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EFFECT OF TRIAZOPHOS-AN ORGANOPHOSPHATE INSECTICIDE ON MICROBIAL POPULATION IN PADDY SOILS

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ABSTRACT

Triazophos is a Thiophosphoric and is the active ingradient of the insecticide Hostathion. The aim of the study is to determine the effects of triazophos at five different concentrations on soil bacterial and fungal count. The effect of triazophos on bacterial and fungal population by incubation in soil for 7 days was determined. Results revealed that triazophos caused significant reduction in the bacterial and fungal populations. By statistical analysis, the concentrations of the pesticides as well as the dilution of soil sample were analyzed by two way ANOVA. Study reveals that both bacterial and fungal population the concentration of triazophos vs sample dilution has been significantly affected. The change in concentration brought significant reduction in bacterial count. We conclude that triazophos insecticide is using in paddy soils highly toxic pollutant to the bacterial and fungal organisms.

Key Words: Triazophos, Soil, Bacteria, Fungi, Two- way ANOVA

INTRODUCTION

India is primarily an agriculture based country with more than 60-70 per cent of its population dependent on agriculture. It's fast growing population is projected to cross 1.3 billion by 2020 (Kanekar *et al*., 2004).Organophosphates are a group of highly toxic pesticides widely used for increasing agricultural productivity in soil. Organophosphates are also reared as non persistent (Racke and Coats, 1988). The reduced persistence of organophosphate insecticides attributed to soil microorganisms has been described by (Chapman and Harris., 1982; Gorder *et al.,* 1982; Sharmila *et al.,* 1989). Triazophos (TAP, O,O-diethyl-O-1-phenyl-1H-1,2,4-triazol-3-yl-phosphorothioate) is one of the broad-spectrum, toxic, nonsystemic contact organophosphorus pesticides (OPs). It has been used on various crops, including rice, cotton, okra and maize to contro pest insects, nematodes and acarids acarids some since the late 1970s (Aungpradit *et al*., 2007; Lin and Yuan., 2005). Pesticides in soil for longer period is undesirable and it leads to the accumulation of the chemicals in soil to highly toxic levels. In addition, these pesticide residues may be assimilated by the plants and get accumulated in edible plant products such as root crops (Babu *et al.,* 2008). 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 hectare (Ranjard and Richaume, 2001).Soil bacsteria play a key role in the global recycling of carbon and other essential elements because of their outstanding range of 10^6 to 10^9 bacteria per gram of soil and metabolic activities to exploit many sources of energy and carbon (Alexander, 1977). The influence of these pesticides on soil bacteria is dependent on physical, chemical and biochemical conditions, in addition to the nature and concentration of the pesticides (Aurelia, 2009).Fungi form a large part of the total biomass of micro flora in soil. Microbial biomass in soil is considered to be agents of breakdown of organic matter, when organic materials are applied to soil (Jenkenson and Ladd, 1981). They are usually abundant in the upper layers of the soil, where aerobic conditions prevail. However, numerically much less abundant (between 10^4 and 10^6 fungal propagules per gram of soil) than bacteria (Alexander, 1977).The present paper deals with impact assessment of commonly used organophosphate insecticide triazophos (40%EC) on soil bacteria and fungal population dynamics.

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MATERIALS AND METHODS

Pesticide

Triazophos was selected for the present investigation in view of it's wide usage in Srikalahasti paddy cultivation for the control of aphids, thrips, midges, beetles, larvaes, cutworms and other soil insects. It is an organophosphorous insecticide as per the international union of pure and applied chemistry (IUPAC),chemical formula of the compound is diethyl o-(1-phenyl-1h-1,2,4-triazol-3-yl) phosphorothioate. It is also known as nicotinamide, and a common insecticide, was obtained from Sudarshan Chemical Industries Ltd., Pune, India.

Collection of Soil

The soil samples were collected from Srikalahasti, Chittoor district a semi-arid region in Andhra Pradesh, India. Samples of about 1 kg were taken from the first 15 cm of depth and then pooled and sieved. Samples were air dried and stored in polyethylene bags at 4 □C. The texture of the soil was Black loam-sandy clay with a pH of about 7.6, maximum water holding capacity 0.224 ml/g, sand 50%, silt 20%, clay 29%, organic matter 0.9%.

Soil incubation studies

The effect of different concentrations of triazophos on microbial population was determined in soil sample.

Effect of Triazophos on soil population

Population of bacteria

The effect of different concentrations of triazophos on bacterial population in agricultural soil sample, in triplicate, was determined. Aliquots (0.05 ml) from stock solutions of the pesticides were applied to five-gram portions of soil contained in test tubes (15 X 150mm). The final concentrations (w/w) of each pesticide concentrations 5, 10, 25, 50 and 100 μg/g soil, which are equivalent to 0.5, 1.0, 2.5, 5.0 and 10.0 kg ha^{-1}. The soil samples receiving only distilled water served as controls. According to Shukla and Mishra (1997), Soil samples were then homogenized to distribute the insecticide, and enough distilled water was added to maintain at 60% water holding capacity (WHC) and incubated at room temperature (28 ± 4^0C). Seven days after incubation, triplicates of each treatment were withdrawn for estimation of bacterial population. Aliquots were prepared from 10^{-1} to 10^{-7} from treated and untreated soil samples by serial dilution plate method on nutrient agar medium and subsequently incubated for 24 hrs in an incubator at 30^0C. The experiments were performed in triplicate.

Population of fungi

The effect of different concentrations of triazophos on fungal population(triplicates) in agricultural soil samples was determined, following the same procedure adopted as in the case of bacterial population. Soil plate method was used to asses fungal propagules developing on rose bengal agar medium and subsequently incubated for five days at 25°C (Shukla and Mishra, 1996).

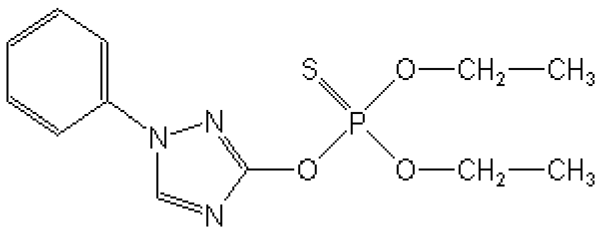
STATISTICAL ANALYSIS

The data on Triazophos impact on microbial bacterial and fungal populations was interpreted by two way Analysis of variance(ANOVA) and duncan multiple range test (DMR).

RESULTS AND DISCUSSION

Chemical structure of Triazophos

Molecular formula of Triazophos is $C_{12}H_{16}N_3O_3PS$



Structure of Triazophos

Physico-chemical properties of soil

The physico-chemical properties of the experimental soil was shown in (Table 1).

Population of bacteria

The effects of triazophos on the number of bacteria cells per gram of soil was shown in (Table 2) and (Figure1). Results reveals that the reduction in the total bacterial count with increase in the insecticide concentrations when compared with control group. The results of the present study doesn't agree with many studies used insecticides on soil microbial communities(Stanlake and Clark, 1975; Digrak and Kazanici 2001) who notice that the presence of pesticides led to an increase in the total number of soil bacteria, nevertheless, the present study shows that the presence of insecticides led to inhibition in the growth rate of soil bacteria. Bacterial population has been significantly affected by the concentration ($F=536.37$; df 5; $p<0.000$) of Triazophos and the sample dilution ($F=19.700$; df 1; $p<0.00$), the concentration vs sample dilution results ($F = 4.979$; df 5; $p<0.03$) as given by ANOVA (Table 3). Treatment of soil with Triazophos brought about a reduction in bacterial population at almost all concentrations (Fig 1). Minimum bacterial count was observed in plate with 100 ppm concentration of triazophos which was significantly very low when compared to control. Short term inhibitory effect on the total bacterial population was observed, which is in accordance with the findings by (Ahmed and Ahmed, 2006; Tawfic *et al.,* 1998 ; Hashem *et al.,* 1999).

Population of fungi

Impact of triazophos on fungi was shown in table 4 and figure 2. The results reveals that decreased in the total fungal count with increasing in the insecticide concentrations compared with control group. Similarly, vapam, captan and nabam at 60 to 400 ppm inhibited fungi in soils (Domsch, 1959;Chandra and Bollen, 1961) demonstrated that soil application of nabam (100 ppm) and mylon (150 ppm) significantly decreased the number of fungal propagules. Fungal population has been significantly affected by the concentration ($F=214.25$; df 5; $p<0.000$) of Triazophos and the sample dilution ($F=10.97$; df 1; $p<0.003$), the concentration vs sample dilution results ($F = 0.497$; df 5; $p<0.775$) as given by ANOVA (Table 5).

CONCLUSION

Our research showed that natural growth of beneficial soil bacterial and fungal species is affected by Triazophos when applied in agricultural farm. The obtained results indicated that triazophos used had adverse effect on bacterial and fungal population. We conclude that triazophos is highly toxic to the soil microbes.

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Table 1: Soil Physico-Chemical properties

Properties	Soil
Sand (%)	50%
Silt (%)	20%
Clay (%)	29%
Organic matter (%)	0.9%
Texture	Black loam-sandy clay
p^H	7.6
Maximum water-holding capacity(ml/g)	0.224

Table 2: Statistical analysis of effect of triazophos on bacterial count

Concentration	10^6 dilution	10^7 dilution	Total
0 ppm	1186.67 ± 36.074	966.67±98.820	1076.67±137.647
10 ppm	234.67±103.737	162.67±12.220	198.67±76.938
25 ppm	105.67±22.898	90.33±6.506	98.00±17.239
50 ppm	72.33±7.371	32.00±5.292	52.17±22.825
75 ppm	46.33±6.028	23.00±2.646	34.67±13.441
100 ppm	32.67±4.163	17.67±1.528	25.17±8.681

Values are the means of triplicates ± SD

Table 3: Summay of Two-way ANOVA for Bacteria count

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Concentration	5070825.222	5	1014165.044	536.37 **	.000
Dilution	37249.000	1	37249.000	19.700 **	.000
Concentration*dilution	47074.000	5	9414.800	4.979 **	.003
Error	45378.667	24	1890.778		
Total	5200526.880	35			

*significant at 5% level

**significant at 1% level

Table 4: Statistical analysis of effect triazophos on fungal count

Concentration	10^2 dilution	10^3 dilution	Total
0 ppm	95.33±6.429	88.33±5.859	91.83±6.706
10 ppm	63.33±5.033	63.33±6.429	63.33±5.164
25 ppm	44.67±6.110	39.00±7.000	41.83±6.646
50 ppm	34.67±5.033	26.67±3.055	30.67±5.750
75 ppm	24.67±3.055	18.33±1.528	21.50±4.087
100 ppm	16.67±3.055	11.33±1.155	14.00±3.578

Values are the means of triplicates ± SD

Table 5: Summay of Two-way ANOVA for Fungal count

Source	Sum of Squares	df	Mean Square	F-value	p-value
Concentration	25502.472	5	5100.494	214.25 **	.000
dilution	261.361	1	261.361	10.97 **	.003
concentration * dilution	59.139	5	11.828	.497	.775
Error	571.333	24	23.806		
Total	26394.306	35			

* significant at 5% level
**significant at 1%level

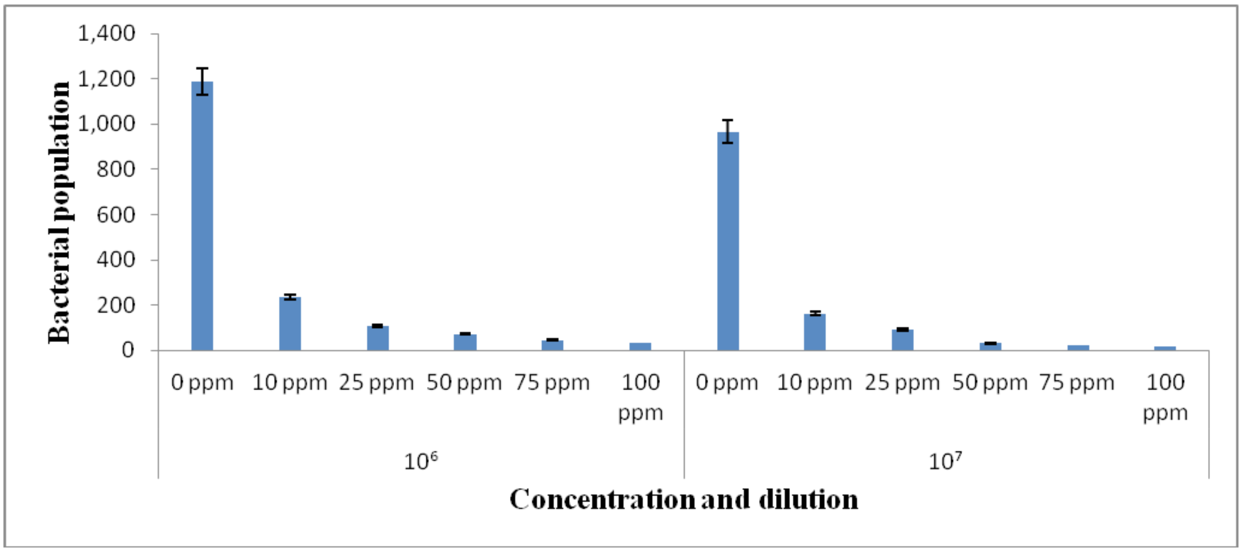

Figure 1: Population of Bacterial count

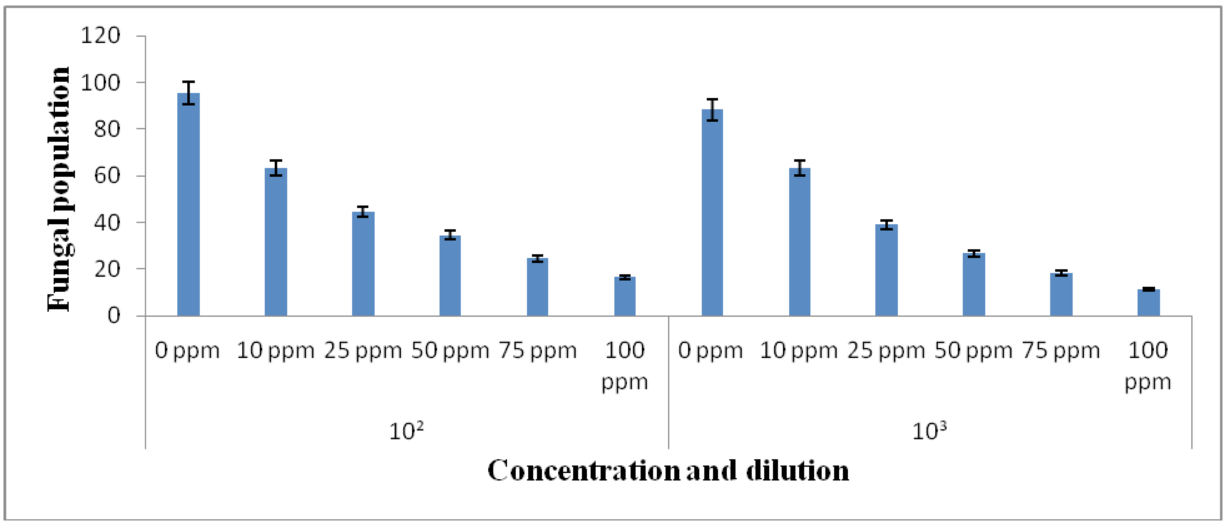

Figure 2: Population of Fungal count