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Studies on the different Formulations of *Azospirillum* and *Paenibacillus* on the Enhancement of Growth and Yield Parameters in Maize (*Zea mays* L.)

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ABSTRACT
The application effect of different formulations of Azospirillum and Paenibacillus cells, viz., single strain inoculation, coinoculation and coaggregates application together with 75% recommended N and P level on the enhancement of growth and yield parameters of maize was studied under in-vitro conditions. It was observed that the application of each formulation of Azospirillum and Paenibacillus cells augmented the growth and yield parameters of maize to a higher level when compared to uninoculated control. However, the application of Azospirillum and Paenibacillus cells, as, natural coaggregates, exhibited the highest performance followed by coinoculation and single strain inoculation of PGPR cells. It was concluded the application of PGPR cells viz., Azospirillum and Paenibacillus as Interbacterial coaggregates, together with 75% recomended N and P level augmented the growth and yield parameters of maize to the highest level when compared to control (100% recommended N and P level without bioinoculation) and thus a saving of 25% recommended N and P level is possible due to coaggregates formulation of PGPR cells.
Key Words: Maize, Azospirillum, Paenibacillus, Formulations, Coaggregates, PGPR

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INTRODUCTION

Maize (Zea mays L.) is the third major crop of the world after wheat and rice which provides more nutrients for humans and animals than any other cereals and the same is grown across a wide range of agro- ecological zones, including, semiarid condition. Numerous biotic and abiotic factors may limit the productivity of that low soil fertility and incidences of diseases are considered to be the major constraints. Phosphorus is generally deficient in semiarid soils and the same is fixed as water insoluble calcium phosphate. Fixation of P in this soil eventually lead to the reduction in BNF (biological nitrogen fixation) and the availability of other nutrients (9,19). Hence, the productivity of maize (yield/ ha) must be greatly enhanced by providing additional nutrient inputs.

Now-a-days maize production management strategies mainly focus on chemical amelioration, including, the use of synthetic chemical fertilizers which are too expensive and also leads to several environmental hazards. In this context, plant growth promotion by free living, beneficial soil microorganisms, as a biological approach, might be an alternative strategy to overcome the biological and environmental hazards posed by the persistent use of synthetic chemicals (27, 28).

Azospirillum and *Paenibacillus* are the two more efficient important PGPR genera which are frequently encountered from the rhizosphere of maize under semiarid condition. *Azospirillum brasilense* has emerged as the biggest, potentially the most promising PGPR group among *Azospirillum* and involved in plant growth stimulation, production of secondary metabolites, such as, siderophores, antibiotics and phytohormones (16, 29). *Paenibacillus polymyxa* (1), a common soil bacterium which possess a wide range of activities, including, plant growth promotion and bio-dissolution of plant nutrients, including, phosphorus in the rhizosphere of many crop plants (17, 31).

Agricultural bioinoculant formulation plays a crucial role in the potential success of the bioinoculants. In the recent years, several new agricultural bioinocula formulations have been proposed of which the EPS mediated Interbacterial coaggregates seems to be a promising one for the production of multipurpose agricultural bioinoculant with multiple benefits (23). However, there were no earlier reports regarding the development and use of Interbacterial coaggregates in maize crop, available. Hence, the present research work has been undertaken with an aim to exploit the positive role of interbacterial coaggregates, comprising the genera of *Azospirillum brasilense* and *Paenibacillus polymyxa*, on plant growth stimulation, nitrogen fixation and phosphate solubilization in maize cv.CO-1 grown under semiarid condition.

MATERIAL AND METHODS

Bacterial strains, the efficient isolates of *Azospirillum brasilense*. (AB-3) and *Paenibacillus polymyxa* (PB-3), isolated from the rhizosphere of maize grown at Keerapalaiyam, Cuddalore district, Tamil Nadu state, India were used in the present study. The *Azospirillum* and *Paenibacillus* cells were maintained in Base-77 and Nutrient glucose agar slants (8), respectively, and incubated at 28 ± 2°C, with monthly transfer.

Preparation of inoculum

Azospirillum brasilense (AB-3) and *Paenibacillus polymyxa* (PB-3) isolates were grown in Base-77 and Nutrient glucose broth, respectively, in shaking bath at 28 ± 2°C for 24 hrs. Then the media were centrifuged separately, at 5000× g for 10 min to harvest the log phase cells and the pellets were washed three times with 0.1M phosphate buffer (pH 6.8), individually. Finally the cells of *Azospirillum brasilense* and *Paenibacillus polymyxa* were resuspended, separately, in the same buffer at a cell concentration of 1×10 7 CFU/mL by measuring OD at 420 nm for *Azospirillum brasilense* and 540 nm for *Paenibacillus polymyxa* and used as inoculum.

Preparation of *Azospirillum* and *Paenibacillus* interbacterial co-aggregates

The coaggregation of *Azospirillum* and *Paenibacillus* isolates were prepared in Co- Ag buffer (11). One ml aliquot of each PGPR cells viz., *Azospirillum* (AB-3) and *Paenibacillus* (PB-3) were mixed together in 10 ml CO-Ag buffer. The mixtures were vortexed for 10 seconds, shaken on a rotary platform shaker for 3 min and left undisturbed at room temperature for 24 hrs. All Co-Ag reactions were performed in triplicate and uninoculated buffer served as control.

Pot culture experiment

The effect of different formulations of *Azospirillum* and *Paenibacillus* cells viz., single strain inoculation, coinoculation and coaggregates application on the enhancement of growth and yield in maize was studied under potculture condition. The study was conducted during June to August 2015 with maize cv CO.1 in the polyhouse of Department of Microbiology, Faculty of Agriculture, Annamalai university, Annamalai Nagar, India.

Rectangular cement pots with 18"×12"×12" size were filled with 45 kg of field soil, flooded with water for two days and brought into fine puddle condition. The maize seeds were soaked for 30 min in the different formulations of PGPR cells viz., *Azospirillum brasilense* (AB-3) cells alone, and *Paenibacillus polymyxa* (PB-3) cells alone, coinoculation of *Azospirillum brasilense* (AB-3) and *Paenibacillus polymyxa* (PB-3) and coaggregates of *Azospirillum brasilense* (AB-3) and *Paenibacillus polymyxa* (PB-3) so as to get a final population of 1×10^7 cells per seed.

The experimental studies were performed in a randomized block design with three replications and the following were the treatments, 1) control + 100% N and P, 2) *Azospirillum brasilense* (AB-3) alone + 75% N and P, 3) *Paenibacillus polymyxa* (PB-3) alone + 75% N and P, 4) *Azospirillum brasilense* (AB-3) and *Paenibacillus polymyxa* (PB-3) Coinoculation + 75% N and P and 5) *Azospirillum brasilense* (AB-3) and *Paenibacillus polymyxa* (PB-3) coaggregates + 75% N and P, application.

During the experimental period, the annual mean minimum and maximum temperature of the experimental area was about 25°C and 39°C, respectively and the mean highest and lowest humidity were 94 and 78 per cent, respectively, the mean rainfall of the area was 1200 mm. A fertilizer schedule of 100:50:50 (100% NPK ha -1) was followed for the control pots, while all other treatments followed with 75% recommended dose of N and P fertilizer.

The crop was given a hand weeding on 30th DAS and well protected against pests and diseases. The experiment was maintained under limited water supply as per the conditions prevailing in semiarid maize ecosystem. Three plant hills in each pot were pegmarked for periodical observation. The plant height, root dry weight, shoot dry weight, nitrogen content, phosphorus and organic carbon content (3, 32) chlorophyll content (20), IAA production (30) was recorded on 45th DAS and grain yield, stalk and cob yield of maize was recorded during the time of harvest.

Growth parameters

Effect of different formulations of PGPR cells on the enhancement of growth and yield parameters in maize

Effect on plant growth

The height of the plant from each treatment was measured at 45th days after sowing (DAS). The mean value of plants from three replications was recorded.

Effect on root and shoot dry weight

The dry weight of the root and shoot was taken at 45th days after sowing (DAS). Three plant samples were drawn, washed, air dried and later dried to a constant weight in an oven at 60°C. The oven dried weight of the root and shoot sample was recorded. The N content of plant was estimated according to Bremner (3) while the P content of plant was done according to Jackson (14). The organic carbon content was estimated following the procedure of Walkley and Black (32) while the total chlorophyll content of maize leaves was estimated according to Mahadevan and Sridhar (20).

Grain and straw yield of maize

The matured crop was harvested, hand threshed, winnowed and sun dried. The dried grains from each treatment were weighed and recorded. After threshing, maize stalk was subjected to sun drying and the weight was recorded.

Cob yield

The number of cobs per plant was recorded during the time of harvest.

Statistical analysis

The experimental results were statistically analyzed in randomized block design (RBD) and in Duncan's multiple range test (DMRT) as per the procedure described by Gomez and Gomez (10).

RESULTS AND DISCUSSION

It was observed that all the formulations of *Azospirillum brasilense* and *Paenibacillus polymyxa* cells viz., single strain inoculation, coinoculation and coaggregates application on the enhancement of growth and yield parameters viz., plant height, root and shoot dry weight, IAA production, nitrogen content, phosphorus content, organic carbon and chlorophyll content, grain yield, stalk and cob yield of maize cv.CO.1 was studied under pot culture condition (Table 1).

Table 1 : Effect of different formulations of PGPR cells on the enhancement of growth parameters in maize

Treatment	Sampling time at 45DAS[a, b]							
	Plant height (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Nitrogen content (in %)	Phosphorus content (in %)	Organic carbon content (in %)	IAA production (mg/g)	Chlorophyll content (in %)
Control*	58.20 ± 0.60[f]	0.295 ± 0.23[f]	0.956 ± 0.27[f]	1.12 ± 0.35[f]	0.52 ± 0.13[f]	0.840 ± 0.26[f]	15.42 ± 0.28[f]	1.05 ± 0.24[f]
Azospirillum**	66.48 ± 0.52[d]	0.326 ± 0.16[d]	1.568 ± 0.36[d]	1.29 ± 0.25[d]	0.64 ± 0.19[d]	0.872 ± 0.18[d]	15.65 ± 0.32[d]	1.35 ± 0.29[d]
Paenibacillus**	61.32 ± 0.56[e]	0.307 ± 0.27[e]	1.142 ± 0.41[e]	1.22 ± 0.27[e]	0.59 ± 0.15[e]	0.864 ± 0.21[e]	15.57 ± 0.38[e]	1.14 ± 0.31[e]
Azospirillum + Paenibacillus Co I**	68.24± 0.54[c]	0.335 ± 0.21[c]	1.624 ± 0.45[c]	1.39 ± 0.29[c]	0.70 ± 0.07[c]	0.946 ± 0.24[c]	15.80 ± 0.33[c]	1.48 ± 0.26[c]
Biofloc of *Azospirillum* + *Paenibacillus* (Natural)**	72.84 ± 0.62[a]	0.351 ± 0.22[a]	1.802 ± 0.36[a]	1.86 ± 0.36[a]	0.92 ± 0.05[a]	1.004 ± 0.20[a]	16.79 ± 0.42[a]	1.88 ± 0.22[a]
Biofloc of *Azospirillum* + *Paenibacillus* (Artificial)**	69.46 ± 0.53[b]	0.339 ± 0.26[b]	1.712 ± 0.40[b]	1.71 ± 0.32[b]	0.74 ± 0.09[b]	0.979 ± 0.16[b]	16.19 ± 0.36[b]	1.61 ± 0.20[b]
LSD (P = 0.05)	**0.32**	**0.28**	**0.041**	**0.018**	**0.02**	**0.02**	**0.02**	**0.08**

a – Average of three replication ± SD
b – Values followed by differed letters are significantly differed at 5% level according to student 't' test.
* at 100% recommended level N and P
** at 75% recommended level N and P

The application of the different formulations of *Azospirillum brasilense* and *Paenibacillus polymyxa* cells was found to augment the growth and yield parameters of maize cv.CO-1 when compared to control (100% N and P level without any bio-inoculation). Among the different formulations, the application of *Azospirillum brasilense* and *Paenibacillus polymyxa* cells, as coaggregates, improved growth and yield parameters of cv.CO.1 maize to a higher level, followed by coinoculation of *A. brasilense* and *P. polymyxa*, *A. brasilense* alone and *P. polymyxa* alone treatment. Interestingly, the application of Interbacterial coaggregates, comprising of *A. brasilense* and *P. polymyxa* cells together with 75% recommended N and P level could augment the growth and yield parameters of maize cv CO.1 to a higher level when compared to maize crop grown in 100% recommended N and P level without any bio-inoculation and thus a saving of 25% recommended N and P fertilizers could be achieved.

The coaggregates application of *A. brasilense* and *P. polymyxa* recorded the maximum plant height, 68.25 cm, root dry weight, 0.342 g/plant, shoot dry weight 1.713 g/plant, nitrogen content 1.41 per cent, phosphorus content 0.84 per cent, indole acetic acid (IAA) production, 16.31 mg/g, organic carbon content 0.714 per cent and chlorophyll content, 1.54 mg/g of leaf on 45th DAS (Table 1) respectively when compared to other formulations and the highest value of grain yield (2.84), stalk yield (3.310) and cob yield (3.18) (Table 2), when compared to other formulations.

Table 2: Effect of different formulations of PGPR cells on the enhancement of yield parameters in maize

Treatment	**Grain yield (t/ha)$^{a, b}$**	**% over control**	**Stalk yield (t/ha)$^{a, b}$**	**% over control**	**No. of cobs /plant $^{a, b}$**	**% over control**
Control*	2.025 ± 0.65^{f}	-	2.325 ± 0.73^{f}	-	3.00 ± 0.26^{f}	-
Azospirillum**	2.296 ± 0.58^{d}	26.43	2.874 ± 0.62^{d}	41.43	3.37 ± 0.19^{d}	37.0
Paenibacillus**	2.272 ± 0.55^{e}	24.09	2.796 ± 0.67^{e}	35.54	3.25 ± 0.14^{e}	25.0
Azospirillum + Paenibacillus Co I**	2.343 ± 0.61^{c}	31.02	2.918 ± 0.61^{c}	44.75	3.50 ± 0.22^{c}	50.0
Biofloc of *Azospirillum* + *Paenibacillus* (Natural)**	2.726 ± 0.63^{a}	68.39	3.142 ± 0.69^{a}	61.66	3.93 ± 0.27^{a}	93.0
Biofloc of *Azospirillum* + *Paenibacillus* (Artificial)**	2.623 ± 0.51^{b}	58.34	2.962 ± 0.65^{b}	48.07	3.60 ± 0.20^{b}	60.0
LSD (P = 0.05)	0.342		0.516		0.386	

a – Average of three replication ± SD; b – Values followed by differed letters are significantly differed at 5% level according to student 't' test;

* at 100% recommended level N and P; ** at 75% recommended level N and P.

The effect of *Paenibacillus* inoculation on the enhancement of growth and yield parameters of maize has already been reported by many researchers (12, 13, 18). The positive effect of Pseudomonas and Bacillus coinoculation has already been reported in wheat (7). The positive effect of *Azospirillum* and *Rhizobium* cofloc on the enhancement of growth and yield in common bean has also been reported (23). In the present study, the coaggregates application of *A.brasilense* and *P. polymyxa* increased the growth and yield in maize to a higher level when compared to other formulations. However, there were no earlier reports regarding the beneficial effect of Interbacterial microbial coaggregates application on growth stimulation not available for discussion. This is the first comprehensive report regarding the beneficial effect of *A. brasilense* and *P. polymyxa* cells, as coaggregates, on the enhancement of growth parameters in maize cv.CO.1.

CONCLUSION

The application of "Intergeneric coaggregates of Azospirillum and Paenibacillus" cells under natural condition together with application of 75 per cent recommended dose of N and P levels in CO-1 maize recorded the growth and yield parameters on par with 100 per cent recommended N and P fertilizers application without any bioinoculation treatment and thus a saving of 25 per cent recommended N and P fertilizers could be achieved through the application of intergeneric PGPR coaggregates (natural) in maize crop. Moreover, the natural co-aggregates application augmented the survival of PGPR cells in maize rhizosphere thereby alleviating soil salinity.

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