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RESEARCH ARTICLE

IMPACT ASSESSMENT OF PESTICIDES ON GROWTH RESPONSE OF BENEFICIAL SOIL BACTERIUM

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ABSTRACT

The experiment was conducted to study the effect of 4 organophosphate (OP) chlorpyrifos, malathion, phorate, dimethoate and 4 bio-pesticides (BP) bio-sanjeevani, biosoft, neemta, karanj extract on growth response of non targeted beneficial soil bacterium. Three strains of bacteria (*Sphingomonas sp. SZL-1*, *Pseudomonas mendocina sp. PC19, Brevundimonas sp. XJ-412)* were isolated from ICAR Research Complex for Eastern Region, Plandu, Ranchi by 16S rDNA gene sequence analysis. In spectrophotometer analysis organophosphate (Conc.1000µl) treated bacteria resulted in the declination of optical density (OD) while biopesticides (Conc.1000µl) treated bacteria showed optimal response as compared to control (0 to 40 hours time interval). Statistical analysis was carried out using Levene's test of homogeneity for analysis of variance and Dunnett's test for comparative mean estimation of better option among organophosphates and bio-pesticides for soil bacteria which play essential role in sustainable soil fertility. Levene's test of homogeneity justified for growth of *pseudomonas* ($F = 10.14$), *sphingomonas* ($F = 8.57$), *brevundimonas* ($F = 11.96$) on treatment with OP and BP. Dunnett's test estimated that, bio-sanjeevani has maximum growth response (0.64 ± 0.4) and critical difference (+0.41), while malathion has minimum growth response (0.26 ± 0.18) and critical difference (-0.04) with respect to control (0.73 ± 0.48) for pseudomonas.

INTRODUCTION

India is primarily an agriculture based country with more than 60-70 % of its population dependent on agriculture. India's fast growing population is projected to cross 1.3 billion by 2020 (Kanekar *et al.,* 2004). In order to reap maximum yields and to meet the demanding population, farmers resort to use of pesticidal application to combat the pest problem. Increase in the consumption of pesticide is likely to be at least two to three times more in the years to come. Pesticides are sometimes non-biodegradable and moreover, toxic to microorganisms with increase in its tolerance level. Soil microorganisms have a great contribution towards soil fertility. Any adverse impact of chemical on soil characteristics and microorganism may lead to ultimate loss of soil fertility (Ubuoh *et al.,* 2012). Chowdhury *et al., (*2008) proposed that microorganism's plays vital role in maintaining soil fertility, degradation of organic matter and pollutants in soils. *Pseudomonas* species good candidates for used as seed inoculants, root dips for biological control of soil-borne plant pathogen and also as antibacterial agents (Rekha *et al.,* 2010). Sphingomonas are having the ability of bioremediation and to use polycyclic aromatic hydrocarbons (PAH) as the sole source of carbon and energy purposes (Leys *et al.,* 2004).

Brevundimonas vesicularis is a potential bio-absorbent of heavy metals and good source of bioremediation (Resmi, *et al.,* 2010).Concern for pesticide contamination in the environment in the current context of pesticide use has assumed great importance (Zhu *et al.,* 2004). Pesticide that disrupt the activities of the soil microorganisms could be expected to affect the nutritional quality of soils and would therefore, have serious ecological consequences (Handa *et al.,* 1999). Ideally a pesticide must be lethal to the targeted pests, but not to non-target species. Unfortunately, this is not the case, so the controversy of use and abuse of pesticides has surfaced. Pesticides might affect microorganisms by reducing their numbers, biochemical activity, diversity and changing the microbial community structure (Martinez-Toledo *et al*. 1998, Smith *et al.,* 2000, Chen *et al.,* 2001a, Cycoń and Kaczyńska 2004). There is now a growing concern among consumers toward food safety and environmentally sound practices, giving more and more importance on the use of bio-pesticides (bio-agents) as an alternative to farm chemicals. Bio-pesticides generally affect only the target pests and are safer to use than conventional pesticides as an integrated pest management (IPM) programs (Gupta and Sen 2012). The study aimed at assessing the pesticidal (organophosphates and bio-pesticides) applications on soil microbial spectrum in order to check its growth response against three different bacterial strains of *Sphingomonas sp. SZL-1*, *Pseudomonas*

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mendocina strain PC19, Brevundimonas sp. XJ-41

MATERIALS AND METHODS

Soil samples were collected from the surface layer (0-15 cm) of the agricultural land of ICAR, Research Complex, Eastern Region, Plandu, Ranchi (Dutta, *et al.,* 2010). The samples were taken to the laboratory and stored at temperature of 4^0C until the analysis were conducted and completed. Microbiological analyses of the soil samples were made following spread plate technique, (Ahmad *et al.,* 2006) pure colonies streaked out and sub-cultured on Nutrient Agar. Bacterial strains of *Sphingomonas sp. SZL-1*, *Pseudomonas mendocina strain PC19, Brevundimonas sp. XJ-41* were identified using 16S rDNA method of genome sequencing (Sultan *et al.,* 2012). Stock solution were prepared on the basis of recommended dose for four different Organophosphate Malathion (2.5 ml in 1 liter of dH_2O), Chlorpyrifos (1 ml in 250 ml dH_2O), Dimethoate (2.5 ml in 1 liter dH_2O), phorate (1g in 10ml dH_2O) and four different bio-pesticides bio-sanjeevani (5 ml in 1 liter dH_2O), Biosoft (5 gm in 1 liter dH_2O), Neemta (5 ml in 1 liter dH_2O), Karanj extract (5 ml in 1 liter dH_2O). Bio-pesticides (Bio-sanjeevani and Biosoft) having formulation of (*Pseudomonas + Trichoderma viride*, *Beauveria bassiana,* respectively) Pure isolated bacterial culture was inoculated in 20 ml broth was kept overnight at 37^{o}C in (Chromas Biotech Pvt. Ltd.) incubator shaker. 1000µl from overnight grown pure bacterial culture was added in different test tubes containing 20 ml nutrient broth in a set of triplicate. 1000µl of various organophosphates and Bio-pesticides were added from stock solutions in all culture added test tubes except one test tube as control and one test tube left as blank with only nutrient broth. Growth curve of three different bacterial colony (*Sphingomonas sp. SZL-1*, *Pseudomonas mendocina strain PC19, Brevundimonas sp. XJ-41)* with Organophosphates and Biopesticides treatment were analyzed using Systronic UV-VIS spectrophotometer-118 at 600 nm for a time duration of (0-40) hours at a regular gap interval of 1 hour .

Levene's test for equal variance using SAS Enterprise Guide 4 was used to tests the null hypothesis, that the population variances are equal called homogeneity of variance. If the resulting p-value of Levene's test is less than some critical value (typically 0.05), the obtained differences in sample variances are unlikely to have occurred based on random sampling. Thus, the null hypothesis of equal variances is rejected and it is concluded that there is a difference between the variances in the population (Scasta *et al.,* 2012). Dunnett's test of comparative analysis is performed for predicting comparison between treatment groups to a single control for predicting better growth option among organophosphates and bio-pesticides for three different soil bacterial species.

RESULTS AND DISCUSSION

This study was initiated to determine the growth curve of three different beneficial soil bacterial cultures individually under pesticidal treatments. Three soil bacteria were isolated and identified (*Sphingomonas sp. SZL-1*, *Pseudomonas mendocina sp. PC19, Brevundimonas sp. XJ-41)* using 16S rDNA method followed by BLAST with the nr database of NCBI gene bank. MEGA4 was used for phylogenetic analysis based on nucleotide homology (Sultan *et al.,* 2012). The result was obtained from measuring optical density of *Sphingomonas* bacterial culture from consortium of four different organophosphates and four different bio-pesticides in the observation period of 40 hour at 600 nm. Growth curve of *Sphingomonas* resulted delay in inclination of growth slope (12-16 hour) and early declination in slope or stationary phase (36 hour) on organophosphates treatment, while bio-pesticides showed early inclination in slope (4-12 hour) and declination (36 hour) as compared to control (Fig.1). Similar observations of growth curve response towards organophosphates and bio-pesticides treatments were also reported in *Pseudomonas* and *Brevuendimonas* (Fig. 2 & 3). Toxic effect of chloropyrifos has been reported by Pablo *et al.,* (2008). Gilani *et al.,* (2010) was also reported that, chlorpyrifos has significant effect on soil microorganisms.

Table 1(a) Levene's test for homogeneity of OD variance ANOVA of squared deviations from group means *Pseudomonas mendocina strain PC19* at 0.05% significance

Source	D.F.	Sum of squares	Mean square	F Value	Pr > F
Organophosphates &BI	8	1.2820	0.1602	10.14	<.0001
Error	180	2.8433	0.0158		

Table 1(b) Levene's test for homogeneity of OD variance ANOVA of squared deviations from group means *Sphingomonas sp. SZL-1* at 0.05% significance

Source	D.F.	Sum of squares	Mean square	F Value	Pr > F
Organophosphates &BI	8	1.2446	0.1556	8.57	<.0001
Error	180	3.2674	0.0182		

Table 1 (c) Levene's test for homogeneity of OD variance ANOVA of squared Deviations from group means *Brevundimonas* at 0.05% significance

Source	D.F.	Sum of Squares	Mean Square	F Value	Pr > F
Organophosphates &BI	8	1.4149	0.1769	11.96	<.0001
Error	180	2.6621	0.0148		

Laboratory statistical methods included the calculation of the mean per cent which were subjected to the relationship between growth curve of soil bacterial species under different pesticidal (OP and BP) treatments along with control.

Certain bacterial species like that *Pseudomonas* aeruginosa are good alternative for degradation of Chlorpyrifos reported by Latifi *et al.,* (2012) which is contradictory to our result this may be due variation in species strain. Dimethoate (0.2%), Fenitrothion, Lindane @ 3.5 to 15 kg/ha, 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 (Lopez *et al.,* 1993).

Table 2(a) Dunnett's test for homogeneity of OD variance ANOVA of standard deviations from group means *Pseudomonas mendocina strain PC19.*

Level of organophosphates and bio-pesticides	N	OD	
		Mean	Std Dev
Biosanjeevani	21	0.64047619	0.45813182
Biosoft	21	0.62761905	0.46586377
Chlorpyrifos	21	0.29190476	0.23674077
Control	21	0.73047619	0.48163758
Dimethoate	21	0.58380952	0.33396820
Karanj Extract	21	0.61761905	0.47934231
Malathion	21	0.26523810	0.18688550
Neemta	21	0.6401905	0.42310520
Phorate	21	0.49428571	0.24673004

Malathion and Chlorpyrifos effect on three bacterial species was similar to screening technique for accessing effect of pesticide on population of soil microorganism (Tu 1970). Aquas extract of neem bio-pesticides showed no inhibitory growth of bacteria (De & Ifeoma 2002 and Mahmood *et al.,* 2010). In a similar observation *Trichoderma* strain with and without pathogen did not affect existing beneficial population results in increased of bacterial population (Shanmugaiah, 2009).

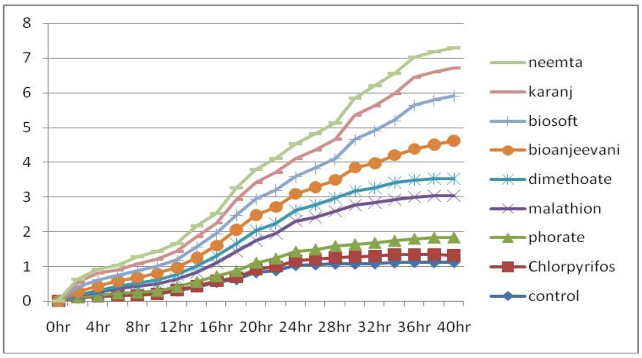

Fig. 1 Graph showing (OD Vs Hour) growth curve of *Sphingomonas sp. SZL-1*

The data obtained from spectrophotometer analysis were statistically analyzed using Levene's test of homogeneity resulted for justification in difference between the variances for growth of *Pseudomonas* (P = <0.001, F = 10.14), *Sphingomonas* (P = < 0.001, F = 8.57), *Brevundimonas* (P = < 0.001, F = 11.96) on treatment with organophosphate and bio-pesticides (Table 1a, b & c).

Dunnett's test estimated that at (0.05% significance) Bio-sanjeevani (BP) has maximum growth response mean (0.64 ± 0.4) and critical difference (+ 0.41 type I test) followed by neemta (0.64±0.4) and (+ 0.41 type I test), respectively. Similarly, Malathion was found minimum growth response mean (0.26 ± 0.18) and critical difference (- 0.04 type I test) with respect to control (0.73 ± 0.48) for Pseudomonas (Table 2a & b).

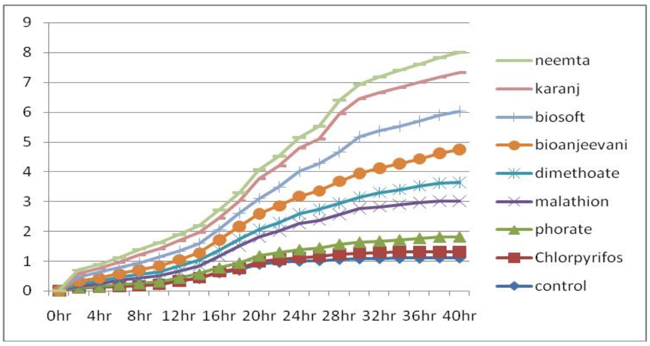

Fig. 2 Graph showing (OD Vs Hour) growth curve of *Pseudomonas mendocina strain PC19*

I test), respectively. While, Phorate was found minimum growth response mean (0.09 ± 0.08) and critical difference (- 0.18 type I test) with respect to control (0.68 ± 0.42) (Table 3a & b)

Table 3(a) Dunnett's test for homogeneity of OD variance ANOVA of standard deviations from group means of *Sphingomonas sp. SZL-1*

Level of organophosphates and bio-pesticides	N	OD	
		Mean	Std Dev
Biosanjeevani	21	0.55095238	0.43862176
Biosoft	21	0.65285714	0.47720166
Chlorpyrifos	21	0.09142857	0.08603986
Control	21	0.68095238	0.42643762
Dimethoate	21	0.26000000	0.18387496
Karanj Extract	21	0.65619048	0.44663717
Malathion	21	0.32523810	0.26485126
Neemta	21	0.31857143	0.16356301
Phorate	21	0.22904762	0.16714978

Dunnett's test estimation (at 0.05% significance) for *brevundimonas* resulted karanj extract (BP) having maximum growth response mean (0.68 ± 0.4) and critical difference (+ 0.47 type I test) in comparison to biosanjeevani (0.49 ± 0.3) and (+ 0.46 type I test), respectively. While Chlorpyrifos was found minimum growth response mean (0.08 ± 0.08) and critical difference (- 0.12 type I test) with respect to control (0.67 ± 0.43) (Table 4a & b).

Table 2(b) Comparisons significant (at 0.05% level) based on calculated value of (OD) variance ANOVA of standard deviations of *Pseudomonas mendocina strain PC19* at different levels of organophosphates & biopesticides

Organophosphates and bio-pesticides comparison	Difference between means	Simultaneous 95% confidence limits	
Control - Biosanjeevani	0.0900	-0.2390	0.4190
Neemta - Biosanjeevani	0.0271	-0.3019	0.3561
Biosoft - Biosanjeevani	0.0271	-0.3019	0.3561
Karanj Extract - Biosanjeevani	0.0171	-0.3119	0.3461
Dimethoate - Biosanjeevani	-0.0567	-0.3857	0.2723
Phorate - Biosanjeevani	-0.1462	-0.4752	0.1828
Chlorpyrifos - Biosanjeevani	-0.3486	-0.6776	-0.0196
Malathion - Biosanjeevani	-0.3752	-0.7042	-0.0462

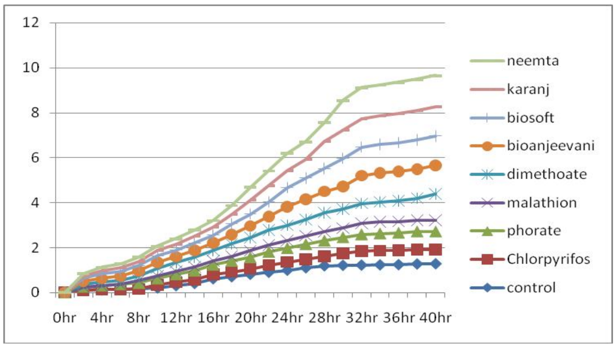

Fig. 3 Graph showing (OD Vs Hour) growth curve of *Brevundimonas sp. XJ-41*

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Table 3(b) Comparisons significant (at 0.05% level) based on calculated value of (OD) variance ANOVA of standard deviations of *Sphingomonas sp. SZL-1* at different levels of Organophosphates & Biopesticides

Organophosphates and Bio-pesticides comparison	Difference between means	Simultaneous 95% confidence limits	
Control - Biosanjeevani	0.1300	-0.1410	0.4010
Karanj Extract - Biosanjeevani	0.1052	-0.1658	0.3763
Biosoft - Biosanjeevani	0.1019	-0.1691	0.3729
Malathion - Biosanjeevani	-0.2257	-0.4967	0.0453
Neemta - Biosanjeevani	-0.2324	-0.5034	0.0386
Dimethoate - Biosanjeevani	-0.2910	-0.5620	-0.0199
Phorate - Biosanjeevani	-0.3219	-0.5929	-0.0509
Chlorpyrifos - Biosanjeevani	-0.4595	-0.7305	-0.1885

Table 4(a) Dunnett's test for homogeneity of OD variance ANOVA of standard .deviations from group means of *Brevundimonas sp. XJ-41*

Level of organophosphates and bio-pesticides	N	OD	
		Mean	Std Dev
Biosanjeevani	21	0.49476190	0.33407513
Biosoft	21	0.65047619	0.45115935
Chlorpyrifos	21	0.08571429	0.08958635
Control	21	0.67809524	0.43114521
Dimethoate	21	0.29000000	0.18697593
Karanj Extract	21	0.68666667	0.48364588
Malathion	21	0.62190476	0.48917910
Neemta	21	0.31571429	0.18375449
Phorate	21	0.22904762	0.16714978

Table 4(b) Comparisons significant (at 0.05% level) based on calculated value of (OD) variance ANOVA of standard deviations of *Brevundimonas sp. XJ-41* at different levels of Organophosphates & Biopesticides

Organophosphates and Bio-pesticides comparison	Difference between means	Simultaneous 95% confidence limits	
Karanj Extract - Biosanjeevani	0.1919	-0.0943	0.4781
Control - Biosanjeevani	0.1833	-0.1029	0.4695
Biosoft - Biosanjeevani	0.1557	-0.1305	0.4419
Malathion - Biosanjeevani	0.1271	-0.1591	0.4133
Neemta - Biosanjeevani	-0.1790	-0.4653	0.1072
Dimethoate - Biosanjeevani	-0.2048	-0.4910	0.0814
Phorate - Biosanjeevani	-0.2657	-0.5519	0.0205
Chlorpyrifos - Biosanjeevani	-0.4090	-0.6953	-0.1228

CONCLUSION

The result of this study showed that organophosphates are showing less growth response to beneficial soil microbes which play essential role in bioremediation and maintaining soil fertility as compared to bio pesticides. The result showed that bio pesticides like bio-sanjeevani, biosoft, baranj extract, neemta are better alternative of future and play important role in sustainable development of agro-ecosystem.

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