ISSN: 0973 - 7049

The Bioscan: Special issue, Vol. 1; 239 -246; 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

EFFECT OF ORGANOPHOSPHORUS INSECTICIDE ON SOIL BACTERIA

Nazia Sultan *et al.*

Soil

Pesticides

Bacterial population

Paper presented in International Conference on Environment, Energy and Development (from Stockholm to Copenhagen and beyond) December 10 - 12, 2010, Sambalpur University

The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

NAZIA SULTAN*, BHARTI S. RAIPAT[1] AND M. P. SINHA[2]
Centre for Biotechnology, Marwari College, Ranchi – 834 001
[1]Depatment of Zoolgoy, St. Xavier's College, Ranchi - 834 001
[2]Depatment of Zoolgoy, Ranchi University, Ranchi - 834 008
E mail: naz.cherry@gmail.com

ABSTRACT

Laboratory experiments were conducted to study the toxic effects of Organophosphorus insecticides on soil bacterial population. The cropland soil from 5-15 cm depth were treated with four different Organophosphates (Endosulphan, Chlorpyrifos, Polytrin and Phorate) at four different concentrations (100, 1000, 2000 and 10,000 ppm) and their effect on the total number of bacterial population was investigated. Results obtained revealed that Chlorpyrifos caused significant reduction in the bacterial colonies. Polytrin treated soil on the other hand showed slight increase in the bacterial colony number, maximum at 100 ppm concentration ($8.9X10^6$), over the control ($5.2X10^6$) after 10 days of incubation in laboratory. Endosulphan with increase in concentration had negative impact on bacterial population. Phorate also acted as an inhibitor but had comparatively less impact on bacterial colony count than Endosulphan. A 2 way ANOVA was carried out and the data so obtained showed that both the concentrations of the pesticides as well as the duration of treatment brought about significant change in bacterial number {[Endosulphan $F=8.700417$; df4,4; $p<0.01$; (concentration); $F=43.76176$; df4,4; $p<0.01$; (duration of treatment)]; [Chlorpyrifos :- $F=12.04944$; df4,4; $p<0.01$; (concentration); $F=36.18957$; df4,4; $p<0.01$; (duration of treatment)]; [Phorate :- $F=4.284723$; df4,4; $p<0.01$; (concentration); $F=7.799454$; df4,4; $p<0.01$; (duration of treatment)} except for Polytrin, {$F=2.816548$; df4,4; $p>0.01$; (concentration); $F=9.546841$; df4,4; $p<0.01$; (duration of treatment)}; where the change in concentration did not brought significant reduction in bacterial count.

*Corresponding author

INTRODUCTION

Pesticides are frequently used in the field to increase crop yield and has become an integral and economically essential part of modern agriculture. Besides combating insect pests, these also affect the population and activity of beneficial microbial communities in soil (Pandey and Singh, 2004).

Since the Organochlorine insecticides cannot be attacked by micro-organisms, they have been gradually replaced by Organophosphates and Carbamates (Francisco *et al.*, 1979). A variety of treatments during agricultural practices introduce pesticides into soil (Audus, 1964).

The wide use of organophosphorus pesticides has created numerous problems including the pollution of the environment. Organophosphorus pesticides are regarded as non-persistent (Racke and Coats, 1988). 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). Low concentrations of Lannate stimulated the growth of soil microbial communities and did not represent much danger (Bakalivanov, 1990; Cernakova, 1993). Similarly Cypermethrin at different time durations had no adverse effects on soil microbes (Binner *et al.*, 1999), whereas all doses of Carbofuran has been found to have stimulatory effect under laboratory conditions (Dordevic *et al.*, 1998; Das and Mukherjee, 1998).

The reduced persistence of organophosphate insecticides attributed to soil microorganisms has been described (Chapman and Harris, 1982; Gorder *et al.*, 1982; Sharmila *et al.*, 1989). These pesticides generally get accumulated in top soil to a depth of approximately 10-15 cm, which is the zone of maximum activity of soil microflora and fauna. There they may exert effect on saprophytic soil microbes and their activities (Suneja *et al.*, 2008). The biodegradation of organophosphorus insecticides by microorganisms in soil has been widely reported (Digrak and Elazig, 1994; Digrak *et al.*, 1995; Lal and saxena, 1982; Motosugi and Soda, 1983), however the effects of Organophosphorus insecticide on soil bacteria have received less attention. Therefore there is an urgent need for further studies on the effect of these organophosphorus insecticides on non target soil bacteria from environmental stability, bioaccumulation and toxicity to non target species view point. The present paper deals with impact assesement of four commonly used organophosphates (Phorate (10% CG), Chlorpyrifos(20% EC), Polytrin (C44 EC) and Endosulphan (35%EC) of Jharkhand on soil bacterial population dynamics.

MATERIALS AND METHODS

The response of bacterial populations in soil after the application of four organophosphorus insecticides at different concentrations was examined.

The soil samples of known record were taken with the help of sterilized equipment from a depth of 5-10 cm from the agricultural field of Horticulture and Agroforestry Research Program (HARP), ICAR Research Complex for Eastern Region, Palandu, Ranchi, (Jharkhand). All the soil samples were collected in plastic bags, taken to the laboratory and stored at 4°C until used for the study.

In the laboratory, the plant material and soil microfauna were removed manually and the soil samples were sieved (<2mm).The experiments were set up in sterilized test pots and each pot contains 1 kg of soil sample.

The pesticides (Mfd: Agritech Ltd and Syngenta India Ltd.) were obtained from market. Soil sample in each pot were treated with four different organophosphorus insecticides viz: Phorate, Polytrin, Chlorpyrifos and Endosulphan at four different concentrations (100, 1000, 2000 and 10,000 ppm) and incubated. Control containing only soil were included in all tests. The temperature of incubation for both mixed and control sample was set at 30°C. Samples for analysis were taken at intervals of 05 days upto 20 days. For each treatment, samples were examined in triplicate. Enumeration of soil bacterial population was done by pour plate method on nutrient agar medium. (Johnson and Curl, 1972). On different days (0, 5, 10, 15 and 20) during the incubation period 1 g of the test sample were taken from the treated and control pots and each were suspended in 9 mL of autoclaved distilled water. Dilutions of 10^{-7} were prepared and plated on nutrient agar medium. Petriplates were incubated at 37°C for 48 hr for bacterial growth. The colonies were counted on colony counter and the results were evaluated as number of bacterial colonies in 1 g of oven dried soil.

RESULTS AND DISCUSSION

Edaphic characteristics of the experimental soil:

pH	5.16
% Organic matter	0.49134
% Organic carbon	0.285
Phosphorus (kg/ha)	4.9056
Potassium (kg/ha)	103.936

Bacterial counts in insecticide treated petriplates in the laboratory are given in Fig. 1, 2, 3 and 4 for four different pesticides.

From Fig. 1, it is clear that bacterial population at different concentrations of Endosulphan has significant difference from control. Number of total bacteria at 10,000 ppm concentration of Endosulphan ($1.8X10^6g^{-1}$) was significantly different from that of control ($6.1X10^6g^{-1}$) on 20^{th} day of incubation. Similar results for Endosulphan are present in the findings of Digrak and Ozcelik(1998) and Nasim *et al*. (2005).In the control soil, decrease in the number of total bacteria occurred during the first ten days of incubation period, which recovered later on. Bacterial population has been significantly affected by both, the concentration ($F=8.700417$; df 4, 4; $p<0.01$) of Endosulphan and the duration of treatment ($F=43.76176$; df 4, 4; $p<0.01$), as given by ANOVA, shown in Table 1.

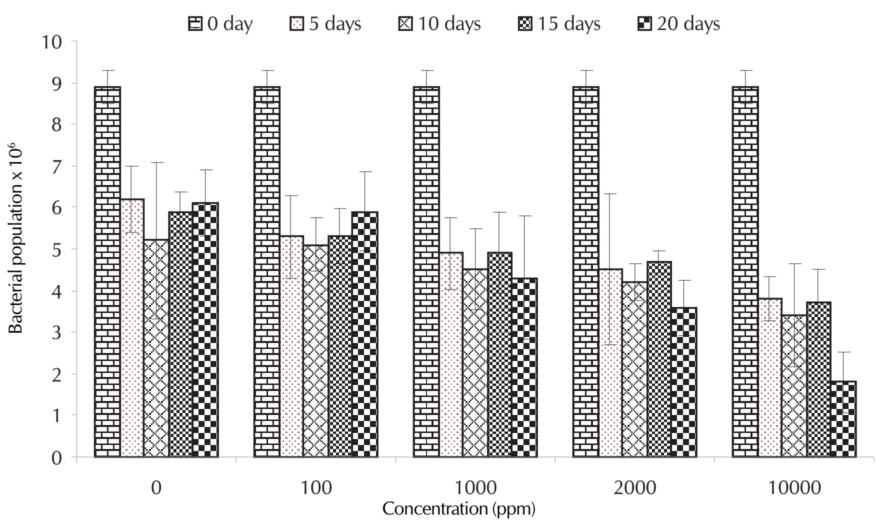

Figure 1: Effect of different concentration of endosulphan at different period of incubation

Table 1: Analysis of variance (2-way) for Endosulphan

Source of variation	SS	df	MS	F	p-value	F critical
Concentration of Endosulphan	14.608	4	3.652	8.700417	0.000627	3.0069173
Duration of treatment	73.476	4	18.369	43.76176	2.02E-08	3.0069173
Error	6.716	16	0.41975			

Treatment of soil with Chlorpyrifos brought about a reduction in bacterial population at almost all concentrations (Fig. 2). Minimum bacterial count was observed in plate with 10,000 ppm concentration of Chlorpyrifos ($0.3X10^6$ g^{-1}) which was significantly very less from control ($5.1X10^6$ g^{-1}) on 5^{th} day of incubation.

Short term inhibitory effect on the total bacterial population was observed, which is in accordance with the findings of Ahmed and Ahmed (2006); Tawfic *et al*. (1998) and Hashem *et al.* (1999). 2-way ANOVA (Table 2) in case of Chlorpyrifos revealed that change in concentration of this pesticide as well as different duration of treatment, both proved to be significant in bacterial population dynamics ($F=12.04944$; df 4, 4; $p<0.01$; $F=36.18957$; df 4, 4; $p<0.01$).

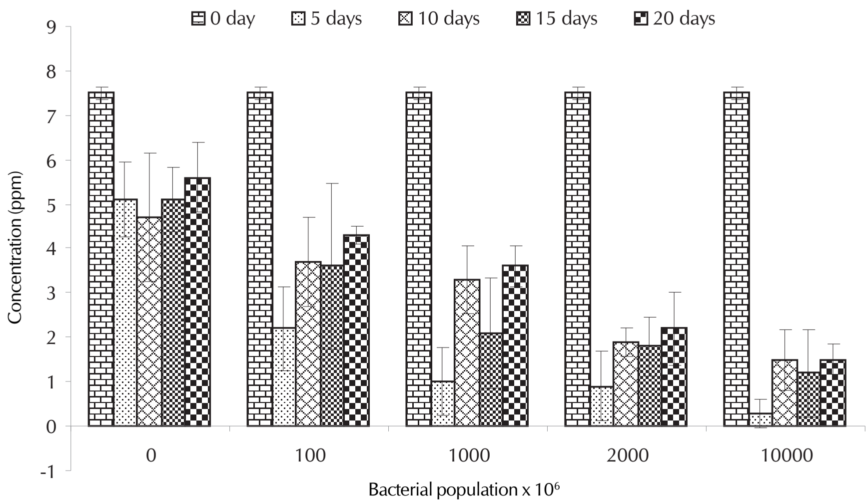

Figure 2: Effect of different concentrations of Chlorpyrifos at different period of incubation

Table 2:Analysis of variance (2-way) for chlorpyrifos

Source of variation	SS	df	MS	F	p-value	F critical
Concentration of Chlorpyrifos	31.7816	4	7.9454	12.04944	0.000104	3.006917
Duration of treatment	95.4536	4	23.8634	36.18957	7.9E-08	3.006917
Error	10.5504	16	0.6594			

The bacterial number determination as in Fig. 3 indicates that Polytrin had slight negative effect on the total number of bacteria, which is confirmed by the results of Rangaswamy and Venkateshwarlu (1992). However the bacterial number increased in many combinations being maximum at concentration of 100 ppm ($8.9X10^6$ g^{-1}) on 10th day of incubation. The increase in the total number of bacteria after Polytrin application can be explained by assuming that soil microorganism can synergistically metabolize this pesticide as Gunner and Zukerman (1968) demonstrated for Diazinon. The number of bacteria decreased in control with the advancement of days. The bacterial count at 100 ppm ($7.6X10^6$ g^{-1}) is significantly different from control ($2.3X10^6$ g^{-1}) on 20th day of incubation. High concentrations of pesticide however caused reduction in the number of bacteria but very negligible. This is similar to the results of Binner *et al.*, 1999, who reported that Polytrin (Cypermethrin) had no adverse effect on soil microbes. The bacterial population at 100 ppm of Polytrin (4.1X10g) was statistically at par with control (4.2X10g) on 15th day of incubation. Some of the insecticides are found non toxic to soil microbes (Martinez-toldo *et al.*, 1993; Gonzalez-lopez *et al.*, 1993; Mandic *et al.*, 1997; Digrak and Kazanici, 2001). ANOVA for Polytrin (Table 3) showed that different concentrations of the pesticide did not brought significant change in bacterial population whereas duration of treatment was significant ($F=2.816548$; df 4, 4; $p>0.01$; $F=9.546841$; df 4,4; $p<0.01$).(Fig.3).

Table 3: Analysis of variance (2-way) for Polytrin

Source of variation	SS	df	MS	F	p-value	F critical
Concentration of Polytrin	14.1064	4	3.5266	2.816548	0.060533	3.0069173
Duration of treatment	47.8144	4	11.9536	9.546841	0.000384	3.0069173
Error	20.0336	16	1.2521			

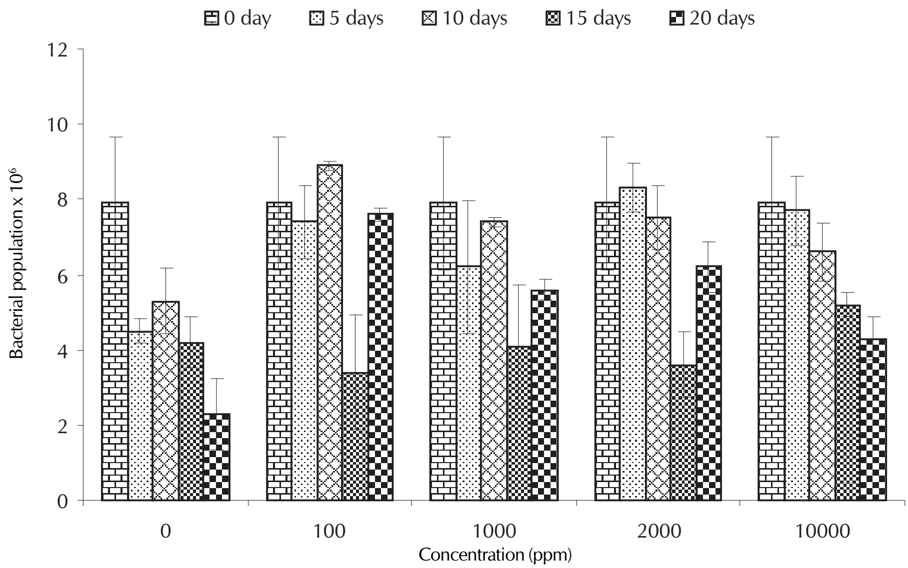

Figure 3: Effect of different concentrations of Polytrin at different period of incubation

It is found that Phorate had slight inhibitory effect on bacterial count as the number of bacteria only slightly varied with that of control (Fig. 4). However the bacterial population is decreased with the advancement of days, being lowest at the concentration of 2000 ppm ($1.2X10^6$ g^{-1}) as compared to that of control ($4.8X10^6$ g^{-1}) on 20^{th} day of incubation. This is supported by the similar results obtained by Digrak and Kazanici (2001). According to ANOVA for Phorate, bacterial population was significantly affected by both the concentration of Phorate and the duration of treatment ($F=4.284723$; df 4, 4; $p<0.01$; $F=7.799454$; df 4, 4; $p<0.01$)

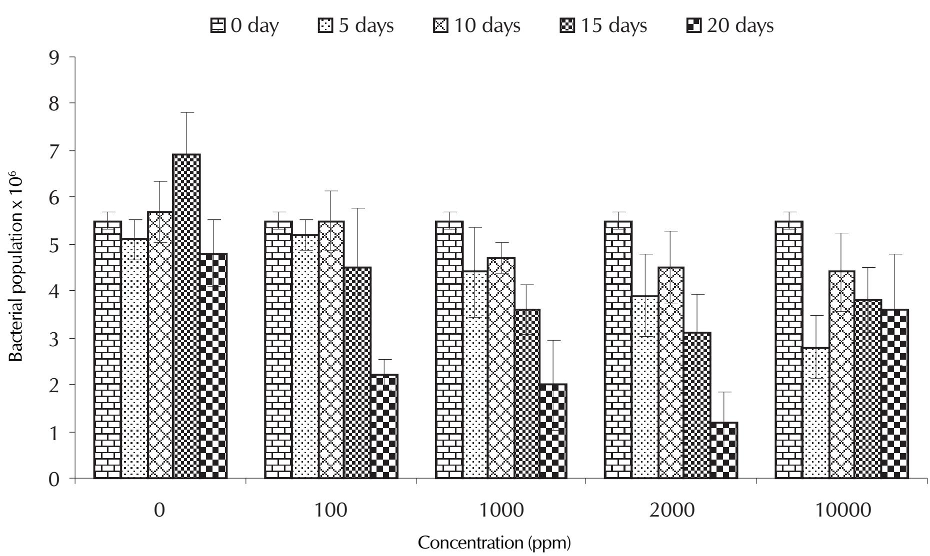

Figure 4: Effect of different concentrations of Phorate at different period of incubation

Table 4: Analysis of variance (2-way) for Phorate

Source of variation	SS	df	MS	F	p-value	F critical
Concentration of Phorate	11.6056	4	2.9014	4.284723	0.015171	3.0069173
Duration of treatment	21.1256	4	5.2814	7.799454	0.001097	3.0069173
Error	10.8344	16	0.67715			

Among the four organophosphorus insecticides used, Chlorpyrifos proved to be the most destructive on soil bacteria. Short term inhibitory effect on total bacterial population was observed after Chlorpyrifos treatment (Ahmed and Ahmed, 2006). On the other hand, Polytrin proved as stimulator for bacterial count except for some values ($3.4X10^6$ g^{-1} at 100 ppm and $3.6X10^6$ g^{-1} at 2000 ppm). Endosulphan brought reduction in bacterial population with the advancement of days of incubation as well as with the increase in concentration. Reduction in total bacterial population in comparision to control was recorded in the Phorate treated soil also after 10-15 days of organophosphate application. Similar impact of pesticide application on microbes has also been reported by Das *et al*. (1995), Saxena and Rai (1999) and Ekundayo (2003).

CONCLUSION

The results so obtained indicated that organophosphorus insecticide used had least adverse effect on bacterial population. It is therefore concluded that all tested insecticides can be safely used as soil treatments when intended to control termites. These effects are not drastic but minor in nature. After overall impact assesement, the four organophosphates are arranged as follows in the descending order of their destructiveness: Chlorpyrifos > Endosulphan > Phorate > Polytrin.

ACKNOWLEDGEMENT

I would like to thank Dr. M.P.Sinha, Reader, P.G.Dept. of Zoology, Ranchi University Ranchi, for his criticism and valuable suggestions on this manuscript, and Dr. Bharti Singh Raipat, H.O.D., Dept of Zoology, St. Xaviers College, Ranchi, for her innovative thoughts and conversations concerning this research.

REFERENCES

Ahmed, S. and Ahmed, M. S. 2006. Effect of Insecticides on the total number of soil bacteria under laboratory and field conditions. *Pak Entamol*. **28(2):**63-67.

Audus, L. J. 1964. The physiology and biochemistry of Herbicides. *Academic Press Inc., London*. p. 176.

Aurelia, O. 2009. Study of the effect of some pesticides on soil microorganisms. *International Symposia Risk Factors for Environment and Food Safety & Natural Resources and Sustainable Development*, Faculty of Environment Protection, Nov. **6-7.** University of Ordea.

Bakalivanov, D. 1990. Side effect of the insecticide Lannate on some soil microorganisms. *Pochvoznanie-i-Agrokhimiya*. **25(5):** 56-61.

Binner, R., Berendes, K. H., Felgentreu, D., Friesland, H. and Glitschka, M. 1999. Cypermethrin in bark and coniferous forest soil after pesticide treatment of single specimen of barked round wood in forests: persistence, distribution of diastereomers and effects on soil microorganisms. *Nachrichtenblatt-des-Deutschen-Pflanzenschutzdienstes*. **51(9):** 227-237.

Cernakova, M. 1993. Effect of insecticide, neramethrine EK-15, on the activity of soil microorganisms. *Folia Microbiol.* **38(4):** 331-334.

Chapman, R. A. and Harris, C. R. 1982. Persistence of isofenphos and isazophos in a mineral and an organic soil. *J. Environ.Sci. Health.* **B17:** 355-361.

Das, A.C., chakravarty, A., Shukul, P. and Mukherjee, D. 1995. Insecticides: their effect on micro-organisms and persistence in the rice soil. *Microbiol. Res.* **150**: 187-194.

Das, A. C. and Mukherjee, D. 1998. Insecticidal effects on soil microorganisms and their biochemical processes related to soil fertility. *World J. Microbio.* **14(6):** 903-906.

Digrak, M. and Ozcelik, S. 1998. Effect of some pesticides on soil microorganisms. *Bull. Environ. Contam. Toxicol*. **60(1):** 916-922.

Digrak, M. and Kazanici, F. 2001. Effect of some organophosphorus insecticides on soil microorganisms. *Turk J. Biol*. **25:** 51-58.

Digrak, M., Elazig.1994. yoresinde yaygÝn olarak kullanÝlan pestisitlerin Bacillus sp., Pseudomonas sp. karÝßÝk k.lt.r ve toprak mikroorganizmalarÝ tarafÝndan par•alanmasÝ. Doktora Tezi, FÝrat .niversitesi Fen Bilimleri Enstit.s., ElazÝÛ,1994.

Digrak, M., Ozelik, S. and Celik, S. 1995. Degradation of ethion and methidation by some microorganisms. *35 th IUPAC Congress, Istanbul*. p. 84.

Dordevic, S., Sestovic, M., Raicevic, V. and Dordevic, A. 1998. Fluctuation of the abundance of microorganisms in the carbofuran treated soil. *Pest*. **13(4):** 281-288.

Ekundayo, E.O. 2003. Effect of common pesticides used in Nigar Detta basin of southern Nigeria on soil microbial populations. *Envior. monit. Assess*. **89(1)**: 35-41.

Francisco, C., Dolores, S. P. and Antonio, J. 1979. Effect of two organophosphorus insecticides on phosphate dissolving bacteria. *Appl. and Env. Microbio*. **37(1):** 169-171.

Gonzalez-Lopez, J., Martinez-Toledo, M. V., Rodelas, B. and Salmeron, V. 1993. Studies on the effects of the insecticide, phorate and malathion, on soil microorganisms. *Env. Toxic. Chem*. **12(7):** 1209-1214.

Gorder, G. W., Dahm, P. A. and Tollefson, J. J. 1982. Carbofuran persistence in cornfield soils. *J. Econ. Entomol*. **75:** 637-642.

Gunner, H. B. and Zuckerman, B. M. 1968. degradation of “Diazinen” by synergistic microbial action. *Nature* (London) **216:** 1183-1184.

Hashem, F., Hafes, H. and El-Mohandes, M. A. O. 1999. Isolation and identification of pyrethroid insecticides-degrading bacteria from soil. *Ann. Agric. Sci. Cairo*. **44(1):** 123-137.

Johnson, I. F. and Curl, E.A. 1972. Methods for Research on ecology of soil borne plant pathogens. Burges Publishing Co. Minneapolis.

Lal, R. and Saxena, D. M. 1982. Accumulation, metabolism and effects of organochlorine insecticides on microorganisms. *Microbiol. Rev*. **46:** 95.

Motosugi, K., Soda, K. 1983. Microbial degradation of synthetic organochlorine compounds. *Experientia*. **39:** 1214-1220.

Mandic, L., Dukic, D., Govedarica, M. and Stamenkovic, S. 1997. The effect of some insecticides on the number of amylolytic microorganism and azotobacters in apple nursery soil. *Jug. Voc*. **31(2):** 177-184.

Martinez-Toldo, M. V., Salmeron, V., Rodelas, B., Pozo, C. and Gonzalez-Lopez, J. 1993. Studies on the effects of a chlorinated hydrocarbon insecticide, lindane, on soil microorganisms. *Chemosphere*. **27(11):** 2261-2270.

Nasim, g., IIyas, N. and Shabbir, A. 2005. Study of effect of organic pesticides: Endosulfan and Bifenthrim on growth of some soil fungi. *Mycopath*.**3(1&2)**:27-31.

Pandey, S. and Singh, D. K. 2004. Total bacterial and fungal populations after chlorpyrifos and quinalphos treatments in groundnut (Arachis hypogaea L.) soil. *Chemosphere*. **55(2):** 197-205.

Racke, K. D. and Coats, J. R. 1988. Comparative degradation of organophosphorus insecticides in soil : Specificity of enhanced microbial degradation. *J. Agric. Food Chem*. **36:** 193-199.

Rangaswamy, V. and Venkateswarlu, K. 1992. Degradation of selected insecticides, monochrotophos, quinalphos, cypermethrin and femalerate, by bacteria isolated from soil. *Bull. Env. Contam. Toxic*. **49(6)** :797-804.

Saxena, H. and Rai,J. P.N.1999. Effect of endosulfan application on microbial population and biochemical processin in tarai soil. *Ind. J. Ecol*. **26:** 136-142.

Sharmila, M., Ramanand, K. and Sethunathan, N. 1989. Effect of yeast extract on the degradation of organophosphorus insecticides by soil enrichment and bacterial cultures. *Can J. Microbiol*. **35**: 1105-1110.

Suneja, S., Kukereja, K., Goyal, S. and Bansal, R.K. 2008. Effect of Nematocides on soil microflora in cotton.*Indian J. Ecol*. **35(1)**: 25-27.

Tawfic, M. A., Ismail, S. M. M. and Mabrouk, S. S. 1998. Residues of some chlorinated hydrocarbon pesticides in rainwater, soil and ground water and their influence on some soil microorganisms. *Env. Int*. **24(5-6):** 665-670.