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CARRIER DEVELOPMENT FOR PGPR-BIOFERTILIZER AND ITS EXPLOITATION UNDER CADMIUM AND LEAD STRESSED CONDITION

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ABSTRACT

Worldwide overpopulation needs progressive crop yield creating environmental pollution. Low cost and easily available biofertilizer development is the best alternative for a sustainable agricultural system. In this study different agricultural waste such as wheat bran, sesame bran and bagasse were used as a carrier for the isolated PGPR. Two most potent cadmium and lead tolerant PGPR were isolated from Indian agricultural soil and identified as *Lysinibacillus varians* strain KUBM17 (accession number MG976681) and *Pseudomonas putida* strain KUBM18 (accession number MG976684). PGPRs were inoculated in different possible combinations of carrier materials. The growth and survivability of PGPRs were checked with plate count technique frequently up to 120 days. The best carrier was selected for PGPR application on radish and gram plants to check their plant growth-promoting ability. Sesame bran showed higher CFU count (7.8×10^6/gm) for the PGPRs from the early days. Whereas, wheat bran and bagasse individually showed slower but sustainable nutrient availability for both the PGPRs. Wheat bran, sesame bran and bagasse in 1:1:1 ratio produced decent performance throughout the time period. The carrier mixture as biofertilizer showed higher plant biomass, germination percentage, chlorophyll content and other growth parameters on radish and gram plant under cadmium and lead stress. Carrier grown *Lysinibacillus varians* and *Pseudomonas putida* were able to nullify the toxicity of cadmium or lead and significantly ($p < 0.05$) enhanced almost all the plant growth parameters of both the plants. So, wheat bran, sesame bran and bagasse can be used for cheap PGPR-biofertilizer development.

Keywords: PGPR; Cd and Pb toxicity; carrier development; radish plant; gram plant

INTRODUCTION

Worldwide increasing population needs more and more crop yield for their existence. Industrialization and urbanization have posed to excessive land pollution especially with different heavy metals. Massive use of chemical fertilizer and pesticides to increase agricultural yield and to control pathogens are often effective but at the same time, causing adverse ecological, economic and social problems **(Choure and. Dubey, 2012).** Different agrochemicals, anthropogenic sources, urban waste and effluents from Ni-Cd battery, tannery and leather industry cause heavy metal contamination in agricultural system. In sustainable agricultural system bacterial fertilizers and bio-pesticides can be a good alternative for chemical fertilizer and chemical pesticides because fungal pathogens are prone to be chemical resistant easily **(Prikryl and Vancura, 1980).** On the other hand chemical compounds may be dangerous for other beneficial microorganisms. These bacteria that promote plant growth and yield are called as plant growth-promoting rhizobacteria (PGPR) **(Ningthoujam *et al.*, 2009).** Several materials were used as carrier for developing biofertilizer like peat soil, coal, FYM, compost, charcoal, lignite, talc, cellulose powder, bagasse, teak leaf meal, sedge peat, press mud, coconut shell powder, **(Tilak *et al.*, 1979)** fly ash etc. **(Samra *et al.*, 2003; Ajaya and Chhonkar, 2000).** Materials, which make available nutrients and habitable micro-pore to the microorganisms especially for bacteria, are generally used as carrier.

Biofertilizers are the carrier-based inoculants which contain microorganisms of various specific capabilities.The usage of carrier materials for the biofertilizer as microbial inoculants is beneficial to protect the bacteria and its effectiveness **(Fuentes-Ramirez and Caballero-Mellado, 2005; Ardakani *et al.*, 2010).** Among various types of carrier materials, peat is the most commonly used material due to its high moisture holding capacity and large surface area. Peat can support high number of microorganisms with long survivability. But it was reported to have toxic contaminants that affect the viability of bacteria **(Bashan, 1998).**

There are different agricultural bi-product such as rice hull (husk), sesame bran, bagasse, wheat bran etc. which are usually discarded after harvesting of the yeild or some time burned that are the main concern of air pollutionin India and other developing countries. The substrates rice husk, rice bran, wheat bran, maize bran, khesari bran, soybean bran, saw dust and mustard oilcake (MOC) were already studied for bio-fungicide production **(Faruk *et al.*, 2014).**

In this study, three different types of agricultural waste were considered as carrier substrate such as sesame bran, wheat bran and Bagasse **(Sugarcane waste).** Bagasse is a rich source of carbohydrates (sucrose, fructose etc.) that can be utilized as nutrients of different bacteria **(Dotaniya *et al.*, 2016).** Wheat bran contains almost all of the B-group vitamins: thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folates and different essential amino acids such as lysine, arginine glycine etc. **(Fardet, 2010)** with different carbohydrates. According to **Ravindran and Jaiswal, (2016),** by-products from different grains have high nutritional value. Sesame bran contains several minerals like potassium (4.6–5.3 g/kg), phosphorus (1.7–2.3 g/kg) magnesium (0.018–0.052 g/kg), calcium in high concentration (9.6–12.8 g/kg), amino acid like tryptophan and some carbohydrates **(Alyemeni *et al.*, 2011; Zouari *et al.*, 2016).**

In this study a proper carrier development for the isolated PGPR has been discussed. Moreover, carrier grown, Cd and Pb tolerant PGPRs were exploited on two plants such as radish and gram under heavy metal stressed condition.

MATERIALS AND METHODS

Collection of Bacteria

Two potent cadmium and lead tolerant plant growth promoting rhizobacteria were isolated from Indian agricultural soil by standard microbiological techniques. These bacteria were characterized and identified as *Lysinibacillus varians* (NCBI GenBank accession number MG976681) and *Pseudomonas putida* (NCBI GenBank accession number MG976684). Characterization and identification were previously published earlier **(Pal and Sengupta, 2019).**

Characterization of Bacteria

The collected Cd and lead tolerant PGPR were characterized with morphological and biochemical properties such as amylase, catalase, methyl red, citrate utilization, gelatine hydrolysis, indole production, urease and different carbohydrate utilization ability by standard protocol **(Pal and Sengupta, 2019).** Plant growth promoting abilities such as IAA production, Phosphate solubilization, ammonia production, HCN production, nitrogen-fixing ability and siderophore production were determined **(Pal and Sengupta, 2019).**

Carbohydrate utilization ability of PGPR

Carbohydrate utilization efficiency was determined by inoculation of isolated bacteria in synthetic broth medium (NH_4Cl 5.0 g; K_2HPO_4 3.0 g; Na_2SO_4 2.0 g; KH_2PO_4 1.0 g; NH_2NO_3 1.0 g; $MgSO_4$. $7H_2O$ 0.1 g; distilled water 1 lit, pH 7.0 ± 0.2) incorporated with anyone carbohydrate among Glucose, fructose, lactose, galactose, arabinose, cellobiose, dextrose, inositol, mannitol, maltose, raffinose and sucrose (2.0 mg). After 48 h of incubation growth was measured at 600 nm **(Pal and Sengupta, 2019).**

Carrier Formulation

Different agricultural waste such as wheat bran, sesame bran and bagasse were used for the preparation of carrier. Selected PGPRs were inoculated in different combinations of sterile carrier. The growth and survivability of PGPRs were checked with typical microbiological plate count technique subsequently after 60 and 120 days from the inoculation.
The different experimental setups for the carrier development were as follows:

1. WB +B1
2. SB+B1
3. Ba+B1
4. WB+SB+B1
5. SB+Ba+B1
6. WB+Ba+B1
7. WB+SB+Ba (1:1:1) +B1

1. WB+B2
2. SB+B2
3. Ba+B2
4. WB+SB+B2
5. SB+Ba+B2
6. WB+Ba+B2
7. WB+SB+Ba(1:1:1) +B2

N.B. WB- Wheat bran; SB- Sesame bran; Ba- Bagasse; B1- *Lysinibacillus varians*; B2- *Pseudomonas putida*

Exploitation of PGPR within carrier

Collection of Seeds

Radish (*Raphanus sativus*) and Gram (*Cicer arietinum*) seeds were collected from the agricultural seed house Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, W.B. India.

Preparation of Soil

Barren soil was collected, sieved and sterilized at 121^{O}C and 15 lb pressure for 1 hour for consecutive 3 days as performed previously by **Pal, Chakraborty, and Sengupta (2018).** Cadmium (10 ppm) and/or Lead (150 ppm) were determined on the basis of EC50 on seed germination, mixed with soil according to experimental design and left it for 1 week to stabilize.

Experimental Design

The complete experiment was conducted under controlled laboratory conditions. Meanwhile, bacterial inoculums were prepared with best and efficient carrier concentration. The treatment sets were as follows:

1. Control
2. Cadmium (Cd)
3. Cd+B1
4. Cd+B2
5. Cd+B1+B2
6. Lead (Pb)
7. Pb+B1
8. Pb+B2
9. Pb+B1+B2
10. Cd+ Pb
11. Cd+ Pb+B1
12. Cd+Pb+B2
13. Cd+ Pb+B1+B2

B1= KUBM 17 grown in carrier

B2= KUBM 18 grown in carrier

The healthy seeds were surface sterilized and placed in pot for germination and seedling growth. Respective bacterial inoculums prepared within the carrier were applied. Standard agricultural practices were taken and pots were displaced to minimize the position effects. pH of soil or carrier before PGPR inoculation and after 120 days of inoculation was measured by Jackson (1973).

In vitro plant growth promotion experiment

Germination percentage was calculated after 7 days of potting. Root length (cm), Shoot length (cm.), Fresh weight (gm.), Dry weight (gm.), Vigor Index **(Abdul-Baki and Anderson, 1973),** Relative water content and Chlorophyll content (mg/gm of tissue) **(Porra *et al.* 1989)** were recorded after 45 days of growth.
Vigour index = Germination (%) x Total seedling length (cm.)

Relative water content (%) = (FW-DW/TW-DW) x 100

Where FW = Fresh weight
DW = Dry weight
TW = Turgid weight

Statistical analysis

Standard error (SE) of all experimental data was anticipated from triplicates (n = 3) and represented in the respective table in 'value ± SE' format. Differences between the experimental groups were intended by unpaired t-test. Difference between control and respective heavy metal treated groups were denoted by lower case alphabet 'a'. Lower case alphabet 'b' indicates differences between heavy metal-treated groups and carrier grown PGPR inoculated heavy metal-treated groups. Asterisk mark (*) indicates a significant level.

RESULTS AND DISCUSSION

Two potent cadmium and lead tolerant PGPRs were isolated, characterized and identified by the authors. Biochemical characterization, carbohydrate utilization ability and plant growth promoting ability are being discussed in Table 1.

For application of *Lysinibacillus varians* and *Pseudomonas putida* in agricultural field in commercial basis it need to develop a cheap and easily available carrier. WB, SB or Ba showed good CFU (colony forming unit) count of the PGPRs but different combinations of carriers showed very promising outcome (Table 2). WB and Ba individually gave slower but long term nutrient availability for both the PGPRs, whereas, the PGPRs utilized SB vigorously from the initial stage and showed highest CFU count at 60 days. In this study it revealed that the survival ability of *Lysinibacillus varians* and *Pseudomonas putida* were not just maintained but also increased in a 1:1 mixture of WB+SB for at least 120 days. SB complimenting the nutrient availability of WB or Ba for the CFU counts from 60days to at least 120days. Moreover, WB+SB+Ba in 1:1:1 ratio represented a descent survivability of *Lysinibacillus varians* and *Pseudomonas putida* throughout the time period. On the basis of that result WB+SB+Ba can be commercially applied by the farmers as a carrier composition for biofertilizer production.

In vitro plant growth promotion experiment by carrier grown PGPR strains on radish and gram plant under cadmium and lead stress.

Application of cadmium and lead tolerant PGPRs as biofertilizers developed by using the best proportion of different carrier showed very promising results during the plant growth experiments. Heavy metal hyper-accumulator Radish and non-hyper-accumulator gram plant showed similar plant growth promotion induced by *Lysinibacillus varians* and *Pseudomonas putida* under cadmium and lead stress condition. Different plant growth parameter such as germination percentage, root length, shoot length, fresh weight, dry weight, chlorophyll content, vigor index and turgid weight for radish and gram plant are depicted in Table no 3 and 4 respectively. Cadmium, lead or combination of cadmium and lead significantly ($p < 0.05$) reduced germination percentage, root length, shoot length, fresh weight, dry weight, chlorophyll content, vigor index and turgid weight in both radish and gram plant. Consortium of *Lysinibacillus varians* and *Pseudomonas putida* within developed carrier (WB+SB+ Ba) reduced the adverse effects of heavy metals and different growth parameters were increased significantly ($p < 0.05$). Radish and gram plant showed promising growth enhancement with PGPR application than the non-inoculated Cd and/or Pb treated plants. As the developed carrier were applied in all the setups including control pots and the pots were interchanged in their positions, it can be assume that nutrients within the carrier and position effect are negligible among the treatments. While, the nutrients within the carrier material can be as attributed to extra benefit for the plant growth enhancement in the agricultural field.

Table 1 Colony morphology, Gram nature, Biochemical characterization and PGP ability of the Bacterial isolates

Bacterial isolates	Minimal Inhibitory Concentration of cadmium and lead	Colony Morphology & Gram nature	Biochemical characterization	Carbohydrate utilization ability	Plant growth promoting ability
L. varians	150 ppm (Cd) 450ppm (Pb)	Creamy yellow, circular with serrated margins, opaque with a glossy surface, Gram-positive, rod	Catalase, methyl red, citrate utilization tests positive and amylase, gelatin hydrolysis, indole production, urease tests negative	Glucose, Fructose, Arabinose, Sucrose, Inositol, Manitol, Maltose	ammonia production, IAA production (20.43 ± 0.318 µg/ml), Nitrogen-fixing ability, low phosphate solubilization and low siderophore production positive
P. putida	150 ppm (Cd) 500ppm (Pb)	Creamy white, circular entire margin with a glossy surface, Gram-negative rod	Catalase, methyl red, citrate utilization positive and amylase, gelatine hydrolysis indole production, urease test negative	Glucose, Fructose, Arabinose, Sucrose, Inositol, Manitol, Maltose	phosphate solubilisation, ammonia production, IAA production (20.05 ± 0.266 µg/ml), low siderophore production

Table 2 Survivility of PGPR for different carrier development

Different carrier [a]	Bacterial count (CFU/gm of carrier) [b]			
	KUBM 17		KUBM 18	
	60 days	120 days	60 days	120 days
WB	$0.56x10^6$	$15x10^9$	$0.92 x10^6$	$19.9 x10^9$
SB	$7.8 x10^6$	$9.7 x10^9$	$13.52 x10^6$	$10 x10^9$
Ba	$0.24 x10^6$	$11.8 x10^9$	$0.13 x10^6$	$11 x10^9$
WB+SB	$7.13 x10^6$	$12 x10^9$	$8.96 x10^6$	$11.9 x10^9$
SB+Ba	$8.21 x10^6$	$7.86 x10^9$	$14 x10^6$	$10.8 x10^9$
WB+Ba	$8.44 x10^6$	$12.5 x10^9$	$1.92 x10^6$	$19.98 x10^9$
WB+SB+Ba	$7.6x10^6$	$13.1x10^9$	$9.4x10^6$	$12.8x10^9$

[a] Different carrier was inoculated with broth culture of respective bacterial isolates (WB= Wheat bran, SB= Sesame bran, Ba= Bagassi)
[b] Initial bacterial count was ~10^2 CFU/gm of carrier.

Table 3 In vitro plant growth promotion experiments by carrier grown PGPR strain on radish plant under cadmium and lead stress.

Treatment	Germination percentage %	Root length (cm)	Shoot length (cm)	Fresh weight (gm)	Dry weight (gm)	Chl (a+b)	Vigor index	Turgid weight (gm)
Control	83.33±0.0001	4.94±0.508	5.7±0.634	0.44±0.092	0.03±0.0061	38.04 ±0.577	474.98	0.4 ±0.021
Cd	87.5±2.78 a**	3.4±0.277 a*	4.8±0.428 a*	0.26±0.049 a**	0.02±0.0033 NS	30.25 ±0.203 a***	420 a*	0.24 ±0.022 a***
Cd+ L. varians	94.64±2.06 b***	6.43±0.262 b***	6±0.311 b**	0.59±0.068 b***	0.04±0.0061 b**	37.03 ±0.289 b***	605.7 b***	0.5 ±0.012 b***
Cd+ P. putida	91.67±2.06 b***	5.08±0.383b**	6.11±0.495 b**	0.4±0.141 b**	0.02±0.0043 NS	32.87 ±0.432 b***	559.17 b***	0.36 ±0.018 b**
Cd+ L. varians+ P. putida	96.43±2.38 b***	6.25±0.47 b***	6.38±0.55 b**	1.06±0.222 b***	0.06±0.0104 b***	37.76 ±0.307 b***	617.15 b***	0.83 ±0.032 b***
Pb	55±5.02 a***	4.01±0.404 a*	4.35±0.379 b**	0.31±0.055 a*	0.03±0.0037 NS	24.94 ±0.58 a***	330 a***	0.18 ±0.014 a***
Pb+ L. varians	64.29±0.0001 b***	4.95±0.289 b	5.35±0.148 b*	0.82±0.156 b***	0.05±0.0077 b**	27.69 ±0.61 b*	447.16 b***	0.55 ±0.028 b***
Pb+ P. putida	66.86±7.04 b***	5.32±0.339 b*	4.88±0.33 NS	0.61±0.104 b**	0.03±0.0046 NS	35.56 ±0.27 b***	427.61 b***	0.35 ±0.004 b***
Pb+ L. varians+ P. putida	76.78±7.8 b***	5.96±0.657 b**	6.66±0.0237 b***	0.91±0.124 b***	0.09±0.0156 b***	33.83±0.81 b***	514.43 b***	0.73 ±0.035 b***
Cd+Pb	50±3.92 a***	3.02±0.25 a*	5.11±0.589 NS	0.46±0.049 NS	0.01±0.0046 a*	19.96 ±0.244 a***	260 a***	0.11 ±0.008 a***
Cd+Pb+ L. varians	57.14±0.0001 b**	5.55±0.409 b***	6.2±0.433 b*	0.72±0.077 b**	0.04±0.0023 NS	26.25 ±0.91 b***	388.55 b***	0.43 ±0.017 b***
Cd+Pb+ P. putida	66.07±2.06 b***	5.01±0.512 b**	5.45±0.286 NS	0.55±0.063 b*	0.03±0.0023 NS	25.09 ± 0.56 b***	324.12 b***	0.33 ±0.024 b***
Cd+Pb+ L. varians +P. putida	58.93±8.5 b**	7.66±0.795 b***	6.76±0.387 b**	0.82±0.104 b***	0.06±0.0086 NS	25.72 ±0.399 b***	429.45 b***	0.62 ±0.024 b***

N.B. Difference between control and respective heavy metal-treated groups are denoted by lower case alphabet 'a'. Lower case alphabet 'b' indicates difference between heavy metal-treated groups and PGPR inoculated respective heavy metal-treated groups. Asteric mark (*) indicate significance level. 'NS' means not significant.

Table 4 In vitro plant growth promotion experiments by carrier grown PGPR strain on gram plant under cadmium and lead stress.

Treatment	Germination percentage (%)	Root length (cm)	Shoot length (cm)	Fresh weight (gm)	Dry weight (gm)	Chl (a+b)	Vigor index	Turgid weight (gm)
Control	74±1.095	6.9±0.463	35.23±0.884	1.02±0.108	0.25±0.176	38.04 ± 0.5	1850	0.42 ±0.012
Cd	54±0.57 a***	5.5±0.294 a**	25.23±0.884 a***	0.74±0.142 a*	0.12±0.08 a**	25.09 ±0.5 a***	1035.18 a***	0.23 ±0.031 a***
Cd+ L. varians	90±0.547 b***	7.26±0.762 b***	29.66±2.245 b**	1.17±0.06 b**	0.29±0.01 b**	27.03 ±0.3 b**	2670 b***	0.41 ±0.014 b***
Cd+ P. putida	68±1.341 b**	6±0.935 b*	27±0.707 NS	1.04±0.89 b*	0.17±0.08 NS	32..87±0.21 b***	1896 NS	0.36 ±0.033 b**
Cd+ L. varians+ P. putida	92±0.758 b***	7.76±0.601 b***	29.56±1.493 b**	1.16±0.1 b**	0.36±0.01 b***	44.94± 0.18 b***	2720 b***	0.45 ±0.008 b***
Pb	60±0.707 a*	5.8±0.681 a**	29.3±4.893 a**	0.89±0.089 NS	0.11±0.01 a**	15.72±0.24 a***	1758 NS	0.33 ±0.026 a**
Pb+ L. varians	82±0.414 b***	7.3±0.696 b**	38.46±1.728 b**	1.26±0.08 b*	0.16±0.01 NS	19.96 ±0.51b***	3154.54 b***	0.49 ±0.057 b***
Pb+ P. putida	70±0.866 b**	6.1±0.308 NS	35.76±4.745 b**	1.24±0.112 b*	0.15±0.02 NS	19.25±0.41 b***	2503.9 b***	0.43 ±0.069 b**
Pb+ L. varians+ P. putida	88±0.651 b***	7.66±0.756 b***	41.26±1.995 b***	1.36±0.052 b**	0.21±0.028 b**	33.83 ± 0.72 b***	3631.76 b***	0.63 ±0.088 b***
Cd+Pb	46±1.036 a***	4.7±0.389 a***	14.6±1.384 a***	0.51±0.103 a**	0.1± 0.03 a**	30.76 ±0.37 a***	668.38 a***	0.17 ±0.035 a***
Cd+Pb+ L. varians	78±0.821 b***	7.03±0.842 b***	38.48±1.751 b***	1.22±0.171 b**	0.15±0.004 b*	25.69±0.085 b***	3000 b***	0.42 ±0.074 b***
Cd+Pb+ P. putida	64±0.758 b***	6.93±0.204 b***	35.33±3.048 b***	1.07±0.031 b**	0.12±0.008 NS	35.56± 0.49 b***	2261 b***	0.38 ±0.064 b***
Cd+Pb+ L. varians +P. putida	92±0.651 b***	7.5±0.553 b***	43±0.612 b***	1.27±0.032 b**	0.17±0.012 b**	39.25± 0.91 b***	3956 b***	0.49 ±0.048 b***

N.B.- Difference between control and respective heavy metal-treated groups are denoted by lower case alphabet 'a'. Lower case alphabet 'b' indicates difference between heavy metal-treated groups and PGPR inoculated respective heavy metal-treated groups. Asteric mark (*) indicate significance level. 'NS' means not significant.

The above result indicating that the effect of heavy metal stress has been mitigated by the application of PGPR along with the carrier for a sustainable life span of experimental plants. **Rajkumar and Freitas (2008)** reported that the plant growth parameters were increased by the inoculation of PGPR as compared with non-inoculated plant in the heavy-metal stress condition. Whereas, different heavy metal like Cd, Pb, Ni etc remarkably hampered different growth parameters of different plants **(Pal, Chakraborty, and Sengupta 2018; Pal, Mandal, and Sengupta 2019; Pramanik *et al*. 2016; Pal and Sengupta, 2019). Huang *et al*., (2016); Gholami *et al*., (2009)** also reported that the application of PGPR increased germination percentage under stressed condition. Both radish and gram plant showed increased chlorophyll content under Cd and/or Pb stress condition with PGPR inoculation through carrier than the non-inoculated heavy metal treated condition. **Barnawal *et.al*, (2008)** observed similar type of enhancement in chlorophyll content.

PGPRs not only survived within the carrier but also were able to express their potentiality to increase plant growth parameters for a long time. So, the developed carrier can be used for the application of different PGPRs on a large scale for agricultural development.

Cd , Pb and Cd+Pb significantly ($p < 0.05$) reduced the vigour index of radish plant by 11.39%, 30.37%, 45.14% and 44.05%, 4.97%, 63.89% for gram plant. Application of PGPR under heavy-metal stress enhanced the vigour index of radish plant by 29.93%, 8.30%, 3.04% and for the Gram plant by 47.02%, 96.27%, 113.83% respectively for *L. varians*, *P. putida* and *L. varians+ P. putida* inoculation as compared with non-inoculated Cadmium treated plants. Similar type of results was noted in other heavy metal combinations. The selected PGPRs applied with developed carrier mixture had a great effect to nullify the adverse effects of heavy metals. The results in this study confirmed the previous results obtained earlier **(Aydinalp and Marinova 2009; Pal, Chakraborty, and Sengupta 2018; Pramanik *et al*. 2016; Rajkumar and Freitas 2008).** Moreover, it can be concluded that, the PGPRs within the developed carrier in this study established similar plant growth enhancement in comparison with PGPRs applied with culture media or as water suspension by the previous workers.

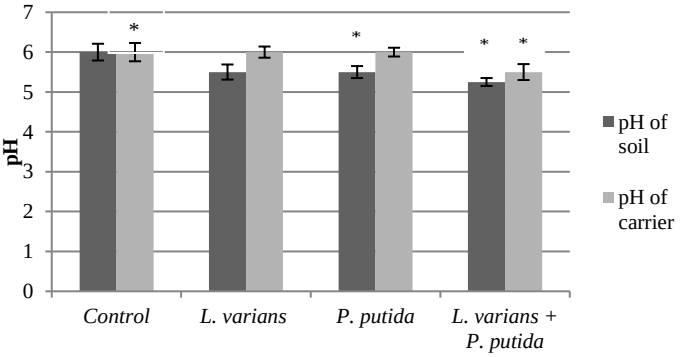

Figure 1 PGPR mediated pH change of soil and carrier.
N.B. Asteric mark above bar (*) indicates significance level between control and respective treatment.

Lysinibacillus varians or *Pseudomonas putida* inoculation reduced pH level by 8.3% than the control soil. Whereas, PGPRs in combination showed 12.5% reduction of pH. Though, PGPR inoculation in carrier showed almost similar type of pH (Figure 1). Lower pH means increased acidity. Some authors showed that PGPR can produce several kind of organic acid which solubilized insoluble phosphates or potassium. The significant decrease of soil pH by Plant growth-promoting rhizobacteria (PGPR) reported by **Stevenson (2005).**

CONCLUSSION

In the present study two Cadmium and Lead tolerant strain were explored with suitable carrier development. Plant growth promoting ability of *Lysinibacillus varians* or *Pseudomonas putida* was previously reported by the authors and in this study they gave similar result while they were grown in a mixture of Wheat Bran, Sesame Bran and Bagasse in 1:1:1 ratio. The carrier ratio was able to provide a good nutrient source for at least 120 days for the PGPRs and showed exceptional CFU count. *Lysinibacillus varians* strain KUBM17 (accession number MG976681) and *Pseudomonas putida* strain KUBM18 (accession number MG976684) was not only survived within the carrier but also significantly ($p < 0.05$) enhanced the plant growth of both radish and gram under cadmium (Cd) and lead (Pb) stress condition throughout their life span. Moreover, the agricultural wastes like Wheat Bran, Sesame Bran and Bagasse can be used for the formulation of a new generation low cost carrier as it contain a good source of carbohydrate and other essential elements for microbial growth. The present study revealed that PGPR could be applied by the carrier as effective bio-fertilizer in almost all soil ecological condition to develop a sustainable agricultural system for the elimination of phyto-toxic effects of cadmium and lead with very little expertise as well as by the farmers themselves.

Author's contribution: AKP conducted the whole experiment, collected the data, performed statistical analysis and written up the whole manuscript. BS supported the experimental works for plant growth parameters. CS hypothesized the paper concept, designed the experiment, supervised throughout the process.

Competing interests: The authors declared that they have no competing interest.

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