ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. 1: 99 - 104: 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.in

TOLERANCE AND BIOTIC STRESS RESPONSE OF SOIL MICROFLORA AGAINST ORGANOPHOSPHORUS PESTICIDES AND BIOPESTICIDES

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KEYWORDS

Organophosphorus
Brevundimonas
Biopesticides
SAS

Proceedings of International Conference on
Anthropogenic Impact on Environment & Conservation Strategy
(ICAIECS - 2012)
November 02 - 04, 2012, Ranchi,
organized by
Department of Zoology, Ranchi University, Ranchi
&
Departments of Zoology and Botany,
St. Xavier's College, Ranchi
in association with
National Environmentalists Association, India
www.neaindia.org

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AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

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ABSTRACT

This research focuses on comparing the impact and toxicity of organophosphorous pesticides and biopesticides at two different concentrations, undiluted (UD) and ten times dilution (10x) on some isolated species of bacteria in soil, which are helpful in bioremediation and maintaining natural fertility of agricultural soil. Pure cultures were subjected to treatment with four organophosphorous pesticides and four Biopesticides and inhibition of growth were studied using agar gel diffusion method. There was considerable change in bacterial growth resulting zones of inhibition, which was calculated for all pesticides using Statistical Analysis Software (SAS) - "The Means/Summary method and Pearson's correlation method". Under this analysis we found that organophosphorous pesticides specially malathion (UD,10x) showed maximum inhibition of bacterial growth (18 mm,12 mm dia) for Pseudomonas, followed by chloripyrifos (16 mm,14 mm dia), dimethoate (18mm,0 mm dia) and phorate (13 mm, 8 mm dia), whereas all the bacteria showed tolerance against biopesticides with no zone of inhibition. The Pearson's correlation stated that *Pseudomonas* and *Sphingo monas* are closely related to each other showing l ess tolerance of (0.88833/<.0001) to overall treatments than *Brevundimonas* (0.70696/ 0.0022). Biopesticides is a sustainable approach reducing biotic stress naturally in long coarse of time.

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INTRODUCTION

As soil is the most important agricultural resource next to water, it is important to study the possible effects of specific practices on soil properties. With the restricted use of most the organochlorine insecticides, the organophosphates are taking the major share of insecticide consumption in India. (Adityachaudhery *et al.*, 1997). Application of pesticides on agricultural crop results in adverse effect on the properties of the soil as well as it alters pH of the soil required for microbial activities of beneficial bacteria to act upon (Rehman and Motoyama 2000).However negative effects of pesticides on non target organisms and their activities on the environment must be recognized (T.U and. Miles 1976).

The important micro flora, beneficial for growth of plants includes nitrogen fixing bacteria and phosphate solubilising bacteria, present in the rhizosphere of the plants. The excess application of these pesticide may adversely effect the function of microorganism. Since the fertility of soil depends upon number and type of organism present in the soil, studies on effect of pesticide application on soil were carried out (Sarnaik *et al*., 2006)

Approximately less than 0.1% of applied pesticide, reaches the target pests, living bulk to effect the environment (Malinowaski, 2000). It has been reported that repeated application of Chloropyrifos organophosphate to the soil did not result in development of microbial population (Ardely, 1999).

In many areas of the world locally available plant materials are widely used to protect stored products against damage by insects infestation. (Golob and Webley 1980; Singh, *et al*., 2003). Botanical products are environmentally safe less hazardous, economical and easily available and can be used as an alternative to chemical pesticide (Talukder *et al*., 1990). The scientists are now working on new source of pesticide which are less harmful and friendly (Chopra *et al*., 1956).

One of the latest discovery methods is the use of the natural enemies of the insects such as bacteria, virus and fungus (Quintela and McCoy, 1997; McCoy, *et al.,* 2000), (Prasad and Syed, 2010). In many countries efforts are being made to minimize the use of harmful insecticide through the use of indigenous plant product, implementation of I. P. M. approaches and use of bio degradable products (Sabbour and Sahab, 2005).

Our present research deals with studying the impact on three soil bacterial species (*Sphingomonas, Pseudomaonas*, *Brevundimonas)* when subjected to treatment with different concentrations of organophosphates and bio pesticides. It has also been observed that a high proportion of the PAH degrading isolates in soil belong to the *sphingomonads* (Reddy *et al.,* 2008) .*Pseudomaonas* are effective root colonizer and bio control agent by production of antibiotic and other anti fungal metabolites. (Sullivan and Gara, 1992). Strains from genera *Pseudomonas, Bacillus, rhizobium* are among most powerful phosphate solubilizer . *Brevundimonas* is involved in degradation of aromatic compounds. Main focus

Table 1: The Means/Summary Method

Organophosphates/ Biopesticides(OP/BP)	N Observations	Variable	Minimum	Maximum	Mean
BIOSANJEEVANI UD*	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
BIOSANJEEVANI 10XD*	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
BIOSOFT 10XD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
BIOSOFT UD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
CHLORIPYRIPHOS 10XD	1	*Sphingomonas*	14	16	15
	1	*Pseudomonas*	12	16	14
	1	*Brevundimonas*	13	15	15
CHLOROPYRIPHOS UD	1	*Sphingomonas*	19	23	20
	1	*Pseudomonas*	14	18	16
	1	*Brevundimonas*	10	12	11
DIMETHOATE UD	1	*Sphingomonas*	10	14	12
	1	*Pseudomonas*	15	19	18
	1	*Brevundimonas*	0	0	0
DIMETHOATE 10XD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
KARANJ UD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
KARANJ 10XD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
MALATHION 10XD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	9	13	12
	1	*Brevundimonas*	11	15	13
MALATHION UD	1	*Sphingomonas*	18	22	20
	1	*Pseudomonas*	17	19	18
	1	*Brevundimonas*	20	24	22
NEEM UD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
NEEM 10XD	1	*Sphingomonas*	0	0	0
	1	*Pseudomonas*	0	0	0
	1	*Brevundimonas*	0	0	0
PHORATE 10X D	1	*Sphingomonas*	8	12	10
	1	*Pseudomonas*	7	8	8
	1	*Brevundimonas*	6	10	8
PHORATE UD	1	*Sphingomonas*	11	13	12
	1	*Pseudomonas*	10	14	13
	1	*Brevundimonas*	0	0	0

*UD – undiluted, 10XD – ten times diluted. *(OP/BP)-Organophosphates/Biopesticides

of our research was towards commercialisation of biopesticides in order of sustainable approach and prevention of environmental pollution.

MATERIALS AND METHODS

In this study, the tolerance of microbial species isolated from soil of agroecosystem, against organophosphates and biopesticides was examined. The soil sample were collected from agricultural fields at I.C.A.R, research complex, from eastern region Palandu, Ranchi on 11th February 2011. Around 1 kg of soil sample was collected from 0-15 cm depth. The bacteria was isolated by spread plate technique (Ahmad and Ahmad,, 2006) and further identified on the basis of 16s rDNA method (Peixoto *et al*., 2002). Malathion 50%EC of century polbounds ltd., Dhanwan-20 for chlorpyrifos, ROGOR insecticide for dimethoate, DHAN-10G for phorate of agritech pvt.ltd were used as a source of organophosphate pesticides. Bio pesticides with common names Biosanjeevani and Biosoft having formulation of *Pseudomonas + Trichoderma viride*, *Beauveria bassiana* respectively and aqueous extract of leaves of two naturally occurring species *Pongamia pinnata (*Karanj*) and Azadarichita indicana (*neem*)* were taken. Species tolerance against all eight pesticides at two different concentration,

Table 2: Pearson's Correlation Co-efficient Method

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0			
Sphingomonas	*Sphingomonas*	*Pseudomonas*	*Brevundimonas*
	1.00000	0.88833 <.0001	0.69335 0.0029
Pseudomonas	*Pseudomonas*	*Sphingomonas*	*Brevundimonas*
	1.00000	0.88833 <.0001	0.70696 0.0022
Brevundimonas	*Brevundimonas*	*Pseudomonas*	*Sphingomonas*
	1.000000	0.70696 0.0022	0.69335 0.0029

undiluted (UD)and ten times dilution (10x) of original concentration were carried out using agar gel diffusion method (Koreman, *et al.,* 1983). The zone of inhibition was measured

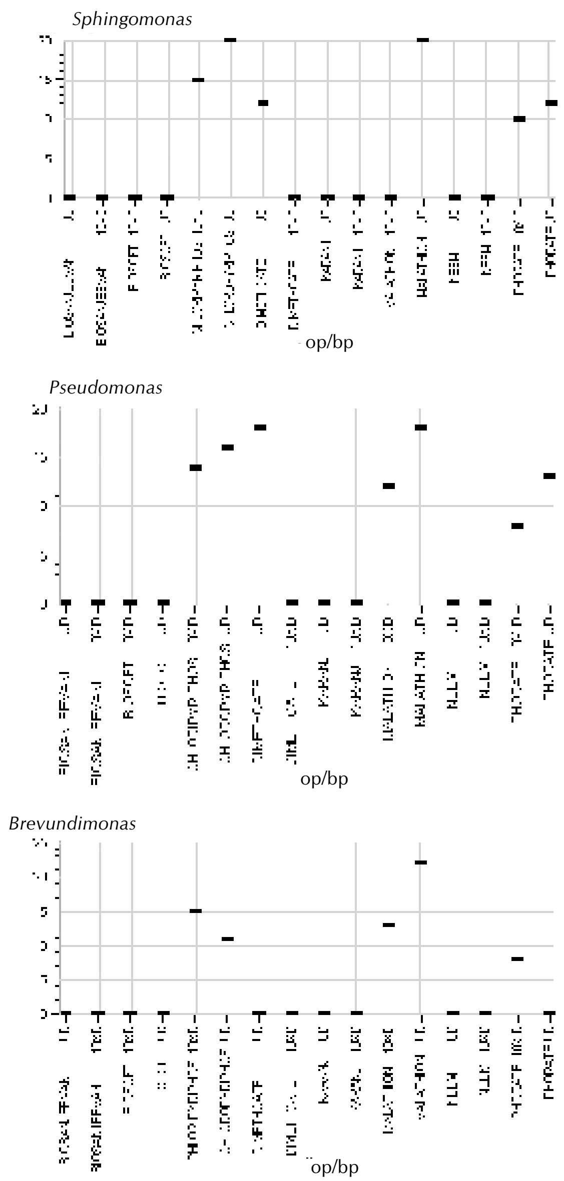

Figure 1: Box- Plot Graph

Table 3:

Row Variable	Column Variable	Pearson's r	Probob>|r|
Sphingomonas	*Pseudomonas*	0.88833	<.0001
Pseudomonas	*Breuvendimonas*	0.70696	0.0022
Breuvendimonas	*Sphingomonas*	0.69335	0.0029

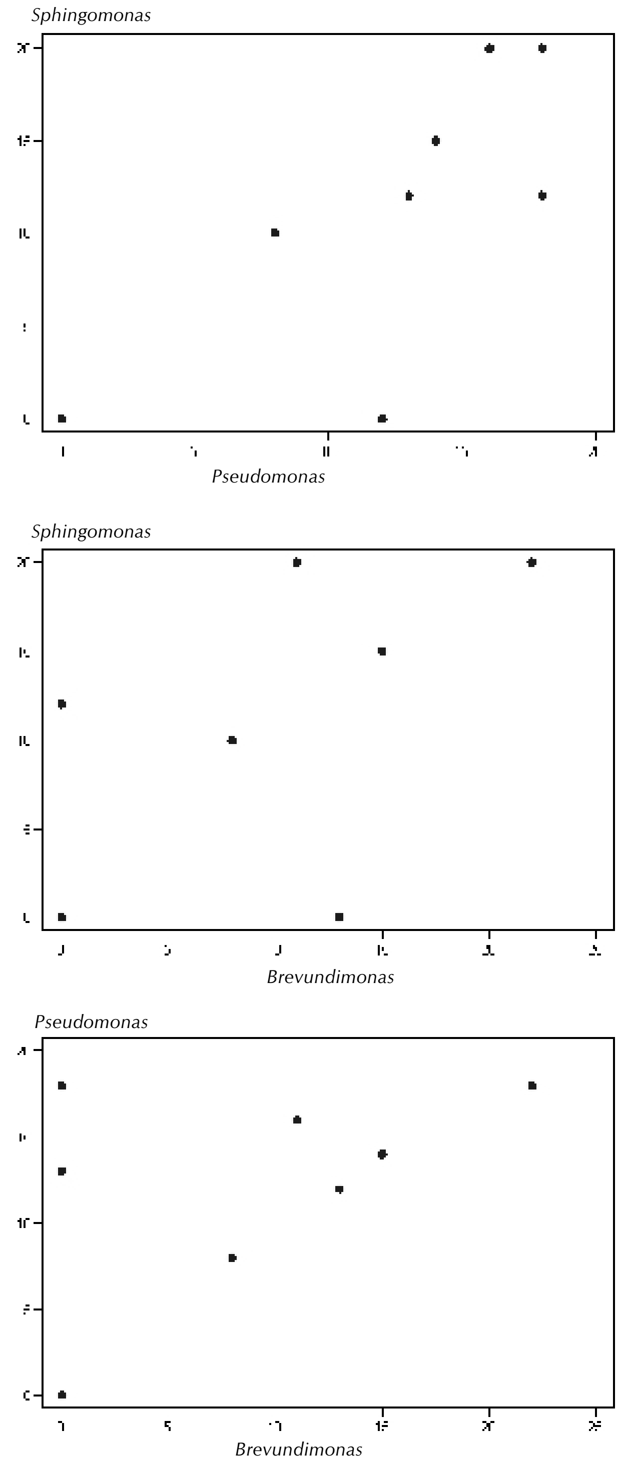

Figure 1: Box- Plot

using sliding calliper with precision of 0.01 mm. Each measurement was taken in triplicates and average of two measurements done for each zone (Siswomihardjo, *et al.,* 2007). Statistical analysis of data was carried out using SAS. The Means/ Summary method followed by Pearson's Correlation method to find maximum impact of organophosphates and biopesticides and the tolerance of species against them. Also the relation of one species *Sphingomonas* was correlated with respect to other two species *Pseudomonas* and *Brevundimonas*.

RESULTS AND DISCUSSION

The result was tabulated and stastically analysed using SAS which showed considerable zone of inhibition on bacterial lawn in response to 4 organophosphate and 4 bio pesticide. The maximum inhibition was observed in case of malathion and chloropyrifos on three bacterial species as shown in table 1 using *"the means/summary method"* which was similar to screening technique for accessing the effect of pesticite on population of soil microorganism (Tu, 1970). Some of the insecticides were not toxic to the microbes under different soil uses (Lopez *et al*., 1993) .However when the water emulsion of malathion fraction or the solvent fraction, aromatic petroleum distillates (ADP) were used on the soil community,(0.44 μL/g of soil, computed from the manufacturer's recommended dosage) no difference in growth were noted for the 2- or 24-h post addition populations plated on Martin agar media (Martin, 1950), or soil extract agar (Allen 1957),when compared to control(Stanlake and Clark 1975). This was similar to our findings when diluted malathion used over bacterial lawn, less zone of inhibition (0 mm, 12mm , 13mm dia) was obtained. Similar toxic effect of chlorpyrifos has been reported by (Pablo *et al*., 2008) while the zone of inhibition produced with addition of dimethoate resulted (UD -18mm,10X -0 mm) in pseudomonas and phorate (UD- 13mm dia,10x- 8mm dia) for Pseudomonas as shown in table no.-1 and box plot graph in Fig 1.

Our results were supported by the findings of (Das and Mukherjee, 2000) who concluded that phorate in particular, stimulated the proliferation of aerobic non symbiotic nitrogen fixing bacteria and phosphate solublizing microorganism and also their biochemical activities,such as N_2 fixing, phosphate solubilising and less persistent in soil for short period of time. Phorate has comparatively less effect than other organophosphate as favoured by the reports of (Das *et al*., 1995) which states that phorate increase the population of bacteria and a stimulation in growth of pseudomonas was observed. Our observation of tolerance of pseudomonas towards phorate can be justified by this. *Breuvendimonas* showed (0 mm dia , with UD Dimethoate) which can be explained as this bacteria was capable to degrade dimethoate, proved when the bacterial isolate which showed the maximum degradation of dimethoate in soil was identified as *Brevundimonas* (Bastos, *et al*., 2002).But in case checking of tolerance of the bacterial lawn against bio pesticides, no zone of inhibition was observed in all three species *Sphingomonas, Pseudomaonas, Brevundimonas* (0mm dia as shown in Table 1 and box plot graph in Fig. 1). In similar studies, it was observed, that aqueous extract of Neem showed no inhibitory growth on bacteria (Natarajan *et al*., 2003) and (De and Ifeoma, 2002) also did not record any antibacterial activity with aqueous extract of leaves and bark extract against test bacteria, but when Neem extracts in hexane and aqueous solvent used showed inhibited growth on both test and control bacteria. (Mahmood, *et al*., 2010). *Trichoderma* strain with and without pathogen did not effect existing beneficial population rather results in increased bacterial population (Shanmugaiah *et al*., 2009), which favour our results.

On the basis of tolerance of bacterial species against organophosphates and bio-pesticides a statistical "Pearson's correlation Co-efficient method" was used which stated that *Sphingomonas* was closely related to *Pseudomonas* as shown in Table 2 and 3, (Stolz 2009) had similar observations, Biochemically, the naphthalene and biphenyl degradation pathways of *Sphingomonas* seem to resemble those in well known *Pseudomonas* and other gram negative bacteria with identical intermediates (Pinyakong, *et al.,* 2003). Maximum effect/impact of organophosphates was found to be on *Sphingomonas*>*Pseudomonas*>*Brevundimonas* as shown in (Table 2 and 3) and graphical relation in (Fig. 2)where *Pseudomonas and sphingomonas* having minimum tolerance results with overall treatments of organophosphates and bio pesticides with respect to *Brevundimonas*. *Sphingomonas* showed (0.88833/<.0001), *Breuvendimonas* showing (0.70696/0.0022) with Pseudomonas and *Sphingomonas* with *Brevundimonas* showing (0.69335/0.0029).

CONCLUSION

The result of this study, revealed that organophosphates are showed more inhibitory effect to soil microbes which play essential role in bioremediation and maintaining soil fertility, as compared to bio pesticides . The result showed pesticides to be destructive in following order Malathion > Chloropyrifos > Dimethoate > Phorate > Biopesticides. Thus Bio pesticides are better alternative of future and play important role in sustainable development of agroecosystem. With comparative study of correlation of all three isolated soil bacterial species, tolerance level order was *Shingomonas*<*Pseudomonas*<*Brevundimonas*. *Brevundimonas* is more tolerant among three species.

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