukherjee, 1998).

Soil microbes had different response to types of insecticides. Cypermethrin and monocrotophos had adverse effects on the total number of soil bacteria in the soil while fenvalerate had very low effect on the soil microbes (Rangaswamy and Venkateswarlu, 1992; Ajaz *et al*., 2005).

Diversity of soil microbes affected variously by insecticides has also been studied. Dimethoate (0.2%), fenitrothion, lindane, @ 3.5 to 15kg per hectare, phorate at 300 fg / g or malathion at 100-300 fg / g had specifically toxic effect on one type of microorganisms but stimulated the growth of another type. Some of the insecticides were not toxic to these microbes under different soil uses (Martinez-toldo *et al*., 1993; Gonzalez-Lopez, *et al*., 1993; Mandic *et al*., 1997; Digrak and Kazanici, 2001). However, field doses of many insecticides (gamma-HCH, phoxim, cypermethrin and chlorfluazuron) had inhibitory effect on soil microorganisms (Amirkhanov *et al.*, 1994).

Reduction in number of microorganisms in different soil types and at various depths was investigated and insecticides and /or their residues inhibited the growth of microorganisms (Digrak and Ozcelik 1998; Tawfic *et al*., 1998; Hashem *et al*., 1999).

The present studies were undertaken to determine the effect of currently used insecticides (=Termiticides) on soil bacterial communities. Chlorpyrifos 40EC, imidachloprid 200SL, cypermethrin 10EC, endosulfan 35EC, carbofuran

20EC, and bifenthrin 10EC were, the insecticides, commonly used in termite control in Pakistan. The effect was seen in the laboratory as serial aqueous concentrations and in the field at control application rates.

MATERIALS AND METHODS

The bioassay and estimating bacterial communities of the soil samples were done in the Laboratories of Institute of Agricultural Microbiology and Biotechnology, Ayub Agricultural Research Institutue, Faisalabad, Pakistan.

Laboratory Experiments

Soil solution preparation

In laboratory experiment, soil samples were taken with the help of sterilized spatula at a depth of 15-20 cm from different agricultural fields of Ayub Agricultural Research Institute, Faisalabad, Pakistan. Soil organic matter and moisture was determined then other plant debris was removed manually and soil was sieved with 4mm mesh. 1 gram of soil was mixed with 9 ml of sterilized water and shook it 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^{-7} were prepared to determine the appropriate dilutions to count bacterial population conveniently. A stock solution of each pesticide was made for further dilutions i.e., 125, 250, 500, and 1000 ppm.

Field experiments

Soil sampling

Soil samples were taken at random by a soil auger from 8-10 places from different fields and mixed thoroughly to prepare one composite sample. Plant material and other debris were removed from the sample by hand and the soil was sieved using 4 mm mesh. The soil samples were collected from top 15-20 cm, largely under sugarcane cultivation. Soil samples were collected at repeated intervals before and after each insecticide treatment. The last sampling was done 28 days after the treatment. Samples were brought to the laboratory and stored at 4°C till analyses were conducted. Soil moisture and organic matter of the soil were recorded at the time of sampling.

Soil bacterial counts

20 grams of Nutrient Agar (Lerk) was put in 1 litre graduated flask and the volume was made up to the mark by adding sterilized water. pH of media was adjusted with the help of conc. NaOH or conc. H_2SO_4 by a Electro processor pH meter. The medium was then sterilized in autoclave at 15 psi and 120°C for 20 min and cooled to a pouring temperature of about 37°C. Serial soil dilutions were prepared for appropriate counting of the bacterial population. One ml of the required dilution was spread evenly on an agar-medium petri plates to determine number of populations per gram soil. In case of the laboratory assay, insecticide solution from stock solution was mixed in the soil solution to get the desired concentrations. Incubation of the petri plates was done in a room set at 30±2^{o}C and 68±2%, temperature and relative humidity, respectively.

RESULTS

Evaluation of effect of insecticides on soil bacterial populations in Laboratory

Bacterial counts in insecticide treated Petri plates in the laboratory are given in Table 1. Addition of chlorpyrifos in plates brought a reduction in bacterial population at different concentrations, which were significantly different from control (8.60). Minimum number (3.62) of populations was found at 1000 ppm, which was non–significantly different from 250 ppm (4.12). Bacterial population at 250 ppm of imidacloprid (11.86) was non-significantly different from control (11.07). At other concentrations the bacterial population had shown reduction as compared to control, of which 500 ppm (6.02) and 1000 ppm (8.62) were significantly different from it. Cypermethrin had shown an increase (13.3) in bacterial populations over control (11.93) at 1000 ppm and two had significant difference between each other. Bacterial population at different concentrations of endosulfan had significant difference from control. Bacterial population at 125 ppm (6.26) and 1000 ppm (5.33) were significantly different from each other. Carbofuran had demonstrated little change in bacterial populations when compared at different concentrations and control, however, populations at 125 ppm (7.67) and 1000 ppm (6.21) had significant difference between each other. Bifenthrin had significantly increased populations (50.97 and 41.69) respectively at 250 ppm and 500 ppm. The populations at 1000 ppm of bifenthrin (16.90) were statistically at par with control (15.52).

Evaluation of effect of insecticides on soil bacterial populations in field

Soil from chlorpyrifos treated fields showed significant changes in bacterial populations at different post-application intervals over pre-treatment counts (11.93). There was reduction in populations at post-application day 1 (6.33), 7 (9.07) and day 14 (8.23), latter two had non-significant difference between each other. Imidacloprid registered a reduction in bacterial populations on post-application day 1 (8.53), day 7 (9.87) and day 14 (8.60) over control (10.90), latter was statistically similar to population at day 21 (10.00). Cypermethrin showed similarity in population at pre-treatment (11.23) and after day 14 (11.03). Endosulfan brought reduction in population after day 1 (7.27) over pre-treatment (13.70) and then at different post-treatment intervals, populations had non-significant difference among them. Pre-treatment bacterial populations (10.43) had statistical similarity to post-treatment intervals except at day 28 (8.13), which was significantly different from populations at other days in case of carbofuran. Bifenthrin had registered an increase in bacterial populations at day 7 (13.97), day 14 (12.10) and day 21 (12.43) over pre-treatment populations (11.30) (Table 2).

Table 1. Comparison of effect of insecticides on soil bacterial populations in the laboratory

Conc.	Number of bacterial population					
(ppm)	chlorpyrifos	imidacloprid	cypermethrin	endosulfan	carbofuran	bifenthrin
0	8.60±0.75a	11.07±0.77a	11.93±0.86bc	7.74±0.80a	7.31±0.90a	15.52±1.82d
125	5.26±0.41b	9.57±0.77ab	10.9±0.82d	6.26±0.69b	7.67±0.79a	10.79±0.90c
250	4.93±0.43 bc	11.86±0.92a	12.24±0.95b	5.90±0.67bc	6.93±0.87ab	50.97±4.32a
500	4.12±0.41cd	6.02±0.44c	11.02±0.83cd	5.78±0.65bc	6.67±0.74ab	41.69±4.22b
1000	3.62±0.41d	8.62±0.62b	13.3±0.86a	5.33±0.63c	6.21±0.71b	16.90±1.82d

Values are Means ± SE. Means were compared by Duncan's Multiple range test using LSD at P=0.05. Means sharing similar letter in a column are not significantly different from one another.

Table 2. Comparison of effect of insecticides on soil bacterial populations in the field

Intervals of soil sampling	Number of bacterial population					
Days	chlorpyrifos	imidacloprid	cypermethrin	endosulfan	carbofuran	bifenthrin
Pre- treatment						
	11.93±1.21b	10.90±121a	11.23±1.83a	13.70±1.83a	10.43±1.65ab	11.30±1.57b
Post-treatment						
Day 1	6.33±0.69d	8.53±0.99c	8.57±1.18d	7.27±1.02c	10.43±1.55ab	11.77±1.54ab
Day 7	9.07±0.71c	9.87±1.14ab	9.37±1.36c	10.33±1.34b	11.30±1.53a	13.97±1.78a
Day 14	8.23±0.79c	8.60±1.14bc	11.03±1.43a	11.63±1.45ab	9.10±1.29b	12.10±1.68a
Day 21	11.37±1.03b	10.00±1.13ab	10.27±1.43b	10.67±1.56b	10.17±1.40b	12.43±1.66a
Day 28	13.73±1.38a	9.03±1.33b	10.30±1.40b	11.06±1.74b	8.13±1.37c	11.83±1.68ab

Values are Means ± SE. Means were compared by Duncan's Multiple range test using LSD at P=0.05. Means sharing similar letter in a column are not significantly different from one another.

DISCUSSION

The results of present study revealed that cypermthrin had negative effect on total number of soil bacteria and this effect was offset after 14th day of treatment but at slow rate, which is conformity with Rangaswamy and Ventakeswarlu (1992) who reported that above cited effect of cypermethrin and monocrotophos on bacteria while fenvalerate had very low effect on soil microbes. Our results do not support the work of Binner *et al*. (1999) who reported that cypermrehtirn had no adverse effect on soil microbes.

In case of endosulfan, significant reduction in soil microbes occurred at high concentration (1000 ppm) and this is supported by the finding of Digrak and Ozcelik (1998) and Nasim *et al*. (2005) who also confirmed the same result for endosulfan.

Carbofuran has acted as stimulator for the population growth of bacteria which was confirmed by the finding of Dordevic *et al*. (1999) and Das and Mukherjee *et al*. (1998). They reported that carbofuran significantly stimulated the populations of bacteria as well as N2 fixing bacteria in the agricultural soil while other tested insecticides reduced proportions of Micrococcus and Rhizopus in the soil.

Imidacloprid also suppressed the soil bacteria communities but at slow rate and normally after 21 days of application its negative effect was vanished. Bifenthrin, too, increased the bacterial population at certain concentrations and at certain days after application over pretreatment counts. We have not found any reference relating to effect of imidacloprid and bifenthrin in the literature.

Among the six pesticides applied chlorpyrifos proved to be the most destructive on soil bacteria. Short-term inhibitory effect on the total bacterial population was observed after chlorpyrifos and quinalphos applications in the groundnut fields, which recovered within 60 days after seed treatment and by 45 days of soil treatment (Pandey and Singh, 2004). Chlorpyrifos hyper resistant bacteria have been isolated from cotton cultivated soil using conventional and API kit methods (Ajaz *et al*., 2005). In the present study, reduction in bacterial populations was counted up to 14 days after field treatment of chlorpyrifos. The appearance of population after 14 days may be due to some of resistant bacteria that had not appeared in the laboratory conditions when the assay was terminated up to six days as there was overlapping of population and it had become difficult to count the population individually. The less number of bacterial populations may also indicate the quantity of organic matter in soils of Pakistan where value of 0.5% is considered as standard for organic matter. It is, therefore, concluded that all tested insecticides can be safely used as soil treatment when intended to control termites.

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