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Research Paper

Agroindustry By-Product Based Formulations of *Burkholderia gladioli* for Plant Growth

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Despite adequate density of desired microorganisms in the formulation, it is essential to demonstrate plant growth promotion potential of the formulation. Therefore, the work described here focuses on evaluation of paddy husk and press mud based formulations of *Burkholderia gladioli* for the plant growth viz., maize, wheat, jowar and bajra which was studied by the pot experiment. The carrier based formulations showed increase in the germination and vigor index of maize, wheat, jowar and bajra. Significant increase in the plant growth viz., root length, shoot length and dry weight with paddy husk and press mud based formulations of *Burkholderia gladioli* was observed. The use of paddy husk and press mud based formulations of *Burkholderia gladioli* for plant growth promotion will be a new herald in blossoming of agriculture and soil biotechnology. This will be very eco-friendly, non-toxic, cheap and also reduce the use of chemical fertilizers.

Keywords: Agroindustry by-product; Eco-friendly; Renewable; Formulation; Vigor index; Agriculture

Introduction

Carrier Based Formulations for The Plant Growth

In order to protect the environment and make nutrients available to plants, use of biofertilizers for the farm under cultivation is necessary. Biofertilizers settle on the root after addition to the seed, plant surface and soil and improve the plant growth and increase product yield by increasing availability of nutrients (Woyessa and Assefa, 2011; Gandhi and Sivakumar 2010; Vessey, 2003).

There are reports where carrier based formulations of plant growth promoting rhizobacteria (PGPR) have shown increase in plant growth and yield. Talc based formulations of *Pseudomonas fluorescens*, (Kloepper and Scroth 1981; de Freitas and Germida, 1992; Vidhyasekaran and Muthamilan, 1995; Vidhyasekaran *et al.,* 1997; Viswanathan and Samiyappan, 1999) *B. subtilis* and *Pseudomonas chlororaphis* (PA23) (Kavitha *et al.,* 2003); vermiculite based formulations of *Pseudomonas fluorescens* (Moenne-Loccoz *et al.,* 1999) and *B. subtilis* (Amer and Utkhede, 2000); chitin based formulations of *B. subtilis* strain GB03 + *B. pumilus* strain INR7 (LS256) and *B. subtilis* strain GB03 + *B. subtilis* strain IN937b (Kokalis-Burelle *et al.,* 2002) have shown increase in the plant growth.

Due to their water-soluble nature, the chemical fertilizers find their way into natural water bodies. As a result, there is eutrophication of water bodies and nitrate poisoning. The soils lose their porosity and become saline.

Agroindustry by-product based formulations viz., paddy husk and press mud for the plant growth are very eco-friendly; cheap; non-toxic and non-polluting. These agroindustry by-products are lignocellulosic in nature and also available in very large amount. Rice milling industry processes about 85 million tons of paddy per year. Similarly, in Maharashtra about 4 million tons of press mud is produced as a by-product from the sugar industries.

There are several reports on study of survival of *P. fluorescens* and *Bacillus* sp. in carriers' viz., lignite, peat, vermiculite, farm yard manure (Amer and Utkhede, 2000; Vidhyasekaran and Muthamilan,

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1995). Therefore, *B. gladioli* was selected here for the study.

Till date there are very few reports on paddy husk and press mud based formulations for the plant growth promotion (Amer and Utkhede, 2000; Vidhyasekaran and Muthamilan, 1995). The paper focuses on paddy husk and press mud based formulations of *Burkholderia gladioli* for the plant growth of maize, wheat, jowar and bajra.

Materials and Methods

Sampling

The root and soil samples of maize (*Zea mays*) were collected from a field at Uralikanchan, Pune, India for the isolation of PGPR. The samples were stored properly in the sterile plastic bags, labeled and stored at low temperature until use.

Enrichment of PGPR

The semisolid LGI containing following composition (g/l) (K_2HPO_4 0.2, KH_2PO_4 0.6, $MgSO_4 \cdot 7H_2O$ 0.2, $CaCl_2 \cdot 2H_2O$ 0.04, sucrose 12, $Na_2MoO4 \cdot 2H_2O$ 0.02, $FeCl_3 \cdot 6H_2O$ 0.01, bromothymol blue 5 ml, pH 6.0 and agar 30) and Jenson's nitrogen free (JNF) media containing following composition were used (g/l) (sucrose 20, K_2HPO_4 0.6, KH_2PO_4 1.8, $MgSO_4 \cdot 7H_2O$ 0.2, $CaCl_2 \cdot 2H_2O$ 0.2, NaCl 0.1, Fe-EDTA 0.06, KOH 4.5, $FeCl_3 \cdot 6H_2O$ 0.01, pH 5.0 and agar 30) for the enrichment of the PGPR. The maize roots were washed with tap water and disinfected with 70% ethanol for 30 sec followed by washing with sterile distilled water (DW). They were again disinfected with 0.1% mercuric chloride ($HgCl_2$) for 1 min. The root sample (1 g) was homogenized using a blender in 9 ml sterile saline and 1 g of rhizosphere soil sample was suspended in 9 ml sterile saline. The supernatant of both the samples was serially diluted in sterile saline. An aliquot (0.1 ml) of each dilution was inoculated separately into 10 ml semisolid LGI and JNF media, incubated at 28ºC for 3 days. The tubes showing formation of thick yellow pellicles were considered as presumptive PGPR.

Isolation, Purification and Maintenance of the Isolate

A loopful of growth from each positive tube was streaked on respective solid medium and incubated at 28ºC for 3 days. Typical colonies were subcultured on respective medium to purify the isolates. The pure cultures were maintained on Glucose Yeast Extract Calcium Carbonate (GYC) containing following composition (g/l) (D-glucose 50, yeast extract 10, $CaCO_3$ 30 and agar 30) slants at 4ºC, respectively.

Characterization and Identification of PGPR

The isolates were subjected to morphological, physiological and biochemical characterization according to the Bergey's Manual of Determinative Bacteriology (1992). The biochemical tests performed were indole production, Voges-Proskauer, citrate utilization, oxidase, catalase, gelatinase, hydrogen sulphide (Vashist *et al.,* 2013), urease production (Schaad, 2001) and oxidative-fermentative (Minana-Galbis *et al*., 2002). The isolate was confirmed by 16S rRNA sequencing.

Plant Growth Promoting Traits of B. gladioli

The initial studies of the plant growth promoting traits of *B. gladioli* have been carried out (Gunjal and Kapadnis, 2013). The activity against the fungal phytopathogens was also studied by the dual culture method. The effect of bacterization on the seed germination, plant growth and vigor index was also performed (Gunjal and Kapadnis, 2013).

Carrier Based Formulations for the Plant Growth

Preparation of the Inoculum

The plant growth promoting rhizobacteria, *Burkholderia gladioli* was selected for developing carrier based formulations. *B. gladioli* was grown in 100 ml GYC broth in 250 ml conical flasks on a shaker at 28ºC (165 rpm) for 3 days. The initial density was 2.0×10^5 cfu/g, respectively.

Selection and Disinfection of the Seeds

The seeds viz., maize, wheat, jowar and bajra were selected. The seeds were disinfected with 95% ethanol for 5 min and then by 0.2% $HgCl_2$ for 3 min. The disinfected seeds were further washed 5 times with sterile DW to remove any traces of the $HgCl_2$ (Sachdev *et al.,* 2009).

Sources of Agroindustry By-Products

The agroindustry by-products, paddy husk was purchased from Surve rice mill located at Karad, Maharashtra and press mud from Theur Sugar Factory, Pune, Maharashtra (Fig.1A and 1B).

Processing of the Agroindustry By-Products

The agroindustry by-products viz., paddy husk and press mud were finely powdered using the mixer. One kg of each material was passed through a sieve of mesh size of 72 µm and packed in polythene bags (bag size: 100 x 150 mm) and used for the study.

Preparation of Paddy Husk and Press Mud Based Formulations

A quantity of 50 g each of the powdered agro-industry by-products were sterilized twice in 250 ml conical flask in triplicate in an autoclave at 121°C, 15 min for 3 days, dispensed in 250 ml sterile DW, mixed with the carriers uniformly and inoculated with 10 ml of *B. gladioli* culture.

Slurry Preparation and Bacterization of Seeds with Carrier Based Formulations

The carrier based formulations (10 g) were mixed with 0.25 g $CaCO_3$ to neutralize the pH and 0.50 g carboxy methyl cellulose (CMC) as an adhesive. Ten disinfected seeds each of maize, wheat, jowar and bajra were suspended in the resultant slurry and the seeds were pelleted for 5 min.

Pot Experiment for Evaluation of the Carrier Based Formulations

The pot experiment was carried using the garden soil which was sterilized in an autoclave at 121°C, 15 min for 3 consecutive days. The plastic pots (size: 18 x 15 cm) were disinfected with 95% ethanol and filled with 1.8 kg sterilized garden soil. The seeds without any treatment served as control. The treatments were randomized to eliminate bias. The treated seeds were sown at equal distance (depth 1 cm) in the plastic pots. The pots were kept in the sunlight and the seedlings were watered daily. After 25 days of sowing, the plants were carefully uprooted and studied for the following plant growth parameters viz., seed germination, vigor index, root length, shoot length and total dry weight.

Statistical Analysis

The data was statistically analyzed to see if the treatment has significant effect on plant growth (Significance level = 0.05) using the Microsoft Excel software.

Fig. 1A: Agroindustry by-product paddy husk

Fig. 1B: Agroindustry by-product press mud

Table 1: Effect of paddy husk based formulation of *B. gladioli* on seeds and plant growth

Plants	Avg root length (cm)		Avg shoot length (cm)		Dry weight (mg/plant)		Germination (%)		Vigor index	
	C	T	C	T	C	T	C	T	C	T
maize	3.25±2.50	3.50± 2.73(0.44)^{c}	3.75±1.03	5.00±1.18 (0.27)^{c}	3.93±0.82	2.23±0.98 (0.04)^{a*}	90±1.41	80±0.00	630.00	680.00
wheat	1.60±0.89	2.00±0.63 (0.20)^{c}	2.60±1.14	3.67±2.65 (0.21)^{c}	2.00±0.93	3.17±1.47 (0.07)^{ab}	80±0.00	90±0.00	336.00	510.30
jowar	2.40±1.14	4.00±2.60	2.40±1.14 (0.11)^{c}	3.00±1.41	2.96±2.25 (0.23)^{c}	2.30±1.31	70±0.00 (0.27)^{c*}	100±0.00	336.00	700.00
bajra	2.50±0.70	3.20±2.16 (0.27)^{c}	2.50±0.93	2.00±1.22 (0.50)^{c*}	2.68±1.24	3.96±0.53 (0.005)^{a}	80±0.00	85±0.70	400.00	700.00

C: Control, T: Test

Results and Discussion

Characterization and Identification of the PGPR

The isolate was motile, Gram negative, optimum salt concentration was 2.50%, pH 5.0-7.0 and temperature 37^{o}C for the growth of the isolate. The isolate did not show hydrogen sulphide (H_2S) and indole production. It was oxidative, showed citrate utilization and positive for Voges-Proskauer (VP) test. The isolate was oxidase and catalase positive and could liquefy gelatin and also showed urea degradation. The 16S rRNA sequencing confirmed the isolate as *Burkholderia gladioli* with 92% similarity (Accession Number: AY297695.1).

Plant Growth Promoting Properties

The plant growth promoting properties were shown by *B. gladioli*. It was found to solubilize phosphorus with solubilization index 2.42 ± 0.02, produced indole acetic acid (IAA) (6.48 ± 0.01 µg/ml), gibberellins (5.57 ± 0.03 µg/ml) and also exopolysaccharide (EPS) (0.98 ± 0.00 g). *B. gladioli* also showed antifungal activity against *Fusarium oxysporum*, *Sclerotium rolfsii* and *Alternaria solani* with % inhibition 15.84, 17.40 and 65.03, respectively (Gunjal and Kapadnis, 2013). The antifungal activity against the fungal phytopathogens indicated strong biocontrol potential. Bacterization of seeds with *B. gladioli* improved the growth of maize, wheat, jowar and bajra plants (Gunjal and Kapadnis, 2013). Bacterization of seeds with *B. gladioli* enhanced the root length of maize and jowar plants by 48.71 and 65.22%, respectively and shoot length of wheat and bajra by 29.41 and 6.25%, respectively (Gunjal and Kapadnis, 2013).

Effect of Paddy Husk Formulation of B. gladioli on the Seeds and Growth

Increase in the vigor index was found by paddy husk formulation of *B. gladioli* of maize plant (Table 1). The seed germination and vigor index of wheat, jowar and bajra plants were increased by paddy husk formulation of *B. gladioli* (Table 1). Significant increase in the dry weight of bajra plant was found by paddy husk formulation of *B. gladioli* (Table 1). The weak significant increase in dry weight of wheat plant was also observed.

Effect of Press Mud Formulation of B. gladioli on the Seeds and Growth

The increase in vigor index of maize plant was found by press mud formulation of *B. gladioli* (Table 2). Significant increase in the root length of maize, wheat and bajra plants by press mud formulation of *B. gladioli* was found (Table 2). The seed germination and vigor index of wheat, jowar and bajra plants were also increased by press mud formulation of *B. gladioli*.

Roy *et al*. (2010) showed vermiculite based inoculant of *Azotobacter chroococcum* significantly improved the plant height, nitrogen uptake, dry matter production, grain yield, protein and oil content by 48, 45, 44, 43, 33 and 33%, respectively of summer (ahu) rice *cv*. IR-36 in acidic soils under water logged conditions of South Assam. Vermiculite carrier based formulations of fluorescent *Pseudomonas* strains R62 and R81 has also shown significant increase in the dry root and shoot weight, shoot length, no. of branches per plant of *Vigna mungo* and increase in

Table 2: Effect of press mud based formulation of *B. gladioli* on seeds and plant growth

Plants	Avg root length (cm)		Avg shoot length (cm)		Dry weight (mg/plant)		Germination (%)		Vigor index	
	C	T	C	T	C	T	C	T	C	T
Plants	Avg root length (cm)		Avg shoot length (cm)		Dry weight (mg/plant)		Germination(%)		Vigor index	
	C	T	C	T	C	T	C	T	C	T
maize	2.60±1.94	5.25±0.95 (0.02)[a]	3.20±1.30	4.00±2.70 (0.28)[c]	3.58±1.79	3.12±2.17 (0.37)[c*]	75±0.70	75±0.70	435.00	693.75
wheat	2.83±1.46	6.25±0.95 (0.001)[a]	3.00±1.67	2.75±1.50 (0.40)[c*]	2.50±1.41	1.87±1.02 (0.23)[c*]	70±0.00	100±0.00	408.10	900.00
jowar	2.25±1.18	3.33±1.52 (0.16)[c]	3.25±2.25	5.33±2.08 (0.13)[c]	3.50±2.12	1.83±0.28 (0.12)[c*]	60±0.00	90±0.00	330.00	779.40
bajra	2.50±0.91	3.67±0.57 (0.05)[a]	3.00±1.46	2.66±1.15 (0.38)[c*]	2.75±1.03	3.41±1.23 (0.23)[c]	60±0.00	85±0.70	330.00	538.05

C: Control, T: Test

*Vigor index = (mean root length + mean shoot length) x % germination (Abdul-Bakki and Anderson, 1973). Each data point represents average of triplicate ± s.d.

Figures in parenthesis, *P* value of t-test. [a]Significant increase; [ab]weakly significant increase; [c]no significant increase. [a*]Significant decrease; [ab*]weakly significant decrease; [c*]no significant decrease. Effects were significant if $P \leq 0.05$; weakly significant if $0.05 < P \leq 0.1$ and not significant if $P > 0.1$.

the pods yield by 300% through field trials (Sarma *et al.,* 2009). Fly-ash based formulation of *A. chroococcum* showed increase in % seed germination, plant height and plant biomass of wheat plant (Kumar and Gupta, 2010). Studies have been done which showed high increase in the root length, dry matter and phosphorus uptake of the maize plant by the carrier material prepared using lignite enriched with 1% soybean powder (SP) + 2% Mussoorie rock phosphate (MRP) (Menaka and Alagawadi, 2007). There are reports on enhanced growth and dry matter content of different crop plants due to amendment of carrier materials (Sharma and Verma, 1979; Tilak and Subba Rao, 1978; Mudenoor, 2002). Application of vermicompost carrier based formulation of phosphate solubilizing bacteria (*P. fluorescens*) has shown increase in growth of maize plant (Shariati *et al.,* 2013). Perz-Murcia *et al*. (2006) also reported significant increase in shoot phosphorus content of cucumber, tomatoes and strawberries due to vermicompost carrier based formulation of PSB.

Kanawade and Kapadnis (2002) have reported use of whey for propagation of actinomycetes with plant growth promoting potential and also yield improvement of groundnut with actinomycetes and *Pseudomonads* mixed bioinocula through bioconversion of whey.

Islam *et al*. (2006) evaluated the effect of carrier based formulation inoculated with *Bradyrhizobium* on the growth of summer mungbean. There is a report where sawdust based formulation inoculated with *P. fluorescens* ERN2$^{amp+,\ strep+}$ and *Rhizobium leguminosarum* AV1$^{amp+,\ tet+}$ have shown increase in the growth and productivity of *Trifolium repens* (Arora *et al.,* 2008).

Several laboratory and field level application of vermicompost carrier based formulations of PGPR have shown higher growth rate of plants, increased uptake of nutrients and increased rate of yield in crops like paddy, tomato, green gram and cow pea (Karmegam *et al.,* 1999; Manivannan and Thilagavathy, 2009).

There is a report on charcoal and talc based formulations of *Bacillus* species, where the charcoal based formulation promoted plant growth promoting activity of various crops (Pahari *et al.,* 2017). The study on optimization of PGPR formulation of *Azotobacter chroococcum*, *P. putida*, *B. cenocepacia* and *P. fluorescens* on the growth of brinjal plant and soil fertility has been done (Babu and Balasaravanan, 2018). The bioformulations of *Bacillus cereus* (MTCC 8297) and *P. rhodesiae* (MTCC 8299 and MTCC 8300) have been reported to increase the shoot height, no. of leaves, early fruiting

and biomass content of tomato, cauliflower, chili and brinjal plants (Kalita *et al.,* 2015). The study has been done on chitin based bioformulation of *P. fluorescens* and natural insecticide (*Vitex trifolia*) against Indian tomato leaf curl virus and its whitefly vector (Vasanthi *et al.,* 2017). The *matka khaad* liquid manure with trehalose has shown to maintain the viability of PGPR viz., *Azotobacter*, *Azospirillum* and *Pseudomonas* at different storage conditions (Manimekalai and Kannahi, 2018).

Conclusion

Paddy husk and press mud carrier based formulations of *Burkholderia gladioli* were found to improve the growth of maize, wheat, jowar and bajra plants. This will be very eco-friendly, non-toxic, cheap and also reduce the use of chemical fertilizers.

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