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Dual inoculation effect of Azospirillum and P-solubilizing fungal isolates on growth, nutrient uptake and biomass of maize (*Zea mays* L.)

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ABSTRACT
Pot culture experiments revealed that there was a significant increase in all growth parameters, nutrient parameters, enzymatic activity and microbial population of Maize due to seed treatment with Azospirillum sp. and P-solubilizing fungal isolates when compared to untreated plants. Among the five isolates of P-solubilizing fungi T_4 (RPP + PSF – 1(Aspergillus sp.) + Azospirillum sp.) resulted in highest values in all the parameters. The treatment with these microorganisms resulted in increase in the plant growth and the nutrient uptake of maize plant. It also increased in the microbial population of rhizosphere and root growth. Thus, the present investigation clearly brought out the potential of Azospirillum sp. and P-solubilizing fungi in augmenting growth and nutrient uptake of maize.
***Key words**: dual inoculation, Azospirillum, Phosphorus solubilizing fungi, Maize*

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INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop next to wheat and rice in the world. It is used as food, feed and for other products and it provides more human food than any other cereal. In India, it ranks fourth in area and production. It is originated from Central America having been domesticated about 7000 years ago. Since Maize is a nutrient exhaustive crop the nutrient management is one of the most important factors that affect the growth and yield of maize [5]. Application of inorganic fertilizers along with biofertilizer significantly increases maize yield [1]. Manure application also increases the nitrogen and exchangeable cation levels in the soil [3]. Among the various nutrients, nitrogen and phosphorus are the principal nutrients for a good harvest of maize. Since the chemical fertilizers deplete the soil productivity, the use of biofertilizers instead of chemical fertilizers to supply the required nutrients to plants is highly essential.

Nitrogen is a vital plant nutrient and a major yield determining factor for maize production. Its availability in sufficient quantity throughout the growing season is essential for optimum growth of maize whereas, phosphorus is closely concerned with many growth processes in crop plants. It is involved in many biochemical reactions and concerned with the metabolism of carbohydrates, fats and proteins and play roles in the breakdown of carbohydrates. It is essential for cell division and carbohydrate synthesis. It also helps in improving the quality and strengthens the plant against lodging.

Since most of the applied phosphorus is fixed into the soil and immobilized it is not available for the plant. Thus, its deficiency becomes an important chemical factor restricting plant growth in soils. Hence the application of phosphorus solubilizers to the rhizosphere makes the phosphorus available for plant. While nitrogen is the most limiting nutrient generally in soil, the deficiency of soil phosphorus reduces the efficiency of nitrogen use by crops. The availability of nitrogen to the crop can be made through the application of *Azospirillum* sp. which is a free-living nitrogen-fixing bacteria closely associated with grasses. In connection with numerous symptoms of a beneficial action of *Azospirillum* sp. on plants, attempts have been made to use them practically in agriculture through inoculation to crops.

MATERIAL AND METHODS

A pot-culture experiment was conducted in the greenhouse of Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, to study the dual inoculation effect of *Azospirillum* sp. and phosphorus solubilizing fungi on growth and biomass of maize. Five efficient P-solubilizing isolates were selected from the previous study [2] and were evaluated in pot culture experiment on maize crop to assess the ability of isolates to enhance growth, biomass and nutrient uptake. The material used and the methods followed in the present investigation have been outlined below. The experiment was laid out in a completely randomized design (CRD) with 8 treatments and 5 replications.

Treatment details

Treatment 1: RPP only
Treatment 2: RPP + *Azospirillum* sp.
Treatment 3: RPP + *Pseudomonas striata* + *Azospirillum* sp.
Treatment 4: RPP + PSF – 1 (*Aspergillus* sp.)+ *Azospirillum* sp.
Treatment 5: RPP + PSF – 2 (*Penicillium* sp.)+ *Azospirillum* sp.
Treatment 6: RPP + PSF – 3 (*Penicillium* sp.)+ *Azospirillum* sp.
Treatment 7: RPP + PSF – 4 (*Aspergillus* sp.)+ *Azospirillum* sp.
Treatment 8: RPP + PSF – 5 (unidentified)+ *Azospirillum* sp.

NOTE: The microbial culture *Azospirillum* sp. is a mixed culture of two isolates such as ACD-15 and ACD-20. *Pseudomonas striata* is a standard culture used in the study has been procured from Department of Agricultural Microbiology, UAS, Dharwad.

Maize hybrid (GH-0727) was used for the pot culture experiment. The selected efficient phosphorus solubilizing fungal isolates and *Azospirillum* sp. were treated with the seeds and seeds were sown in pots @ 5 seeds per pot. After 15 days of sowing, thinning was done to retain one plant in each pot. The recommended dose of $N:P_2O_5:K_2O$ were applied and plant protection measures were carried out as per the package of practices.

Plant growth parameters such as germination percentage, plant height, number of leaves per plant, leaf length, chlorophyll content (SPAD readings), Dry matter accumulation and nutrient parameters such as Plant nitrogen content and uptake, Phosphorus content, Potassium content were measured at 30 and 45 DAS. Enzyme activities such as dehydrogenase, urease, and phosphatase; root parameters and microbial population in the rhizosphere soil was also enumerated at 30 and 45 DAS.

RESULTS AND DISCUSSION

Maize is a very important crop in cereals and is a staple food in many countries. Since maize is an exhaustive crop it requires high amount of nutrients for its growth and development. Among the essential nutrients required by the crop, nitrogen and phosphorus are of much importance. Mainly, P-solubilizing microbes (PSM) belong to bacteria, fungi and actinomycetes. Among these, fungi are more important since they produce higher amount of organic acids compared to other PSMs. *Aspergillus* and *Penicillium* form the important fungal genera and are more efficient P-solubilizers compared to other fungal genera [4, 6]. *Azospirillum* sp. is an associative symbiotic nitrogen fixing bacteria which fixes nitrogen mainly in cereals and the application of *Azospirillum* sp. to plants results in improved growth yield and nutrient uptake. The inoculation of plants with nitrogen fixing and phosphorus solubilizing microorganisms can help in improvement of maize growth and yield.

The effect of dual inoculation of PSF and *Azospirillum* sp. on growth and biomass of maize was carried out in pot culture experiment (Plate 1). The results of experiment were recorded at 30 and 45 DAS. Significantly higher growth of plant, chlorophyll content (Table 1), microbial (Table 3), and enzymatic activity (Table 4), root growth (Table 5, Plate 1) and also higher nutrient content (Table 2), with high biomass was observed in inoculated treatments compared to control. And among the inoculated treatments highest results in all parameters were observed in T_4 i.e., inoculation of isolate PSF-1 and *Azospirillum* sp. with RPP.

The performance of these isolates in consortia was better than control. The strains were efficient in expressing multifunctional properties such as providing nutrients in available form to the plants in addition to the production of plant growth promoting leading to the overall increase in the growth nutrient uptake and biomass of maize. These efficient PSF isolates based on all the functional properties and nutrient uptake by the plants clearly indicates that they play an important role in plant growth and development by fixing the biological nitrogen, phosphorus solubilization and production of phytoharmones.

This study has clearly brought out the potential of P-solubilizing fungus and Azospirillum sp. in augmenting the growth and nutrient level in plant, which can help in improvement of overall growth and development of maize plant.

Table 1: Plant growth parameters and chlorophyll content at 30 and 45 days after sowing (DAS) as influenced by PSF and *Azospirillum* sp.

Treatment	Details	Plant height (cm)		Number of leaves per plant		Leaf length (cm)		Dry matter accumulation (g/plant)		Chlorophyll content at different interval (SPAD value/plant)	
		30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS
T_1	RPP only	34.47	85.27	9.00	10.33	47.73	58.90	8.57	17.62	30.57	34.30
T_2	RPP + *Azospirillum* sp.	36.53	87.30	9.67	11.00	50.70	61.37	8.73	23.54	31.57	36.59
T_3	RPP + *Pseudomonas striata* + *Azospirillum* sp.	37.40	90.87	10.33	11.33	52.30	63.97	9.06	26.58	33.57	37.65
T_4	RPP + PSF-1 + *Azospirillum* sp.	42.87	95.73	11.33	12.67	57.23	70.07	9.33	34.72	36.37	40.28
T_5	RPP + PSF-2 + *Azospirillum* sp.	40.57	94.60	11.00	12.33	56.17	68.97	9.27	33.82	35.43	39.79
T_6	RPP + PSF-3 + *Azospirillum* sp.	37.77	90.03	9.33	11.33	51.27	64.67	9.13	29.23	33.43	38.16
T_7	RPP + PSF-4 + *Azospirillum* sp.	38.93	92.03	9.67	11.67	52.33	65.57	9.22	30.12	34.10	38.48
T_8	RPP + PSF-5 + *Azospirillum* sp.	39.63	92.10	10.00	11.67	54.90	68.23	9.23	32.15	34.40	38.94
S. Em. ±		0.638	0.780	0.152	0.180	0.158	0.391	0.007	0.013	0.368	0.303
C. D. at 1 %		2.633	3.224	0.628	0.744	0.653	1.613	0.028	0.055	1.522	1.250

Table 2: Nitrogen, phosphorus, potassium content and uptake by the plants at 30 and 45 DAS as influenced by PSF and *Azospirillum* sp.

Treatment	Details	Nitrogen content of the plant (%)		Nitrogen uptake (g/plant)		Phosphorus content of the plant (%)		Phosphorus uptake (g/plant)		Potassium content of the plant (%)		Potassium uptake (g/plant)	
		30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS
T_1	RPP only	1.05	1.28	0.090	0.226	0.36	0.40	0.030	0.070	0.98	2.22	0.084	0.390
T_2	RPP + *Azospirillum* sp.	1.40	1.66	0.122	0.390	0.42	0.53	0.037	0.124	1.08	2.36	0.094	0.556
T_3	RPP + *Pseudomonas striata*+ *Azospirillum* sp.	1.45	1.75	0.131	0.465	0.61	0.69	0.055	0.182	1.52	2.61	0.138	0.693
T_4	RPP + PSF-1 + *Azospirillum* sp.	1.56	1.87	0.146	0.648	0.66	0.76	0.062	0.263	2.20	2.94	0.205	1.021
T_5	RPP + PSF-2 + *Azospirillum* sp.	1.54	1.84	0.143	0.634	0.65	0.75	0.060	0.259	1.92	2.91	0.203	1.005
T_6	RPP + PSF-3 + *Azospirillum* sp.	1.45	1.70	0.132	0.498	0.62	0.70	0.056	0.205	1.53	2.66	0.140	0.779
T_7	RPP + PSF-4 + *Azospirillum* sp.	1.45	1.77	0.133	0.534	0.63	0.73	0.058	0.219	1.71	2.71	0.158	0.816
T_8	RPP + PSF-5 + *Azospirillum* sp.	1.47	1.80	0.136	0.578	0.64	0.74	0.059	0.239	1.76	2.77	0.163	0.890
S. Em. ±		0.02	0.013	0.002	0.004	0.005	0.003	0.0004	0.001	0.007	0.017	0.001	0.005
C. D. at 1 %		0.08	0.052	0.007	0.015	0.019	0.014	0.0017	0.004	0.030	0.069	0.003	0.019

Table 3: Influence of PSF and *Azospirillum* sp. on population of *Azospirillum* sp. and PSF in the rhizosphere soil of plants at 30 and 45 DAS

Treatment	Details	*Azospirillum* (CFU × 10^4/g of soil)		Phosphorus solubilizing fungi (CFU × 10^4/g of soil)	
		30 DAS	30 DAS	30 DAS	45 DAS
T_1	RPP only	7.33	3.00	3.00	5.33
T_2	RPP + *Azospirillum* sp.	12.33	6.00	6.00	7.67
T_3	RPP + *Pseudomonas striata+ Azospirillum* sp.	15.00	10.00	10.00	11.33
T_4	RPP + PSF-1 + *Azospirillum* sp.	18.33	11.67	11.67	16.67
T_5	RPP + PSF-2 + *Azospirillum* sp.	17.67	11.33	11.33	16.33
T_6	RPP + PSF-3 + *Azospirillum* sp.	14.67	9.00	9.00	12.33
T_7	RPP + PSF-4 + *Azospirillum* sp.	15.67	9.67	9.67	13.67
T_8	RPP + PSF-5 + *Azospirillum* sp.	16.67	10.67	10.67	14.33
S. Em. ±		0.215	0.319	0.136	0.136
C. D. at 1 %		0.889	1.318	0.562	0.562

Table 4: Urease, phosphatase and dehydrogenase enzyme activity in the soil at 30 and 45 DAS as influenced by PSF and *Azospirillum* sp.

Treatment	Details	Urease activity (μg NH_4-N/g soil/day)		Phosphatase activity (μg PNP/g soil/h)		Dehydrogenase activity (μg TPF/g soil/day)	
		30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS
T_1	RPP only	10.11	52.50	47.92	84.56	34.76	44.52
T_2	RPP + *Azospirillum* sp.	15.37	61.63	55.65	87.01	38.99	52.28
T_3	RPP + *Pseudomonas striata + Azospirillum* sp.	19.20	64.12	60.42	91.10	44.32	56.72
T_4	RPP + PSF-1 + *Azospirillum* sp.	35.99	66.93	73.81	96.14	50.33	60.99
T_5	RPP + PSF-2 + *Azospirillum* sp.	35.32	66.22	72.92	94.73	49.57	59.89
T_6	RPP + PSF-3 + *Azospirillum* sp.	22.04	63.39	58.33	93.13	39.91	57.69
T_7	RPP + PSF-4 + *Azospirillum* sp.	22.04	64.79	63.39	93.46	45.79	58.21
T_8	RPP + PSF-5 + *Azospirillum* sp.	29.46	64.87	71.43	93.75	47.63	58.96
S. Em. ±		0.298	0.444	0.505	0.453	0.336	0.457
C. D. at 1 %		1.232	1.835	2.084	1.872	1.389	1.889

Table 5: Root growth and morphology of maize plant at 55 DAS as influenced by the inoculation of *Azospirillum* and PSF (By using root scanner)

Treatment	Details	Average diameter (mm)	Root volume (cm^3)	Length per volume (cm/m^3)	Length (cm)
T_1	RPP only	0.73	1.25	10,455.76	318.03
T_2	RPP + *Azospirillum* sp.	0.73	2.28	19,151.04	592.38
T_3	RPP + *Pseudomonas striata+ Azospirillum* sp.	0.89	2.77	14,541.52	461.80
T_4	RPP + PSF-1 + *Azospirillum* sp.	1.38	8.88	19,981.19	616.52
T_5	RPP + PSF-2 + *Azospirillum* sp.	1.17	5.17	16,762.20	524.35
T_6	RPP + PSF-3 + *Azospirillum* sp.	0.70	2.53	21,970.94	679.19
T_7	RPP + PSF-4 + *Azospirillum* sp.	0.85	3.33	19,796.74	624.07
T_8	RPP + PSF-5 + *Azospirillum* sp.	0.96	3.31	16,003.73	491.13
S. Em. ±		0.007	0.018	79.46	6.86
C. D. at 1 %		0.028	0.074	328.20	28.35

Control

Azospirillum

***Azospirillum*+PSB**

RPP+*Azospirillum*+PSF-1

RPP+*Azospirillum*+PSF-2

RPP+*Azospirillum*+PSF-3

RPP+*Azospirillum*+PSF-4

RPP+*Azospirillum*+PSF-5

Plate 1. Root growth and morphology of maize plants at tasseling stage.

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