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Investigating the Role of PGPM in Assisting Plant Growth Under Stress Caused by Organophosphate Pesticide-Phorate

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ABSTRACT: Farmers across the world face the challenge of compromised agriculture productivity due to abiotic stress. Residual pesticides accumulating in the soil rhizospere are often found to cause a chemical imbalance in the soil microclimate resulting in chemical toxicity and osmotic stress. Plant growth promoting microorganisms (PGPM) are popularly studied for their ability to enhance plant growth by providing stress tolerance to plants. The present study is to investigate the role of PGPM in reducing toxicity caused due to accumulation of organophosphate pesticide (OPP) - Phorate. Isolation, evaluation and selection of best phorate tolerant PGPM strains was done by various rounds of purification and biochemical characterization assays. Isolates of PGPM from the rhizospere of Pearl Millet-PB, Maize PM, Pigeon Pea-PA, Sugarcane-PS and Sorghum-PJ were studied for their ability to tolerate a wide range of OPP (150 ppm to 1050 ppm). Subsequently, *in vitro* plant bioassay was performed to select the isolate that can provide best growth promotion property to host plant when challenged with pesticide phorate. The parameters studied to evaluate growth promotion abilities were percentage germination, seed vigor and total biomass. We were successful in isolating, evaluating and selecting best PGPM strains for providing tolerance to host plant under phorate induced stress. Out of the four isolates tested, isolates PM and PB showed better tolerance to phorate and recorded higher LD_{50} and MIC values as compared to other isolates. When compared with control, OPP stress subjected PM and PB showed best tolerance to OPP with LD_{50} 981 ppm/unit OD and 1249 ppm/unit respectively and MIC values as 1050 ppm. However from the plant bioassay it was evident that between PM and PB, PM was a better bioinoculant as it contributed to plant growth with respect to all studied parameters (*viz.* percentage germination and seed vigor) where as PB stood as second best at providing growth support to host plants when challenged with phorate. Culture isolate PM emerged as the choicest isolate that can be further explored as a potential bioinoculant under pesticide stressed conditions. Isolate PB, with comparable pesticide tolerance (very near MIC & LD_{50} values) as PM, was clearly outcompeted by PM when it came to supporting plant growth (evident from *in vitro* plant bioasaay). A Culture having high pesticide tolerance under *in vitro* conditions, may or may not possess the ability to support plant growth when used as a bioinoculant at high pesticide concentrations. The study clearly emphasizes the importance of host response studies for making selection of rhizobacteria for the purpose of remediating pesticides. PB with comparable pesticide tolerance with PM under *in vitro* conditions was clearly outcompeted by PM when it came to supporting plant growth (as evident from results of plant bioasaay). The study offers proof that, inoculation with carefully selected pesticide tolerant PGPM can have a positive impact on plant growth even under high concentration of organophosphate pesticides.

NOTE: Full length manuscript of Gupta et al. Investigating the Role of PGPM in Assisting Plant Growth Under Stress Caused by Organophosphate Pesticide- Phorate . 2014; 4(3): 142.

INTRODUCTION

Continuous increase in world population has put serious pressure on the existing agricultural land. Farmers across the world encounter severe crop losses due to biotic stresses such as pathogenic attack (bacterial, fungal) and abiotic

stresses like temperature, water (drought, salt), ionizing radiation, chemicals (heavy metals, toxins), nutrient (mineral deficiency/excess) etc. [1]. Using chemical fertilizers and pesticides to overcome these stresses has deleterious after effects on soil quality [2]. These chemicals compounds often persist in the environment and bring about various undesired changes including bioaccumulation in agricultural products.
India is the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally. In India, insecticide usage constitutes 76% of all the chemicals used to treat the agricultural lands as against the rest of the world that uses only 44%. Ideally, pesticides should be target organism specific, biodegradable and should not leach into ground water - unfortunately that is rare. Over-use of these chemicals result in depletion of organic matter, loosening of the soil causing erosion, depletion of micro and macro nutrients, water scarcity in the rhizosphere, surface and ground water contamination, food contamination, acidification and alkalization. It also disrupts number of beneficial soil microbes, increases the salinity of soil, and impacts human directly and/or indirectly [1].

Organophosphates are the most common insecticides that farmers use in India. These have been reported to cause largest number of deaths of non-target organisms. Phorate is one such organophosphate which is used to control suckling and chewing pests, mexican bean beetle, corn rootworm, mites, etc, and used on potatoes, corn, peanuts, cotton, sugarcane, wheat, soybeans, etc. It is a water soluble insecticide, whose dosage to field is 7.5 to 10 kg per acre [3].Usage of phorate has increased from 2630 to 3284 (metric tonnes) between 2005-2010. In India, total production of phorate was estimated to be 4,800 metric tonnes [4]. In a publication by Bano and Musarrat, 2003; LD50 value as low as 2 ppm was reported to be toxic where as residual levels of phorate in Indian soils (north western) are as high as 200 ppm [5]. Phorate can over stimulate the nervous system through acetlycholinesterase inhibition causing nausea, dizziness, confusion, respiratory paralysis and death at high exposures. There are dietary risks from drinking phorate contaminated water and its metabolites in groundwater and surface water. Phorate and its breakdown products persist in soil, plants and it has been reported the usage OPP has lead to decrease the number of helpful bacteria in the soil rhizosphere. So there is a need of using bioinoculants that could help in maintaining the diversity of microbes in soil and remediate the residual pesticides so that they do not contribute to abiotic stress conditions in agriculture soils. .

In order to remediate the soils contaminated with pesticides, their biodegradation using bacteria and/or enzymes has been suggested to be one of the effective measures [6]. Plant growth promoting micro-organisms (PGPM) such as Rhizobium, Azospirillum, Pseudomonas, Flavobacterium, Arthrobacter, Bacillus etc. are free-living bacteria in the rhizosphere, exerting beneficial effects on plant growth by: fixing free atmospheric nitrogen, increasing nutrient uptake from soils, reducing disease susceptibility by other pathogenic bacteria, viruses, nematodes and fungi, increase plant's defense against physical and chemical changes etc. [7]. They also provide 'Induced Systemic Resistance'. Employing PGPM to support plant growth can reduce the use of chemical fertilizers, pesticides, ease the chemical burden in agriculture soils, prevent chemical accumulation, and lessen the toxic effects of irrigation run-off to natural water bodies and ground water reserves [8]. Objective of the present study is to isolate and select PGPM from soil rhizosphere and evaluate their role in protecting plants against pesticide stress while supporting plant growth under elevated pesticide (Phorate) concentrations.

MATERIALS & METHODS

Isolation and biochemical characterization of PGPM isolates
Sampling of soil: Soil sample along with roots (upto 25cm) from Pigeon pea (Canjanus canjan), Pearl Millet (Pennisetum glauccum), Sugar cane (Sacchrum officinarum) and Maize (Zea mays) and Sorghum (Sorghum bicolor) were collected from agricultural fields of six different crops near Narora, Distt. Bulandshahar (U.P, India). The soil samples were air dried and sieved to remove rocks and other debris. 1g each of soil sample from millet, maize, pigeon pea, sugarcane and sorghum rhizosphere were separately inoculated in King's B broth and incubated overnight at 180 r/min, 30oC. Pin head colony obtained were plated and pure culture obtained was carried over for subsequent purification. Pure Isolates obtained from rhizosphere of millet, maize, pigeon pea, sugarcane and sorghum were named as PB, PM, PA, PS and PJ.

For characterization and identification of bacterial isolates biochemical tests were performed based on schematic suggested by Bergey's Manual for Determinative Bacteriology [9]. These included gram staining, triple sugar agar test, tests for fluorescence, production of indole, urease, oxidase and catalase, starch hydrolysis, methyl red test, gelatin hydrolysis, maltose & mannitol fermentation and tests for succinate, malate and citrate utilization. For each test a single pinhead colony of bacterial sample was taken

from an overnight grown culture. The pinhead colonies were inoculated/ streaked in test tubes/petriplates containing respective media and incubated at 30oC overnight with/without shaking at 180 r/min according to protocol as per the demands of the protocol [10]. The tubes/plates were checked for positive/negative reaction as indicated by change in color (starch hydrolysis, methyl red, maltose, mannitol, succinate, malate, citrate, oxidase), yellow green fluorescence or bubble formation (catalase).

Biochemical markers indicating remediation potential of PGPM

Single pin head colonies of PB, PM, PA and PS were inoculated in 50ml King's B media separately and left on a shaker incubator (180 r/min) overnight at 30oC and used as starter culture for each of the following tests. After 16 h of incubation, when 0.6 optical density was achieved, requisite volume of starter cultures was dispensed into test vials as per protocols detailed below.

Biofilm formation: Trypticase soya broth was prepared and 1ml starter cultures PB, PM, PA and PS were inoculated in 9ml trypticase soya broth separately in test tubes and kept at 180 r/min over night at 30oC. After overnight incubation, the contents of the test tubes were emptied by decanting and washed with PBS. Further, the test tubes were rinsed with 0.1% crystal violet and kept for drying. The test tubes were checked for ring formation [11], an indication of biofilm formation ability of seeded cultures.

Emulsification index: 4ml of the starter cultures of PB, PM, PA and PS were dispensed in four test tubes separately and 6ml kerosene oil was layered on each of these test tubes and left on shaker incubator (180 r/min) overnight at 30oC. Post incubation, the tubes were checked for formation of emulsification layer. Lengths of emulsification layer, liquid column were recorded to calculate emulsification index using the formula below and the results were tabulated (Table 2) [12].

Emulsification Index = (Length of emulsification layer)/(Length of total column)X 100

Biosurfactant production: SW agar media was prepared with CTAB and methylene blue and plates were prepared with a control. Single pin head colonies from PB, PM, PA and PS were stabbed on separate plates and kept at30oC for 5 days. The plates were checked for blue color colony formation, an indication of rhamnolipid production [13].

Proline: 1ml of starter culture of PB, PM, PA and PS were inoculated in separate test tubes with 9 ml King's B media and kept on shaker incubator (180 r/min) overnight at 30oC. Pellet was collected separately by centrifugation at 10,000 r/min for 10min. Ethanol and water (70:30) was added to the pellet and the mixture was sonicated and again centrifuged at 10,000 r/min for 10 min. 50µl of supernatant was dispensed in screw cap tube and 100µl reaction mix (ninhydrin 1% w/v in acetic acid 60% v/v and ethanol 20% v/v) was added. Similarly for obtaining standard curve, 100µl of reaction mix was added to 50µl of proline (range = 0.5-6 mM) prepared in 70:30 ethanol and water. The sealed screw cap tubes were kept at 95oC in water bath for 20 min and then (at room temperature) tubes were centrifuged for 1min at 2500 r/min. 100µl of the mixture was transferred into the wells of a microtitre plate, optical density was recorded at 520nm [14].

Standard curve was prepared and proline concentration was determined using formula:

Proline in µmol.g-1FW= (Abs extract-Blank)/SlopeX(Vol extract)/(Vol aliquot)X1/FW

Where Abs extract is the absorbance determined with the extract, blank is the absorbance determined with only ethanol:water (70:30) and slope (calculated from standard curve), Vol extract is the total volume of the extract, Vol aliquot is the volume used in the assay, FW (expressed in mg) is the fresh weight of the culture. Absorbance should be within linear range.

Exopolysacchride: Luria broth was prepared and 1 ml of starter culture from PB, PM, PA and PS were inoculated in 9ml media in separate test tubes and kept on a shaker incubator (180 r/min) at 30oC for overnight incubation. Post incubation, the cultures were collected in separate oakridge tubes and centrifuged at 10,000 r/min for 10 min. Pellets were collected and 10ml of cold isopropnol was added to each. The mixture was transferred to the test tubes and kept overnight at 4oC. The precipitated exopolysacchride was collected, centrifuged, dried and weighed [15]. Results from the experiment are shown in table 2.

Tolerance of PGPM isolates to Phorate: The stock solution of phorate (Phoratox-10, Phorate 10% CG, encapsulated) was prepared by adding phorate granules to 100ml autoclaved distilled water to prepare a stock solution of 1875 ppm. The reagent bottle was kept in shaker incubator

(180 r/min) for 4 hours till all the active phorate was dissolved and remaining debris were filtered out. 1ml of starter culture from PB and PM were separately seeded in test tubes with 9ml of King's B medium with increasing concentration of phorate over a range of 150 ppm – 1050 ppm (0ppm, 150ppm, 300 ppm, 450ppm, 600ppm, 750 ppm, 900ppm and 1050ppm) prepared from phorate stock, inoculated with 1 ml starter culture and overnight kept on a shaker incubator (180 r/min) at 30oC. Optical density was recorded at 595 nm.. Percentage growth reduction in culture isolates upon exposure to phorate was calculated using the formula-

Percentage growth reduction = (OD phorate-OD control)/(OD control) X 100

Where OD phorate was optical density recorded for the culture stressed with phorate and OD control was optical density recorded for the culture free of phorate stress.

LD50 values were calculated based on the trend line and R2 (slope) values (Figure 4). MIC and LD50 of each of the test isolates was documented as shown in table 3.

In vitro Plant bioassay: In this experiment, mung bean seeds were subjected to phorate stress in two sets i.e. with and without PGPM, where the PGPM used were PM and PB strains. The seeds were first surface sterilized by two rounds of 70% ethanol and washed with autoclaved distilled water. One set of petriplates consisted of water with pesticide concentrations from 0ppm, 75ppm, 150ppm, 225ppm and 300ppm made from the stock along with 25 mung seeds each. And the other set consisted of pesticide concentrations from 0ppm, 75ppm, 150ppm, 225ppm and 300ppm along with 25 mung seeds each coated with isolates PM and PB by dipping the seeds in overnight grown culture for 5 min.

After 3 days of germination parameters such as percentage germination, seed vigor and total biomass were studied to assess the effect of inoculation with isolates PM and PB [16].

Percentage germination = (Total number of seeds germinated)/(Total number of seeds inoculated) X 100

Seed vigor = (Mean root length + Mean shoot length) X Percentage germination

Length of radical and plumule were measured using a ruler and average length from all the germinated seedlings was calculated. Total biomass was calculated by keeping the germinated seedlings at 50oC until constant weight was observed from all seeds. The results from the experiment were tabulated (Table 4, 5).

RESULTS & DISCUSSION

We were successful in isolating and purifying five PGPM strains from the collected agricultural soil. Gram staining was performed and it showed that isolates PB, PM, PA and PJ were gram negative rods. Gram negative and aerobic rods/cocci fall in group IV micro-organisms which includes Acinetobacter, Pseudomonas, Beijerinckia and Acetobacter [9]. Members of the genus Pseudomonas (for example, P. aeruginosa, P. chlororaphis, P. cichorii, P. fluorescens, P. putida P. Syringae) are known to give yellow flourescence. Except PJ, probable Pseudomonas isolates PB, PM and PA showed yellow-green fluorescence. Furthermore, Pseudomaonas are reportedly non-glucose fermentors and produce acid in triple sugar test [9, 17]. The isolates PB, PM and PA showed a red butt as well as slant with no H2S production.. However, H2S production was observed in PJ and it was exempted from further studies as it indicated the characteristics of biosafety level 2 or 3 organisms which are not fit for application as bioinoculants (Table 1). Isolates PB, PM and PA tested positive for catalase production, once again a feature of aerobic Pseudomonas spp. where the enzyme catalyzes the degradation of hydrogen peroxide that is harmful for cells and this feature may also help in reducing abiotic stress due to reactive oxygen species [18]. Another test investigated for indole and urease production, the absence of which is another characteristic of Pseudomonas spp. PB, PM and PA showed a negative reaction for indole test. PB and PA were urease negative but PM was urease positive. Gram negative rods that give a positive reaction for oxidase test include Aeromonas, Pseudomonas and Vibrio [9]. When tested for oxidase PM and PB gave positive results with the exception of PA (Figure 2). Gelatinase activity is a property of Pseudomonas by which they are able to liquefy the gelatin [19]. Our isolates PB, PM and PA have shown positive reaction for gelatin hydrolysis. No acid production was observed when methyl red test was performed, a result that contradicts with the characteristic feature of Pseudomonas genus [20] (Figure 1(b)). Pseudomonas genus has been proven to have C4-dicarboxylate transporters which help them to utilize succinate and malate as the only carbon source [21, 22]. The isolates PB, PM and PA were tested positive for utilization of succinate and malate. It is the known property of Pseudomonas genus to produce acid when it utilizes and ferments C- sources like mannitol and maltose, Our results for mannitol and maltose fermentation were positive and

agreeing with the reference [17] (Figure 1(c), 1(d)). Lastly, the isolates PB, PM and PA were also tested positive for citrate utilization, which is another characteristic of Pseudomonas spp. [23]. Isolate PS was tested gram positive and rod shaped bacteria. Based on biochemical features (bergey's manual), isolate PS could possibly be Bacillus or Paenibacillus. Isolate PS was tested positive for yellow green fluorescene, triple sugar agar test, catalase test, oxidase test, gelatine hydrolysis, succinate and malate utilization, mannitol and maltose fermentation, citrate test (Figure 1 (c) and (d), 2). But it was positive for indole test and urease test, which was a negative reaction for Pseudomonas spp (Figure 1(a)). confirming that PS is not belong to Pseudomonas spp. Result of biochemical assays was tabulated and color coding was followed to identify the extent of similarity in the test isolates with Pseudomonas genus (Table 1). A score card was generated in which maximum score referred to maximum similarity to genus Pseudomonas. It was observed that PB had maximum similarity to Pseudomonas genus with the score of 13. After PB, PM and PA scored 12. Isolate PS with least Pseudomonas match score 10 is a precise indicator that it is deviating from reported Pseudomonas genera characteristics.

Proline is a known osmoprotectant that helps plants in combating osmotic stress [24]. It was observed that PM produced maximum amount of proline - 0.42 µmol.g-1FW when compared to PB, PA and PS. It has been reported that organisms capable of producing exoploysaccharides (EPS) and biosurfactants can offer protection to plant from abiotic stress.EPS and biosurfactants are surface active agents that help in degradation of pollutants. EPS also assists in the formation of biofilm which accelerates pollutant degradation, protects resident microbes from desiccation and gaurdfrom other antagonistic bacteria [25, 26, 27]. Biosurfactant could also reduce the surface tension of pollutants and helps in achieving better degradation. Rhanmnolipids are such biosurfactants (observed as blue colour colonies on SW agar plates – Table 2) [28, 29]. In our study, isolates PM and PB showed greater level of exopolysacchride production i.e. 5mg/l and 4mg/l respectively as compared to PA and PS (Table 2). However, contrary to the reports where better EPS contributes to stonger biofilm formation, we have observed that culture isolate with strong EPS (PB) did not show biofilm formation. Biofilm formation was strong in PA with the appearance of blue colour ring, weak in PM (Figure 3). Culture isolate PS was found to be weak EPS producer with no biofilm formation. Presence of rhamnolipids was observed only in PB. Emulsification activity in any organism would assist in solubilizing complex chemicals like pesticides and alleviate plants from abiotic stress [30]. Isolates PM and PB had a higher emulsification index (6.897) than PA (5.172) and for PS it was zero (Table 2). All these properties could be instrumental in contributing to the ability of the isolates to offer stress tolerance under elevated pesticide concentrations.

We have investigated the ability of our isolates PB, PM, PA and PS to tolerate concentrations of phorate within the range 150-1050 ppm. In all the cultures, percentage growth reduction in the presence of pesticide became prominent beyond 150 ppm. At 300 ppm of phorate concentration, the percentage growth reduction of PM and PB were 39% and 22.93% respectively while it was 46.99% and 45.29% for PS and PA isolates respectively at the same concentration of pesticide. This showed PM and PB isolates were more tolerant to pesticide at higher concentration as compared to PA and PS. LD50 value for PM and PB were 981 ppm and 1249 ppm respectively which are higher than that of PS and PA (764 ppm and 658 ppm respectively). MIC values for all except PA were 1050 ppm; for PA it was 750 ppm (Table 3). In an earlier study by Rani et al., 2009, bacterial isolate Ralstonia eutropha was shown to utilize phorate as sole carbon source only till 20 ppm (20µg/ml) concentration [31].

While choosing isolates for remediation purposes, it is important to analyze how they behave in conjunction with the plant. Therefore, keeping in view the biochemical markers indicating pesticide tolerance limit and remediating properties, PM and PB were chosen for in vitro plant bioassay at concentration range of phorate 75 ppm-300 ppm because beyond 300 ppm the percentage growth reduction was drastic for all the isolated strains. Seeds inoculated with PM isolate showed much better percentage germination (92%) than the seeds without PM (88%) at 75 ppm of phorate concentration. The inoculated seeds with PM have shown very promising plant protection ability irrespective of increase in phorate concentration. As observed in table 4, even after increase in phorate concentration till 300 ppm, seeds coated with PM could record 96% germination which is 26.3% higher as compared with uninoculated control seeds which shows that PM isolate was able to alleviate the phorate stress and help the plant by providing support. In study conducted by Sahin et al., 2004, they have observed that with PGPM inoculation on sugar beet and barley there was increase in total yield [32]. Similarly even in seed vigor, seeds coated with PM are consistently better at all phorate concentrations. PM has shown constantly good performance

in terms of all parameters tested that are percentage germination, seed vigor and total biomass. There was no significant difference in percentage germination with and without inoculation with PB isolate at low concentrations of phorate (with PB – 96%, without PB – 100% at 75ppm phorate concentration) and even at high concentration similar trend was seen (with PB – 71%, without PB – 72% at 300ppm phorate concentration) (Table 5). Percentage germination of seeds is mostly a reflection of the inherent seed potential/quality and its affinity with the bioinoculant, because PM was improving percentage germination, it is an indication that mung seeds were preferring association with PM rather than PB. The isolates we are using are helpful rhizobacteria and thus either marginally improve percentage germination (as observed in PM) or leave it unaffected (as in observed in PB). Seed vigor is a factor that is greatly

Table 1 Biochemical characterization of isolated PGPM strains and their similarity to *Pseudomonas* genus.

S. No.	Biochemical tests	Reference Reaction	PA	PB	PM	PS	PJ
1	Gram Staining	N- Gram positive	N- Gram positive	N- Gram positive	N- Gram positive	P- Gram negative	N
2	Fluorescence Test	P	P++	P++++	P++++	P++	Nil
3	Triple Sugar Agar Test	N	N	N	N	N	H_2S Production
4	Citrate Utilization	P	P	P	P	P	Nil
5	Indole Production	N	N	N	N	P	Nil
6	Urease Production	N	N	N	P++	P++	Nil
7	Catalase Production	P	P	P	P	P	Nil
8	Starch Utilization	N	P+	P+	P+	P+	Nil
9	Methyl Red	P	N	N	N	N	Nil
10	Gelatin Hydrolysis	P	P	P	P	P	Nil
11	Maltose Fermentation	N	N	N	N	N	Nil
12	Mannitol Fermentation	N	N	N	N	N	Nil
13	Succinate Utilization	P	P	P	P	P	Nil
14	Malate Utilization	P	P	P	P	P	Nil
15	Oxidase	P	N	P	P+	P	Nil
	SCORE		12	13	12	-	

Positive and Agree	
Negative and Agree	
Positive and Disagree	
Negative and Disagree	
Weak positive/negative in agreement	
Weak positive/negative in disagreement	

Biochemical assays performed on isolated PGPM strains PA, PB, PM, PS and PJ. Reference reaction refers to the reaction *Pseudomonas* genus shows for the respective biochemical assays: P- Positive reaction, N- Negative reaction.

Table 2.Biochemical tests evaluating pesticide remediating properties of PGPM

Biochemical tests for remediation properties	PA	PB	PM	PS
Biofilm formation	N	N	P++	P+++
Emulsification index	5.172	6.897	6.897	Nil
Biosurfactant production	N	P	N	N
Proline concentration (µmol.g^{-1}FW)	0.153	0.133	0.42	0.188
Exopolysacchride production (mg/l)	1	4	5	1

Biochemical tests performed on isolated PGPM strains PA, PB, PM and PS.

Table 3 Percentage decrease in growth, LD_{50} and MIC of isolated PGPM strains under pesticide stress

	PM	PB	PS	PA
150ppm	12.53	-5.93	6.09	1.2
300ppm	39	22.93	46.99	45.29
450ppm	83.71	81.93	90.04	93.7
LD_{50}	981 ppm	1249 ppm	764 ppm	658 ppm
MIC	1050 ppm	1050 ppm	1050 ppm	750 ppm

MIC was the minimum concentration of phorate where no growth of PGPM isolates was seen.

Table 4 Plant bioassay to determine effect of inoculation by PM isolate

Phorate concentration (ppm)	% Germination without PGPM	% Germination with PGPM (PM)	Seed vigor index without PGPM	Seed vigor index with PGPM (PM)	Biomass without PGPM (g)	Biomass with PGPM (PM) (g)
75	88	92	744.48	916.32	0.653	0.693
150	84	96	774.48	880.32	0.598	0.69
225	76	96	612.56	827.52	0.607	0.697
300	76	96	576.08	823.68	0.616	0.695

Percentage germination, seed vigor and biomass of Mung seed with and without PM isolate inoculation.

Table 5 Plant bioassay to determine effect of inoculation by PB isolate

Phorate concentration (ppm)	% Germination without PGPM	% Germination with PGPM (PB)	Seed vigor index without PGPM	Seed vigor index with PGPM (PB)	Biomass without PGPM (g)	Biomass with PGPM (PB) (g)
75	100	96	200.00	236.53	0.602	0.593
150	62	69	161.20	183.33	0.72	0.647
225	58	58	113.42	140.78	0.661	0.689
300	72	71	113.04	65.77	0.64	0.651

Percentage germination, seed vigor and biomass of Mung seed with and without PB isolate inoculation.

influenced by external stress which is exposure to phorate in the present study. It is clear from the results that at higher concentration of pesticide, seed vigor index is significantly higher when treated with PM as compared to seeds challenged with pesticide and unprotected by PM (Table 4). In a study conducted by Lifstiz et al., 1987, they have observed a significant increase in root length of rapeseed after inoculation with Pseudomonas putida and in another study by Jaleel et al., 2007, they have shown increase in yield of Catharanthus roseus under abiotic stress conditions when they were inoculated with PGPM strain Pseudomonas fluorescens [33, 34]. In case of PB seed vigor showed improvement under PB inoculation, the improvement was not as high as in case of PM inoculation (Table 5). Significant biomass increase is observed in seeds treated with PM strain when compared with the untreated stressed seeds. No significant difference in biomass of seeds treated with PB and untreated seeds were observed. Considering significantly high performance of isolate PM inoculated with seeds, the author conclude that PM could be the most promising isolate to offer protection under phorate stress condition [35]. The current study was carried out for a short term. In vitro plant bioassay can be extended to the greenhouse level to correctly assess the effect of the isolate on leaves, fruits and flowers also.
(ref in btw)

CONCLUSION

Isolate PM showed characteristic positive result for Pseudomonas spp. and could tolerate high concentrations of phorate (abiotic stress). It demonstrated most assuring result for plant growth promotion under phorate toxicity. This exhibits that PM is the most capable isolate of all that could provide protection to plant under phorate stress and alleviate it. We would be further proceeding to molecular characteristics and gene identification for further

confirmation of the isolates which is not attempted as a part of this study.

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