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Exploring the Applicability of PGPR to Remediate Residual Organophosphate and Carbamate Pesticides used in Agriculture Fields

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Abstract

To meet the food demand of growing population and to achieve higher crop yield, farmers use excessive amount of inorganic fertilizers and pesticides. In India a major share (up to 20%) of organophosphate and carbamate pesticides are mostly used by five agriculturally rich states *viz*., Andhra Pradesh, Punjab, Tamil Nadu, Karnataka and Gujarat. Amongst those pesticides dimethoate, monocrotophos, carbendazim and methyl parathion consumption is high in India.

Residues of pesticides remain in agricultural produce and migrate into human food chain. Microbial communities of soils play an important role in cycling of elements in ecological systems and provide essential nutrients to plants. Members of these microbial communities are the plant growth promoting rhizobacteria (PGPR). PGPR produce different types of enzymes and metabolites that have an ability to degrade xenobiotic compounds. The aim of the study is to explore the possibility of PGPR to tolerate and remediate organophosphate and carbamate pesticides. Microbial strains isolated from native agricultural fields were named as: PGPR 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In the present study organophosphate and carbamate pesticides used were dimethoate, monocrotophos, carbendazim and carbofuran. Experiments were carried out to determine the tolerance level of PGPR strains with different pesticide concentrations. All the isolates were checked for their viability against these pesticides at various concentrations ranging between 10 - 100 ppm. MTT and respiration assay were done for assessing the viability of bacterial isolates. In agriculture soils, pesticides residue concentration is usually in the

range 0.2 - 2ppm. All isolates have shown 90% survivability at the concentration of 10ppm as compared to control (without pesticide). At the time of application in fields, concentration of respective pesticides would be very high. A better PGPR formulation should tolerate wide range of pesticides so that they can involve in plant growth promotion and degradation of residual pesticides. Thus in our experiment all the isolates were tested for tolerance limits of up to 100ppm concentration of pesticides. In monocrotophos at 100ppm concentration under stressed condition PGPR2 showed 57% viability followed by PGPR1 with 56% viability whereas PGPR6 and PGPR10 were found to be more sensitive. For dimethoate, PGPR3 showed 85% survivability at 100ppm followed by PGPR2 with 69%. Isolates PGPR6, PGPR9 showed less tolerance. PGPR2 isolate proved to be a superior isolate showing consistent growth even in the presence of high organophosphate pesticides across a wide range. For carbendazim PGPR1 exhibited more viability followed by PGPR2 i.e. 61%, 60%. PGPR6, PGPR10 showed less tolerance level to even carbamate pesticides. In case of carbofuran PGPR7 and PGPR8 showed more tolerance with the survivability of 87%, rest all the isolates showed more than 70% survivability at 100ppm concentration of pesticide. In carbamate type pesticides though each isolate responded differently to the pesticide per say, there was a very uniform response to increasing pesticide concentrations by every isolate. Hence we can say that PGPR1, 2 and 3 that exhibited comparably better tolerance to both organophosphate and carbamate type of pesticides would be better option for sustaining plant growth in agriculture soils that are particularly stressed due to heavy chemical inputs. Further studies at our laboratory involve exploration of the degradation pathways of these pesticides by select PGPR isolates.

1. Introduction

A global shift is clearly visible to improve agriculture research and food productivity owing to the scarcity of non-renewable resources. In the past, the main driving force was to improve potential yield and productivity of crops by using more and more chemical inputs *i.e.*, synthetic fertilizers, insecticides and pesticides. But now the objective of productivity is closely associated with the desire of sustainability (Khan et al, 2004). Currently Indian agriculture is at high risk, because of high inputs of pesticides amounting to high quantity of residual chemicals in soil, contaminating land and water resources.In 2005 pesticides worth about Rs.2,700 crores were used in India. Highest consumption was recorded in Andhra Pradesh (20%), followed by Punjab (10%), Tamil Nadu (9%), Karnataka and Gujarat (6%) (Ahemad and Khan, 2009). Due to extensive use of organophosphate and carbamate pesticides, the microbiota of diverse ecological niches is developing improved tolerance to address the adverse

effects of these pesticides and also to degrade these compounds. Organic pesticides applied to soil may be used as substrates by the tolerant microorganisms and undergo degradation. Nowadays research on pesticide resistant and/or degrading bacterial strains is emerging as a healthy option for better environment (Mandal et al, 2003; Wahab, 2009; Ahemad et al, 2011). Hence, it is important to explore the ability of bacterial isolates and use them as a tool in order to clean the environment.

PGPR have been subjected to numerous investigations focusing on biotechnological applications in agriculture, horticulture, forestry and environmental protection. PGPR have diverse taxonomy and include species from the genera *Azospirillum, Azotobacter, Bacillus, Enterobacter, Gordonia, Klebsiella, Paenibacillus, Pseudomonas, Serratia,* etc., amongst others (Myresiotis et al, 2011, Joseph et al, 2007). Microbial inoculants can be used as biofertilizers, biocontrol agents, phyto-stimulators, and bioremediators based on their mode of action in agriculture field (Ashrafuzzaman et al, 2009). PGPR is known for the production of growth hormones, vitamins and other amino acids that influence the growth of the plant by increasing its nutrient availability (Kumar et al, 2011). The success of biocontrol of plant pathogens by PGPR may involve one or more of the metabolites or activities such as production of HCN, siderophore, antibiotics and other biocidal compounds (Ahemad et al, 2011).In the present study, we have investigated the tolerance of PGPR to 2 different pesticides representing organophosphate and organocarbon group of pesticides. Each of the pesticides used have different potential, properties and toxicity limits (Kanekar et al, 2003). Properties of the 4 pesticides used in the study: moncrotophos, dimethoate, carbendazim and carbofuran are tabulated in table no.1. The principal aim of the study is to assess the pesticide tolerance limits in PGPR and to explore the possibility of their application for degrading residual pesticides in soil.

Table 1: Pesticide properties.

Pesticides used	**Nature**	**Consumption (MT)**	**Concentration in agriculture soil**	**Crops**	**Limits of toxicity**
Dimethoate	Organo-phosphate	636	0.914 - 5.18 mg/kg	Pulses, vegetable and fruits.	LD_{50} for rats is 60- 387 ppm
Monocrotophos	Organo-phosphate	1815	-3.92 ng g^{-1}	Pulses, vegetable and fruits.	LD_{50} for rats is 112 ppm
Carbendazim	Organo-chlorine	1992	2.2-9.2 ppb	Pulses, vegetable and fruits.	LD_{50} for rats is >15000 ppm
Carbofuran	Organo-chlorine	NA	NA	Pulses, vegetable and fruits.	LD_{50} for rats is 7- 18 ppm

2. Material and Methods

2.1 Bacterial inoculants

The bacterial strains isolated from agriculture field were named as PGPR1, PGPR2, PGPR3, PGPR4, PGPR5, PGPR6, PGPR7, PGPR8, PGPR9 and PGPR10 respectively and were regularly maintained on KB / nutrient agar.The plant growth promoting properties of these isolates were reported earlier by the authors. In the current study, we are testing their ability to tolerate different pesticides concentrations under *in vitro* conditions.

2.2 Pesticides

In this experiment used pesticides were dimethoate, monocrotophos, carbendazim and carbofuran with concentrations tested in the range: 10,20,40, 80 and 100ppm. All pesticide samples were the ready to use market available formulations as shown in Table 2.

Table 2: Details of pesticides used in this study.

Technical name of pesticides used	Commercial name & company	Purity	Stock concentrations prepared
Monocrotophos	Chetak, Crop Chemical India Ltd	53%	190µL/10ml water
Dimethoate	Anugor, Anu Produts Ltd	30.5%	328µL/10ml water
Carbendazim	Bavistin, BASF	50%	20mg/10ml water
Carbofuran	Furan3G, Crop Chemical India Ltd	3%	2-3 crystals

Design of the experiments for the tolerance level of bacterial cultures: In 2ml culture media, respective concentration of pesticides was added and inoculation was done using100µl of overnight grown PGPR culture broth. After an incubation period of 18hrs, MTT assay was done to check the viability of bacterial isolates (Patel et al, 2013). For each PGPR isolate, five concentration ranges of each pesticide and a control (without pesticide) were studied. All the experiments were carried out with 3 replicates.

2.3 MTT assay

MTT assay was carried out to estimate the living biomass of PGPR after challenging with various concentrations of pesticides. An aliquot of 100 µl overnight grown culture was collected in triplicate from each pesticide concentration and placed in the wells of a microplate. 10 µl MTT (Himedia) stock solution was added to each well to obtain a final concentration of 0.5 mg/ml MTT in the reaction mix. The cells in the 96-well plate were then incubated at 37^0C till blue purple crystals were formed. Crystals thus formed were solubilised using 100 µl organic detergent DMSO (CDH, AR Grade)

during a 2 h incubation period. After incubation, absorbance values were measured with an ELISA reader (Benchmark, Bio-Rad) at 570 nm (Abate *et al*, 1998). All the treatments were carried out in triplicates and values were recorded for statistical analysis. The entire experiment was repeated for observing consistency in the result.

3. Result and Discussions

Plant growth promoting rhizobacterias showed significant tolerance to pesticides studied, indicating their ability to survive under high pesticide stress conditions. For monocrotophos most of the PGPR isolates showed survivability 75 to 90% at the pesticide concentration of 10ppm and they were viable till 100ppm of pesticide concentration. In PGPR1 viability % was really high. Though in initial phases microbial growth was inhibited, after 40ppm it was found that PGPR1 was persistent till 100ppm with the same vigor (66% viability). The second highest growth was recorded in PGPR2 (57% viable) at 100ppm concentration. Though PGPR1, 2 showed growth decrease with increasing concentration of pesticides, even at 100 ppm concentration both of them retained more than 50% viability (Fig. 1). Moderate tolerance was observed in PGPR3, PGPR4 and PGPR5 as they showed comparatively less (<50%) viability at higher concentrations (100 ppm) of pesticide. PGPR6, PGPR7, PGPR8, PGPR9 and PGPR10 were susceptible isolates as even at a concentration of 40ppm their viability decreased to 50 - 60 % and at 100ppm it reached survivability rate as low as 2 - 15%. In dimethoate the survivability of PGPR3 was highest (85%) and 2nd highest survivability was found in PGPR2 (69%) at the concentration of 100ppm. Moderate growth was recorded in PGPR1, PGPR5 and PGPR7. PGPR4, PGPR6, PGPR8 and PGPR9 were found to be most susceptible with their viability falling below 15% at the concentration of 100ppm (Fig. 2).

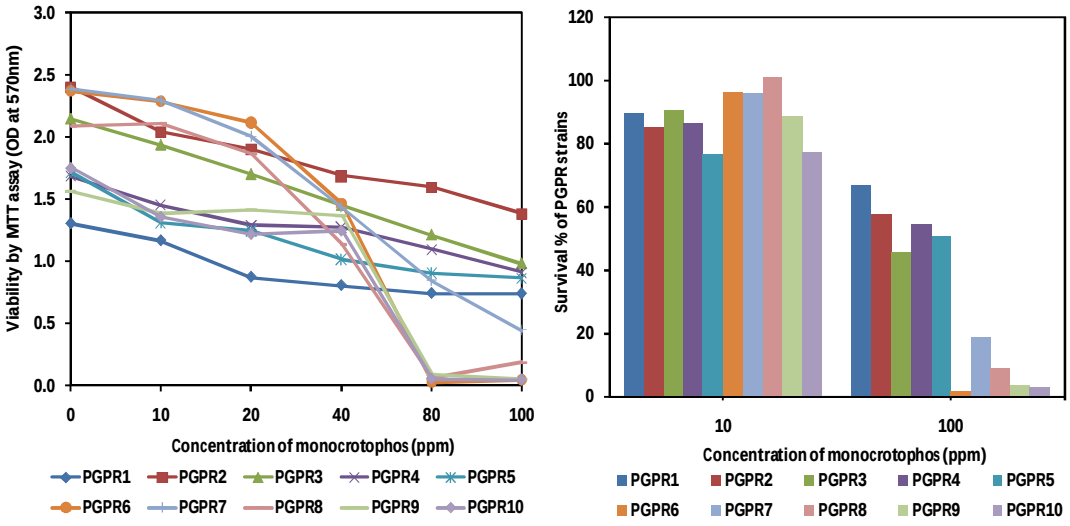

Figure 1: Tolerance of PGPM against monocrotophos.

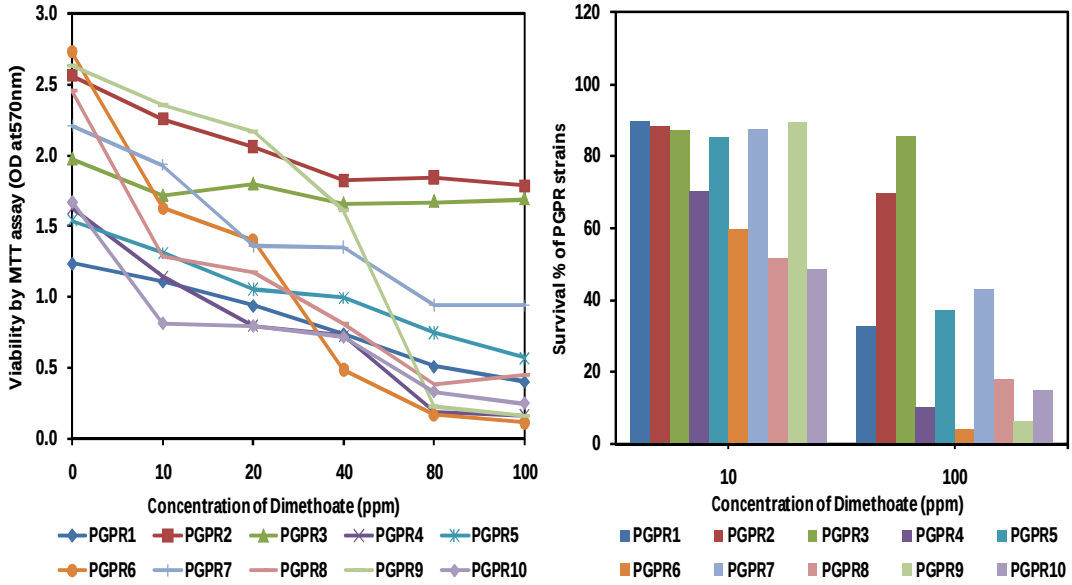

Figure 2: Tolerance of PGPM against Dimethoate.

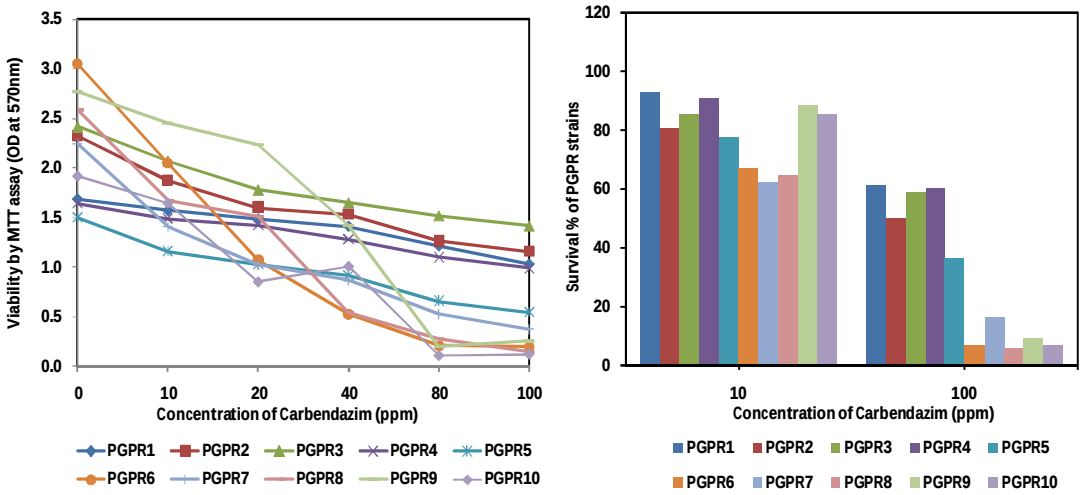

Figure 3: Tolerance of PGPM against carbendazim.

In carbamate type pesticides PGPR response varied not only with the concentration of pesticides but also with the type of pesticide. In carbendazim PGPR1, PGPR3 and PGPR4 were found best, with their survivability as high as 60% or more, even at 100 ppm concentration. Moderate tolerance was recorded in PGPR2 and PGPR5 at 100ppm concentration. The remaining PGPRs showed least growth. In carbofuran all PGPR isolates recorded 80 to 90% survivability even at 100ppm concentration (Fig. 3,4). We may say that of all the pesticides tested, Carbofuran registered comparatively less impact on microbial growth.

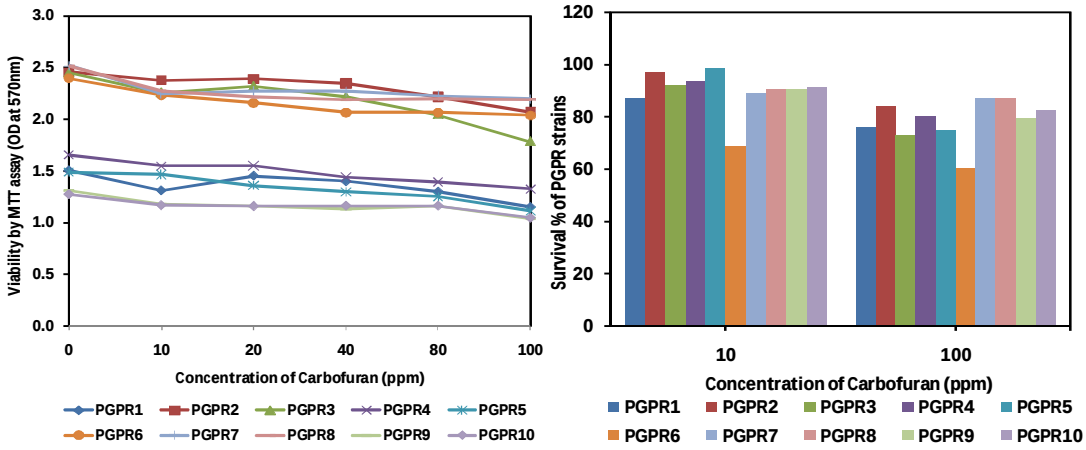

Figure 4: Tolerance of PGPM against carbofuran.

Chemicals inputs adversely affect the rhizospheric organisms including PGPR and associated biotic processes. Studies on the effect of various pesticides have however, largely concentrated on the populations of soil microflora. Literature with regard to the effect of pesticides on activities of PGPR is inadequate. Considering this, the present work was designed to evaluate the toxic effects of fungicides and pesticide at recommended rates on the survival of selected rhizobacteria. In our work we found that all 10 PGPR isolates showed tolerance to pesticides and their limits varied with the concentration and type of pesticides used. At 10ppm concentration all PGPR showed up to 90% survivability because 10ppm of pesticides may not interfere with the metabolic processes of PGPR isolates and hence their growth is not affected so much as compared to control. But at 100ppm concentration PGPR1, PGPR2, PGPR3 and PGPR4 showed more than 50% viability as compared to control. Their response towards pesticides might be due to the native characteristics of agricultural soils from where they have been isolated. The results agree with publication of Ahemad *et al.*, (2011) where pesticide-tolerance and the functional diversity of bacteria in soil was studied with respect to 53 PGPR isolates from fields.

4. Conclusion

PGPR are known for their plant growth promotion capabilities. In this manuscript we have checked the capacity of agriculture field isolates to tolerate pesticide compounds. In this study 10 native PGPR were selected to check their viability against different pesticide concentration. We found that all the isolates showed tolerance level against pesticides and all are responding well at lower concentrations of pesticide stress. However, at higher concentrations (100 ppm), 3 isolates: PGPR2, PGPR3 and PGPR4 recorded the best survival capability with monocrotophos, dimethoate, carbendazim and carbofuran. These PGPR should be further studied to know their contribution in metabolizing the residual pesticides and thus decreasing the toxicity caused due to migration to aerial parts of the plant which means the agricultural produce.

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