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INVESTIGATIONS ON GROWTH OF SOIL BACTERIAL COMMUNITIES WITH RELATION TO PESTICIDES

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ABSTRACT

This review summarizes information on the behavior of organophosphates and their impact on growth of beneficial soil bacterial communities (*Acinetobacter sp*. and *Xanthomonas sp*.). Organophosphates were applied on soil at three different doses (R.D, 2 X R.D, 10X R.D) which had a detrimental effect on microbial population. Available information raises concerns regarding the long term effect of organophosphates. The *Xanthomonas* count in Chlorpyrifos treated soil was found to be (0.00 in RD,),(0.003x10^6, in 2RD),(0.9 x 10^6,in 10RD) on tenth day and (0.0, in RD), (0.00, in 2RD),(0.00, in 10RD) on fortieth day as and when compared with control (2.1 x 10^6) on zero day, (1.9 x 10^6) on tenth day and (3.4 x 10^6) on fortieth day. Further growth curve were plotted for *Acinetobacter* sp. and *Xanthomonas* sp. with respect to four different organophosphates. It was obseverved that *Acinetobacter* in presence of Chlorpyrifos has early log phase (0.01 O.D at 2nd hour) and with Dimethoate has late log phase (0.01 O.D at 16th hour) with respect to control (0.01 O.D at 4th hour).

Keywords: *Organophosphates (Op), bacterial communities, microbial population, R.D (Recommended agricultural dose).*

Number of Tables: 2 **Number of Figures : 3** **Number of References: 17**

INTRODUCTION

India is primarily an agriculture based country with more than 60-70 per cent of its population dependent on agriculture. India's fast growing population is projected to cross 1.3 billion by 2020 (Kanekar *et al*., 2004). The use of chemical pesticides and fertilizers in Indian agriculture has seen sharp increase in recent years. Organophosphates are a group of highly toxic pesticides widely used for increasing agricultural productivity in soil. Organophosphorous is also reared as non-persistent (Racke and Coats, 1988). Pesticides residue may influence the microbial populations which carry out different processes, such as nitrogen fixation, nitrification, ammonification, organic matter decomposition, sulfur oxidation and phosphorous solubilization. The study of the interrelationships between microorganisms and pesticides has increased considerably in recent years.

Soil has large number of microorganisms, which has got to play several different important roles. One such is, *Acinetobacter* which is widespread in nature, and can be obtained from water, soil, living organisms and even from human skins. Species of *Acinetobacter* have been attracting increasing attention in both environmental and biotechnological applications. Some strains of this genus are known to be involved in biodegradation of a number of different pollutants such as biphenyl and chlorinated biphenyl (Abdel-El-Haleem, 2003). *Acinetobacter* has showed that it can utilize a wide range of pollutants as a sole energy and carbon source (Briganti et al., 1997; Chibata and Tosa, 1981). Similarly certain strains of *Xanthomonas* species found in soil can increase plant growth in several ways. They may produce a compound that inhibits the growth of pathogens or reduces invasion of the plant by a pathogen. They may also produce compounds (growth factors) that directly increase plant growth (Ann Kennedy). So, our present research focuses on the study of effect of four different organophosphates viz: Chlorpyrifos, Malathion, Dimethoate and Phorate, at three different doses, on the colony count / number and growth curve of these two bacteria; i.e. *Acinetobacter* sp. SS-2 and *Xanthomonas* isolated from soil ecology.

MATERIALS AND METHODS

The soil samples were collected from experimental plot fields of I.C.A.R research complex, for eastern region Palandu, Ranchi on 16th February 2011.The soil was collected from upper 0-15 cm, other plant debris were removed manually and soil was sieved with 4 mm mesh. Further they were brought to the laboratory and stored at 4^{0} C till analysis was conducted. Four different Organohosphates (Chlorpyrifos, Malathion ,Dimethoate and Phorate) were purchased from the market.

Study was carried out in two parts:

The stock solutions of OPs were prepared for treatment with soil sample, based on calculations for three different concentrations [recommended dose (RD), two times recommended dose (2X RD) and ten times recommended dose (10XRD)]. The recommended dose for different organophosphates is given in table 1 .Then soil samples

were treated with these four organophosphates at three different concentration in twelve different containers and one container was left untreated as control. Enumeration of soil microorganisms was done specifically for two different species , at regular interval of ten days up to forty days as shown in (Table 2, fig.1)

Growth pattern of two bacteria in presence of four organophosphates were studied. Pure cultures of *Acinetobacter sp. SS2* and *Xanthomonas* NML 03-0222 maintained in the lab were used for growth curve analysis. These were previously isolated from soil sample and identified by 16S rDNA method (Peixoto .R.S *et al*., 2002).

Growth kinetics study was conducted with nutrient broth (Medox pvt. Ltd.) media in test tubes. 100µl of pure organophosphates solution and 1000µl of pure bacterial inoculum of *Xanthomonas* and *Acinetob-acter*, each were added to two different test tubes containing 20 ml of nutrient broth .This was done for all four organohosphates with one test tube as control having nutrient broth and inoculum only with no organophosphates, and other test tube left as blank with only nutrient broth. Total pair of six test tubes sets were prepared for both bacterial species. The test tubes were incubated in incubator shaker (Chromas biotech Pvt. Ltd.) at 37^0 C (150 rpm) and O.D.at 530 nm (Madigan *et al*.,1997) was recorded periodically until the growth reached the stationary phase or death phase as shown in (fig. 2 and 3).

RESULT AND DISCUSSION

The microbial number determination (table 2, fig1) indicated that, in the soil treated with four different organophosphates (Chlorpyrifos, Malathion, Phorate, Dimethoate), when compared with control soil ,the total number of bacteria was lower .In the agricultural soil treated with Chlorpyrifos there was decrease in number of *Xanthomonas* colonies showing ($0.0x10^6$ in RD,) ($0.003x10^6$,2RD), ($0.9x10^6$,10RD) on tenth day and (0.0,RD), (0.0×10^6,2RD), (0.0×10^6,10RD) on fortieth day than control (2.1×10^6) on zero day,($1.9x\ 10^6$) on tenth day and (3.4×10^6) on fortieth day (table 2). Our result is supported by (Jiying Ning *et al*.,2010) who concluded that *Xanthomonas* degrade dichlorvos but unable to degrade chlorpyrifos. Similar observation was found by Garg and Tandon (2000) who conducted research on the effect of OP pesticides on bacteria of salt affected alkaline soil of Banasthali region. The variation in *Xanthomonas* population gave an indication that OP pesticides had either stimulatory or inhibitory effects on different microbial groups and had some role to play in the degradation of selected organophosphates. Dimethoate also showed growth inhibitory effect in X*anthomonas* (2.2×10^6 in RD,),(1.9×10^6,2RD),(0.007×10^6,10RD) on tenth day and (3.6×10^6,RD),(0.2×10^6,2RD),(0.004×10^6,10RD) on fortieth day than control (2.1×10^6) on zero day,($1.9x\ 10^6$) ontenth day and (3.4×10^6) on fortieth day (table 2). but has less impact as compared to other organophosphates on long run as shown in (fig.1) as *Xanthomonas* is able to degrade certain organophosphates which is supported

by the findings of (Rosenberg & Alexander, 1979) who concluded that X*anthomonas* which could use phosphorothionate and phosphorodithionate pesticides as a sole source of phosphorus were unable to utilize these compounds as a source of carbon. (Tchelet et al.,1993) observed similar result that, *Xanthomonas* sp. can hydrolyze parathion (organophosphate) and can further metabolize *p*-nitrophenol.

Colony count of *Acinetobacter sp.* showed increase in Chlorpyrifos treated soil (1.6 x10^6 in RD,),(0.02 x 10^6,2RD),(0.007 x10^6,10RD) on tenth day and (3.1 x10^6,RD),(1.3x 10^6,2RD),(0.09 x10^6,10RD) on fortieth day than control (1.7 x 10^{-6}) on zero day,(1.2x 10^6) on tenth day and (2.1 x 10^{-6}) on fortieth day (table 2, fig.1), which is similar to the results of (Chanika *et al*., 2011) who concluded that *Acinetobacter rhizosphaerae* are able to rapidly degrade the organophosphate (OP). *Acinetobacter* was less affected by Malathion showing (2.3 x10^6 in RD,),(1.9 x 10^6,2RD),(0.006 x10^6,10RD)on tenth day and (3.5 x10^6,RD),(2.2 x 10^6,2RD),(1.9 x10^6,10RD) on fortieth day; than control (1.7 x 10^{-6}) on zero day,(1.2x 10^6) on tenth day and (2.1 x 10^{-6}) on fortieth day (table 2, fig.1). which is similar with the reports of (Shan Xie *et al.*,2009), (Hussein *et al.* ,2011) who observed that *Acinetobacter johnsonii MA19*, could degrade malathion..

Table 1: *Recommended agriculture dose of Organophosphates*

Organophosphates	Recommended Dose
Chlorpyrifos	1lit : 250lit water/acre
Malathion	1lit : 250lit water/acre
Dimethoate	1lit : 250lit water/acre
Phorate	10 kg/acre

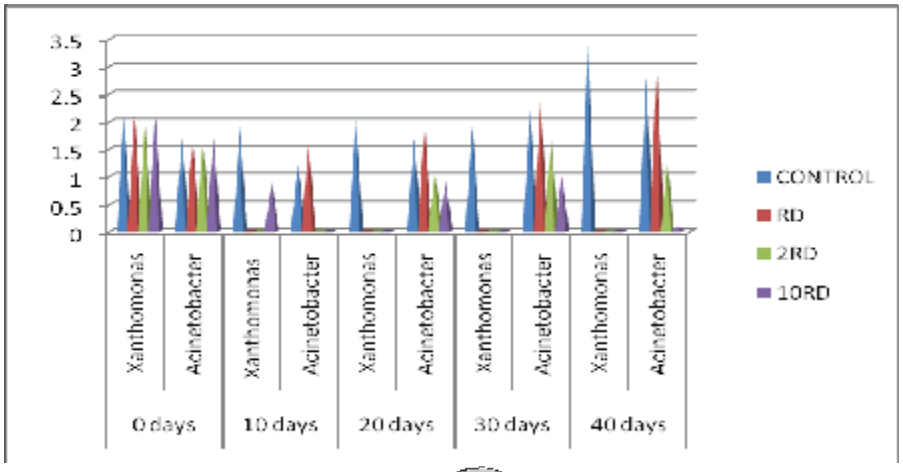
Table 2: *Population density of two bacterial communities*

Chlorpyrifos										
doses	**0 days**		**10 days**		**20 days**		**30 days**		**40 days**	
	Xantho monas	***Acineto bacter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***
CONTROL	2.1	1.7	1.9	1.2	2	1.7	1.9	2.2	3.4	2.8
RD	2.1	1.7	0	1.6	0	2	0	2.4	0	3.1
2RD	2.1	1.7	0.003	0.02	0	1.1	0	1.7	0	1.3
10RD	2.1	1.7	0.9	0.007	0	0.9	0	1	0	0.09
Phorate										
doses	**0 days**		**10 days**		**20 days**		**30 days**		**40 days**	
	Xantho monas	***Acineto bacter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***
CONTROL	2.1	1.7	1.9	1.2	2	1.7	1.9	2.2	3.4	2.8
RD	2.1	1.7	2.3	2.5	0	2.7	0	2.6	0.7	2.9
2RD	2.1	1.7	1.9	2.8	0.008	1.2	0.009	1.5	0.04	1.5
10RD	2.1	1.7	1.5	1.4	0	1	0	1.1	0.009	1
Malathion										
doses	**0 days**		**10 days**		**20 days**		**30 days**		**40 days**	
	Xantho monas	***Acineto bacter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***
CONTROL	2.1	1.7	1.9	1.2	2	1.7	1.9	2.2	3.4	2.8
RD	2.1	1.7	0	2.3	0.05	2.6	0	3.1	0	3.5
2RD	2.1	1.7	0	1.9	0	1.7	0	2.9	1.5	2.2
10RD	2.1	1.7	0	0.06	0	0.9	0	1.2	0	1.9
Dimethoate										
doses	**0 days**		**10 days**		**20 days**		**30 days**		**40 days**	
	Xantho monas	***Acineto bacter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***	***Xantho monas***	***Acinetob acter***
CONTROL	2.1	1.7	1.9	1.2	2	1.7	1.9	2.2	3.4	2.8
RD	2.1	1.7	2.2	0.08	2.3	0.8	2.5	0.09	3.6	0.04
2RD	2.1	1.7	1.9	0.001	2.5	0.9	2.7	0.001	0.2	0.001
10RD	2.1	1.7	0.007	0	2.1	0	1.2	0	0.004	0

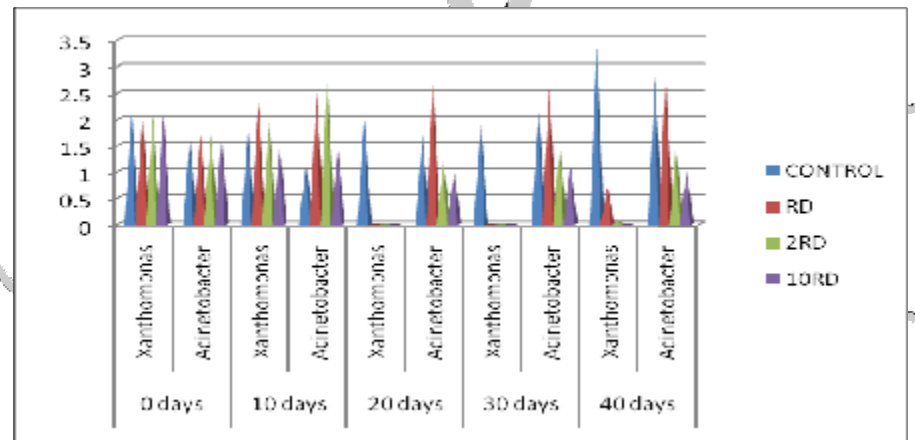
Bacterial count value mentioned in the table corresponds to ***X 10^6*** *per gram of soil*

Fig 1: *Graph showing bacterial count of Xanthomonadaceae bacterium NML 03-0222 and Acinetobacter sp. SS-2*

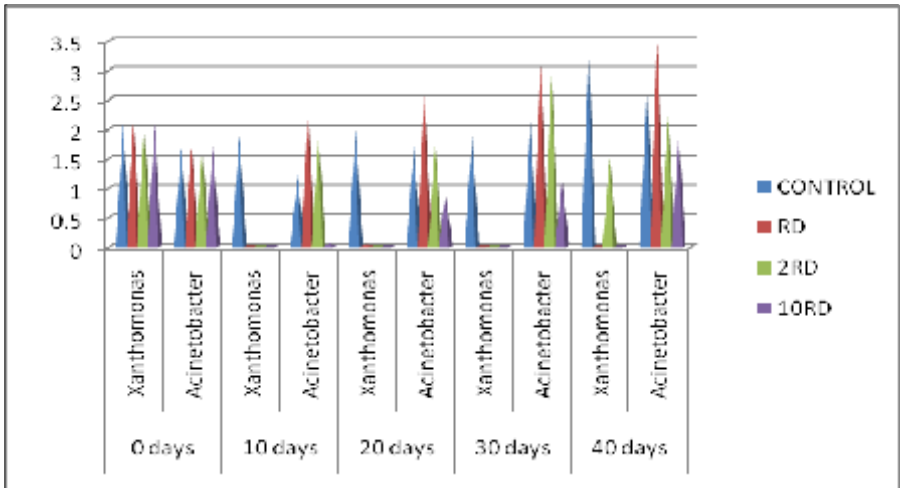
a) Chlorpyrifos



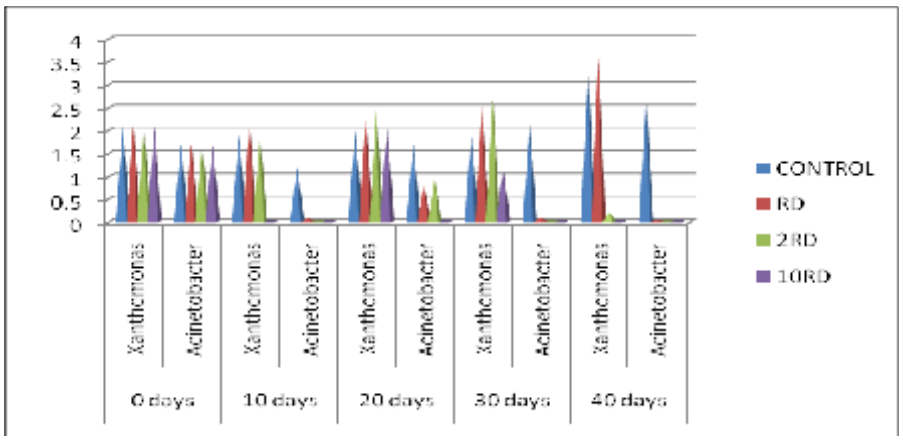
b) Phorate



c) Malathion



d) Dimethoate



Growth curve of *xanthomonas* on treatment with chlorpyrifos showed late log phase (0.01 O.D at 16th hour) followed by malathion (0.03 O.D at 14th hour) as compared to control having log phase (0.02 O.D at 2nd hour) . Dimethoate showed early log phase (0.01 O.D at 2nd hour) and early stationary phase (1.8 O.D at 32nd hour) similar to control as shown in (fig.2)While in case of *acinetobacter*, with chlorpyrifos it has early log phase (0.01 O.D at 2nd hour) and in presence of dimethoate, it has late log phase (0.01 O.Dat 16th hour) with respect to control (0.01 O.D at 4th hour) as shown in (fig.3)

Fig 2: *Graph showing growth curve of Xanthomonadaceae bacterium NML 03-0222 in presence of organophosphates*

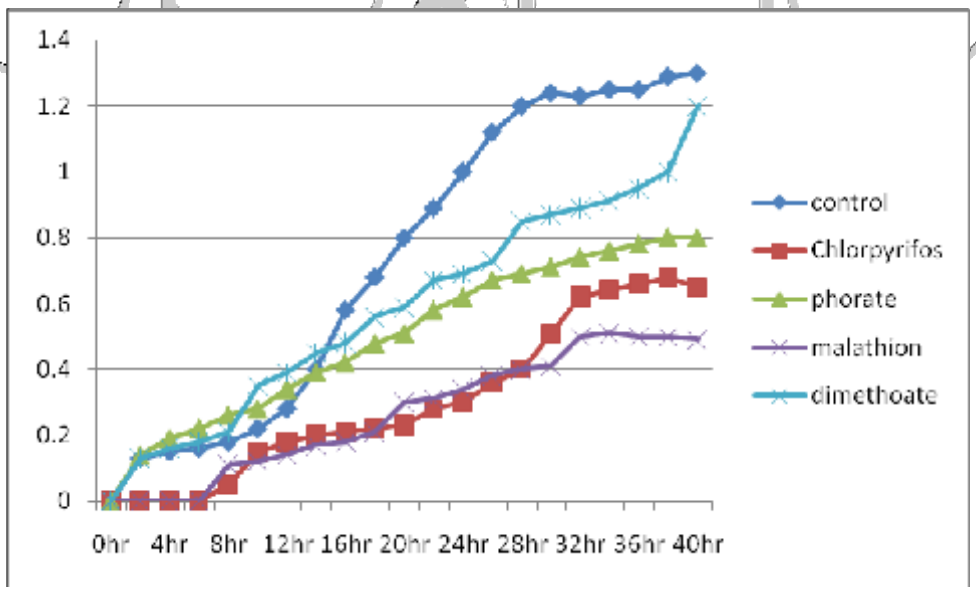
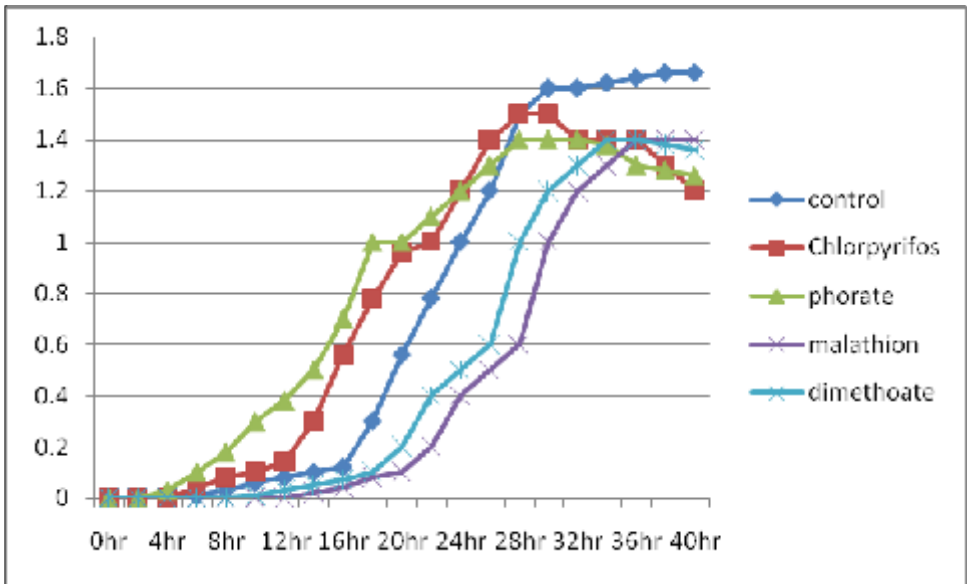


Fig 3 : *Graph showing growth curve of Acinetobacter sp. SS-2 in presence of organophosphates*



CONCLUSION

Our research showed that natural growth of beneficial soil bacterial species (*Xanthomonas* and *Acinetobacter)* is effected by most of organophosphates in long duration of time when applied in agricultural farm , while some organophosphates are also degraded by these bacterial species.

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