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Survival of Plant Growth Promoting Rhizobacteria (PGPR) in Soil under Pesticide Stress

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Abstract

A study was conducted to determine the effects of pesticide application at different doses on the population density of two important plant growth promoting rhizobacteria (PGPR): Methylobacterium spp. and Bacillus spp. These bacteria play an important role in plant growth promotion and as such are associated with the soil's nutritional value. The purpose of the study was to find whether or not pesticides do affect these PGPR and ultimately lead to environmental stress. Four organophosphates and four biopesticides were used for the purpose of the study.

The results revealed that pesticide applications caused a drastic reduction in the microbial population present in the soil, especially Methylobacterium sp. which was completely extinct in soil treated with Chlorpyrifos, Phorate and Dimethoate, except for (count of 0.7 x 106, 30 days, R.D. in Phorate treated soil), (106, 40 days and 0.55 x 106, 30 days, 2 R.D. in Chlorpyrifos treated soil). The same species proliferated well in biopesticide-treated soil except for a reduction in Neem treated plates (2.4 x 106 in R.D as compared to 3.3 x 106 in control). Increased doses of Dimethoate and Chlorpyrifos had profound effects on the Bacillus sp. population. In the case of biopesticides, only Karanj had a negative impact on its count, especially at 10 R.D., maybe as a result of its antibacterial effect. So, our present research focuses on the extensive use of biopesticides from a sustainable agriculture point of view.

Keywords: *Methylobacterium, Microbial population, Organophosphates, PGPR, R.D.(Recommended dose).*

1. Introduction

The extensive use of chemical fertilizers in farming guarantees high yields but causes various environmental problems. In view of this, there has been a recent resurgence of interest in environment-friendly, sustainable and organic agricultural practices (Esitken *et al*., 2006). The relationship between microorganisms and plants is symbiotic since both partners benefit from each other. So far, a considerable number of bacterial species, mostly associated with the plant rhizosphere, have been tested and found to be beneficial to plant growth, yield, and crop quality (Pýrlak and Kose, 2009). Bacteria that colonize the rhizosphere and plant root and enhance plant growth are referred to as plant growth promoting rhizobacteria (PGPR) (Frankenberger and Arshad, 1995). In addition to improving plant growth, PGPR are directly involved in the increased uptake of nitrogen, synthesis of phytohormones, and solubilization of minerals such as phosphorus and iron by the production of siderophores that chelate iron and make it available to the plant root (Renwick and Campbell, 1991; Pal *et al*., 2001; Usha rani et al., 2011). Bacteria of diverse genera such as *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Serratia* (Gray & Smith, 2005), as well as Methylobacterium were identified as PGPR.

Among such inhabitants are the pink-pigmented facultative methylotrophic bacteria (PPFMs), which are members of the Genus *Methylobacterium* and are gram-negative alpha proteobacteria. They are versatile in nature and ubiquitous on plant surfaces, potentially dominating the phyllosphere population. Rice *et al*. (1995) reported that the production of plant growth regulators such as auxins, particularly indole 3-acetic acid (IAA) and indoe-3-pyruvic acid (Ivanova *et al*., 2001), zeatin, zeatin riboside and reacted cytokinins by methylotrophs (Holland and Polacco, 1994) and IAA production and nitrogen fixation by *Rhizobium*, have been reported as factors enhancing the plant growth of legumes, the increase in the vegetative growth of the plant being attributed to the increase in the yield of a crop.

Among the PGPR cluster, *Bacillus* is one of the most potential genera in view of their spore forming ability, thereby increasing the adaptation of the *Bacillus* strain to commercial formulations and field applications (Liu and Sinclair, 1993). *Bacillus* is frequently isolated from rhizosphere, these species are also common plant endophyte. *B.mucilaginous* has been observed for its capacity to solubilize potassium (Wu *et al*., 2005) and phosphate (Idriss *et al*., 2002). *Bacillus cereus* AR156 is a plant growth-promoting rhizobacterium that induces resistance against a broad spectrum of pathogens including *Pseudomonas syringae pv. tomato* DC3000 (Niu *et al*., 2011). *Bacillus cereus* RS87 was previously reported to promote plant growth in various crops in both greenhouse and field trials (Jetiyanon *et al*., 2008).

The continuous application of pesticides may result in pollution processes that put soil at risk, driven by soil microorganisms and thereby affecting soil fertility (Sturz *et al*., 1999, Lopez *et al*., 2002, Cycon *et al*., 2006). Therefore, the present study was undertaken to establish whether or not the survival of the above mentioned two important bacterial species is under threat due to pesticide applications.

2. Materials and Methods

The soil samples were collected on 16th February 2011 from the experimental plot fields of the I.C.A.R research complex, in the eastern region of Palandu, Ranchi. The soil was collected from the upper 0-15 cm, other plant debris was removed manually and soil was sieved with a 4 mm mesh. The samples were then brought to the laboratory and stored at 40 C pending analysis. Four different Organohosphates (Malathion 50%EC of Century Polbounds Ltd, Dhanwan-20 for chlorpyrifos, ROGOR insecticide for dimethoate, DHAN-10G for phorate of Agritech Pvt. Ltd) and the two biopesticides: Biosanjeevani and Biosoft with formulations of *Pseudomonas+Trichoderma viride* and *Beauveria bassiana* respectively were purchased from the market. Leaves of two naturally occurring species *Pongamia pinnata* (Karanj) and *Azadarichita indicana* (Neem) were taken and their extracts were prepared in distilled water following the method of Jaber and Al-Mossawi, (2007). These served as the other two biopesticides for the study.

The dilutions of Ops and Bps were prepared for treatment with soil samples, based on the 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 the soil samples were treated with all these eight pesticides at three different concentrations in twenty four different containers and one container was left untreated as control. Enumeration of the soil bacterial population was done by pour plate method on nutrient agar medium (Johnson and Curl, 1972). On different days (0, 10, 20, 30 and 40) during the incubation period, 1 g of each test sample was taken from the treated and control pots and each suspended in 9 ml of autoclaved distilled water. Dilutions of 10^{-7} were prepared and plated on nutrient agar medium (Medox Pvt. Ltd). Petriplates were incubated at 37ºC for 48 hrs for bacterial growth. The colonies were counted on the colony counter (Chromous Biotech Ltd.) and the results were evaluated as the number of bacterial colonies in 1 g of oven dried soil. Numeration of soil microorganism was specifically carried out for two different species: Bacillus and Methylobacterium. The bacterial counts represented in graphs (***Figure 1*** and ***2***) are expressed as *no. X* 10^{-7} *per gm of soil.*

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

3. Results and discussion

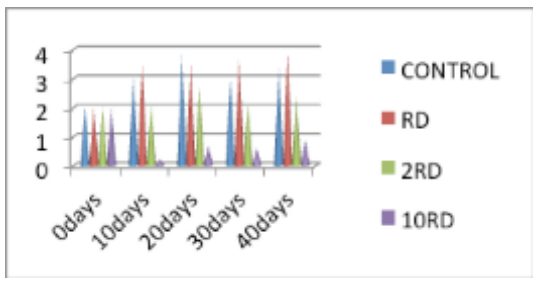

Figure 1 (i). *Graph representing the effect of Chlorpyrifos on the population density of Bacillus*

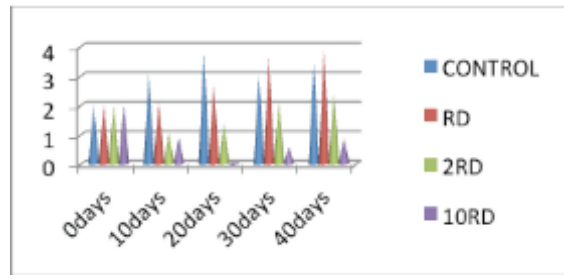

Figure 1 (ii). *Graph representing the effect of Malathion on the population density of Bacillus*

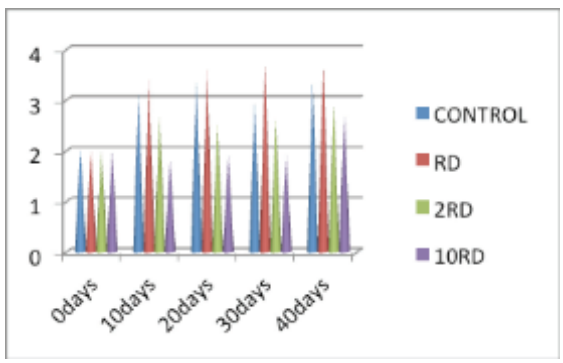

Figure 1 (iii). *Graph representing the effect of Phorate on the population density of Bacillus*

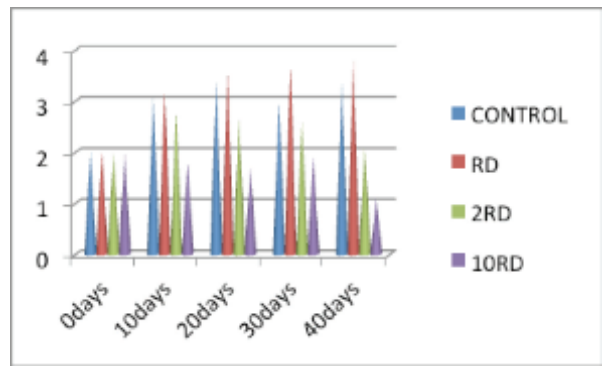

Figure 1 (iv). *Graph representing the effect of Dimethoate on the population density of Bacillus*

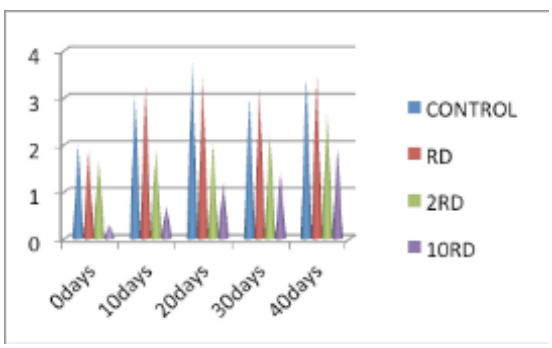

Figure 1 (v). *Graph representing the effect of Bio Sanjeevani on the population density of Bacillus*

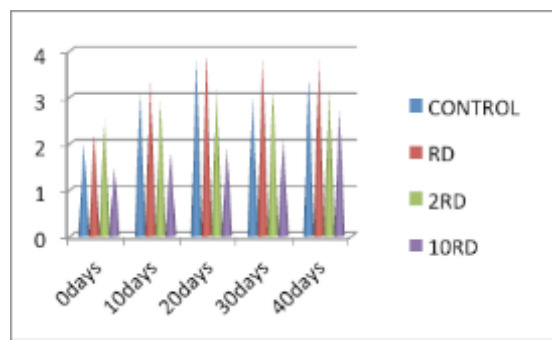

Figure 1 (vi). *Graph representing the effect of Biosoft on the population density of Bacillus*

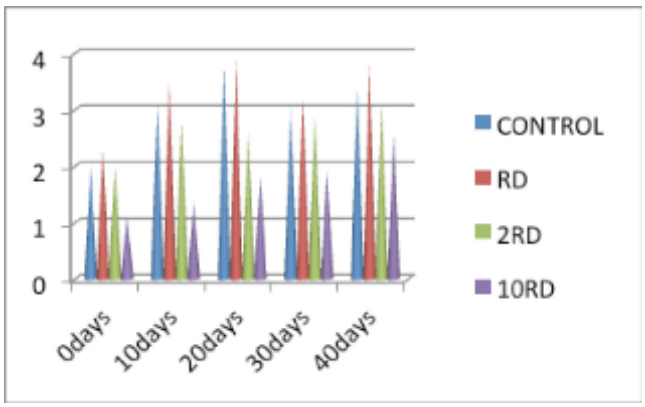

Figure 1 (vii). *Graph representing the effect of Neem extract on the population of Bacillus*

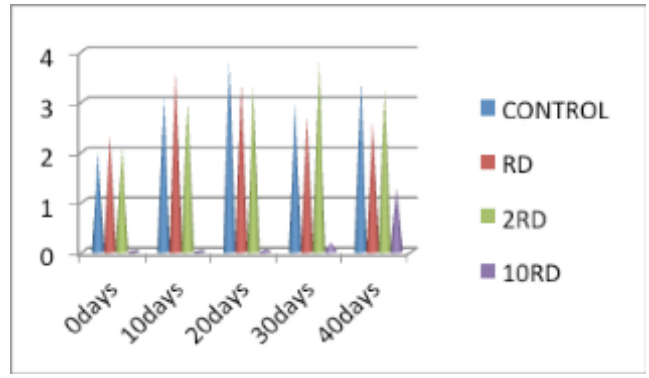

Figure 1 (viii). *Graph representing the effect of Karanja extract on population of Bacillus*

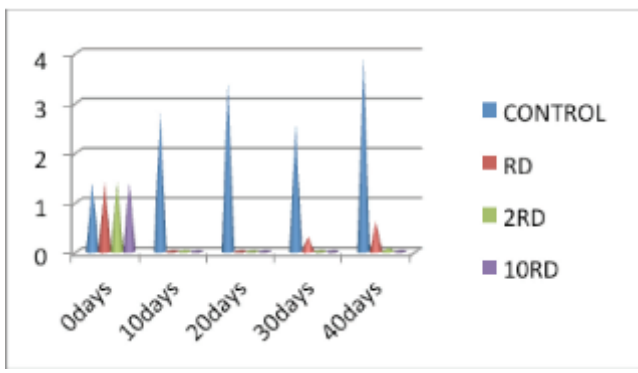

Figure 2 (i). *Graph representing the effect of Malathion on population of Methylobacterium*

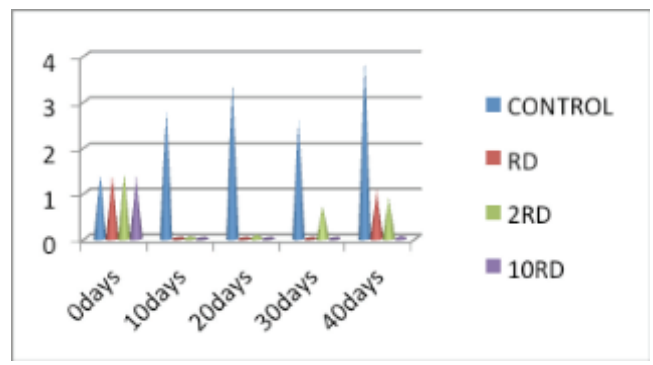

Figure 2 (ii). *Graph representing the effect of Chlorpyrifos on population of Methylobacterium*

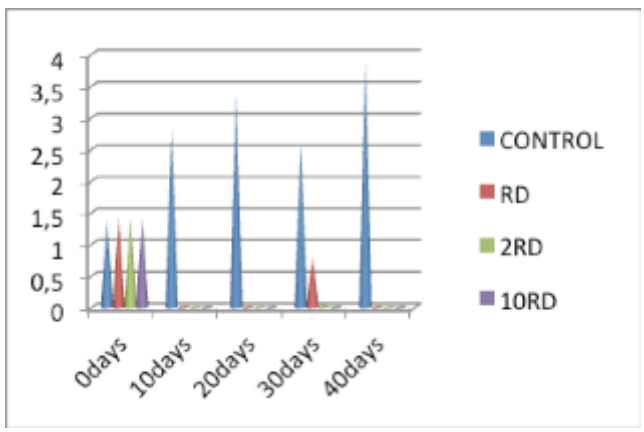

Figure 2 (iii). *Graph representing the effect of Phorate on the population of Methylobacterium*

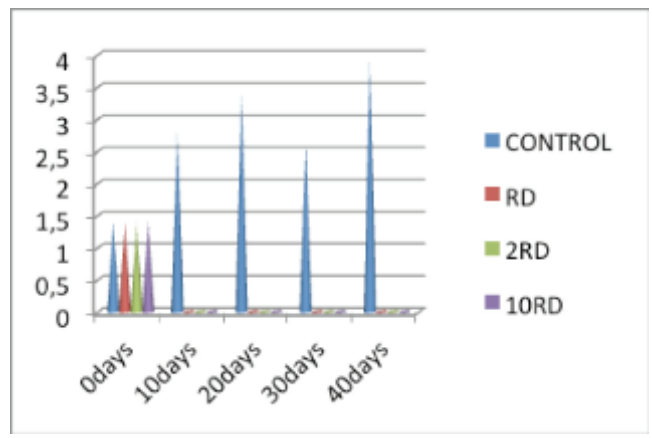

Figure 2 (iv). *Graph representing the effect of Dimethoate on population of Methylobacterium*

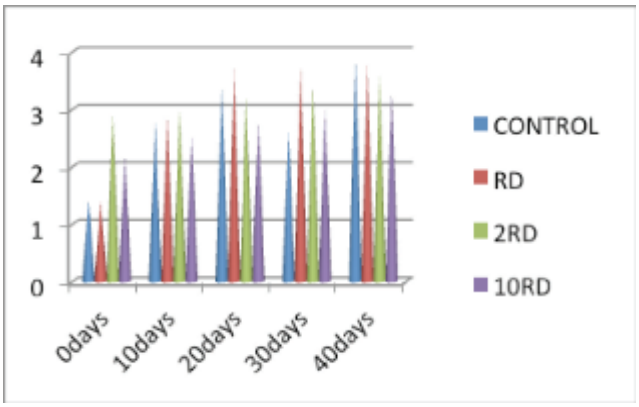

Figure 2 (v). *Graph representing the effect of Biosanjeevani on the population density of Methylobacterium*

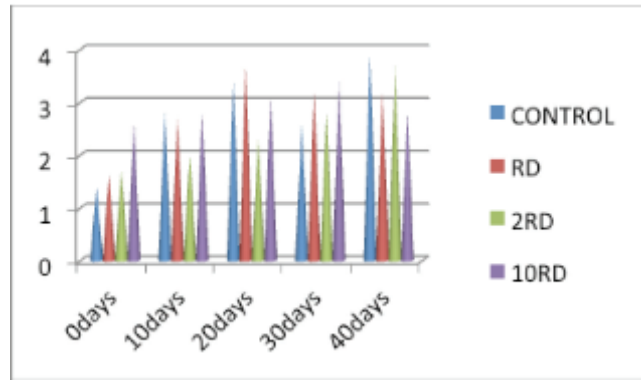

Figure 2 (vi). *Graph representing the effect of Biosoft on the population density of Methylobacterium*

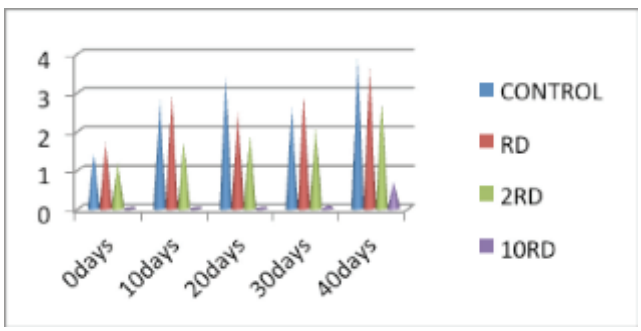

Figure 2 (vii). *Graph representing the effect of Neem extract on the population density of Methylobacterium*

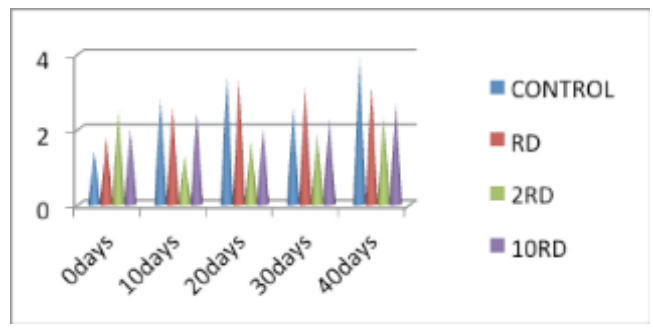

Figure 2 (viii). *Graph representing the effect of Karanja extract on the population density of Methylobacterium*

The study findings revealed that all the four organophosphates had drastic effects on the count of the *Methylobcterium* population as can be seen in ***Figure 2 [(i)*** to ***(iv)]***. With Phorate and Dimthoate, it was completely absent throughout the 40 days analysis when compared with control. In the Malathion and Chlorpyrifos treated soil, the *Methylobacterium* count was absent at all doses and on all days, except on the 40th day when it slightly reappeared but in very low numbers. This could be correlated with the findings of Pandey and Singh (2002) who observed short term inhibitory effects on the total bacterial population after Chlorpyriphos and Quinalphos applications which were recovered within 60 days after seed treatment and 45 days into soil treatment. Similar short term inhibitory effects on the total bacterial population were observed by Ahmed and Ahmed (2006), Tawfic *et al*. (1998) and Hashem *et al*. (1999).

On the other hand, the population density of the *Bacillus sp*. increased in the soil treated with organophosphates, ***Figure 1 [(i)*** to ***(iv)]*** especially, Phorate and Dimethoate showing their stimulatory effects as compared to control. The earlier works (El-Shahaat *et al*., 1987, Das and Mukherjee, 1994) also reported similar observations with different organophosphorous and carbamate insecticides in soil. Regarding the distribution of individual microorganisms in the rhizosphere soils, it was revealed that Phorate stimulated the growth of bacteria such as *Bacillus*, *Escherichia* and *Flavobacterium*, actinomycete like *Micromonospora*.

Higher doses of Malathion reduced the number of Bacillus. Similar effects of high concentrations were reported by Lakshmikantha (2000) who conducted research on the effect of foliar insecticides fenvalerate,

quinalphos and endosulfan on soil microorganisms and found that insecticides at normal recommended doses did not affect the major groups of soil microflora .i.e. bacteria, fungi, actinomycetes, free living nitrogen fixers and P-solubilizers, while four times more than the recommended concentration exerted high depressive effects, followed by two times the recommended concentration.

In Chlorpyrifos and Malathion-treated soil, the number increased significantly at R.D. Our findings are supported by the results of Congregado *et al*. (1979) who found that organophosphorus insecticides Dimethoate and Malathion at 10 and 100 µg/g of soil stimulated total bacterial population. It is also favored by the reports of Tu (1970) who showed that the number of total bacterial colonies increased during weeks 1 and 2 after treatment with Malathion and Dimethoate. The increase in the number of *Bacillus sp*. after OP application can be explained by the assumption that this soil microorganism can synergistically metabolize these pesticides, as Gunner and Zukerman (1968) demonstrated for Diazinon.

The growth of *Methylobacterium sp*. was not at all affected by the application of biopesticides (***Figure 2 [v-viii]***) except in the Neem treated soil at 10 R.D. The count in all setups was same as that in the control soil. This can be explained by the fact that some of the insecticides are found non toxic to soil microbes (Martinez-toldo *et al*., 1993). *Bacillus* number also remained the same in soil treated with biopesticides (***Figure 1 [v-viii]***) as in untreated soil, although there was a slight reduction in its population in Karanja treated soil (10 R.D.), may be due to an antibacterial effect at a high dose. Overall, the biopesticides showed a synergistic effect on the growth of these two bacterial species.

Conclusion

Soil microbial activities can be influenced by the application of agrochemicals, including the population of soil microorganisms. The extent of the impact depends on the dosage applied. The effects of the application of different doses in some degree are different from those of the recommended dose application. In this experiment, the chemicals used were organophosphates and biopesticides. The half-life of the chemical is rather short in soil, so the effect is not significant. If there are any effects, they would disappear quickly and would not cause an ecological problem from a microbial point of view. The results thus obtained indicate that the organophosphorus insecticide used had the least adverse effects on the *Bacillus* population but a significant effect on *Methylobacterium*. These effects are not drastic but minor in nature and the populations recovered over a period of time.

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