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Interactive effect of liquid *formulations* containing available and unavailable forms of phosphorus source on nitrogen efficiency in aerobic rice

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Abstract
Green house experiment was studies to explore the effect of thus developed liquid *formulations* from previous experiments of above authors and the respective lignite based *formulations* in the presence and absence of nutrients and at two forms of available and unavailable form of phosphorous (KH_2PO_4 and North Carolina Rock Phosphate (NCRP)) on aerobic rice variety MAS-26. As a result, higher root nitrogen concentration was observed in *P. fluorescens* liquid *formulation* and also in the plants received P as KH_2PO_4. However, there was significantly higher root N content was observed in plants inoculated liquid *formulation of P.fluorescens*. Whereas, liquid *P. fluorescens* and *Azospirillum* showed highest total nitrogen content.

Keywords: Greenhouse experiment, *pseudomonas fluorescens*, *Azospirillum*, phosphorus forms

Introduction
In rice, phosphorus play an important role in determining productivity. Because root parameters decides the phosphorus uptake capacity. Phosphorus (P) is highly required for the for plant growth and nutrition, though it is least available nutrients among all, because P is become unavailable form once it get complexes with chemical and biological form (Richardson *et al.* 2009) [10]. A key aspect of improving crop performance in low-P soils is improving P acquisition efficiency via improved root traits (Lynch and Brown 2008; Richardson *et al.* 2011) [7, 11]. In the view of future generations one should focus on optimal and uniform utilization of the resource as they fetches more costs (Ditta *et al.* 2018) [3]. Very poor quality and locally deposited phosphates are available but they are fully utilized for the farmers benefit (Tarafdar 2013) [12]. Rock phosphate contains 25% of P content; being insoluble in water cannot be directly applied as fertilizer (Reddy *et al.* 2002) [9]. With this background, liquid inoculant *formulations* developed for *Azotobacter*, *Azospirillum*, *Acinetobacter*, *Bacillus* and *Pseudomonas fluorescens* were used in the study formulated (from previous works) at two forms of available and unavailable form of phosphorous (KH_2PO_4 and North Carolina Rock Phosphate (NCRP)) on aerobic rice variety MAS-26.

Materials and Methods
Laboratory investigations
Beneficial microbes used in the study
Cultures of *Azotobacter sp*, *Azospirillum*, *Bacillus*, and *Acinetobacter* and *P. fluorescens* obtained from the department purified on specific media and subjected to microscopic observation and based on colony morphology compared with the standard cultures and maintained

Green house investigation
A green house experiment was conducted to know the effect of thus developed (from the previous work) liquid *formulations* of selected microorganisms and the respective lignite based *formulations* in the presence and absence of nutrients and at two forms of available and

unavailable form of phosphorous (KH_2PO_4 and North Carolina Rock Phosphate (NCRP)) on aerobic rice variety MAS-26.

Methods of soil processing for pot culture studies

Soil collection and processing were done as per the procedure given by Dayamani and Premalatha during 2025 [2].

Preparation of inoculant *formulations*

Preparation of liquid based and carrier based inoculant *formulations*

Liquid based and carrier based inoculant *formulations* were prepared according to the procedure given by Dayamani and Premalatha during 2025 [2].

Table 1: Liquid *formulations* used for the green house investigation

Bacterial culture	Specific medium	Osmolyte concentration
Azospirillum sps.	Malate medium (Okons media)	PEG 400 (1%) + Glycerol (2%)
B. megaterium	Modified Sperber's medium	PVP K -15 (1%) + Glycerol (2%)
Acinetobacter sps.	Modified Sperber's medium	PEG 6000 (2%) + Glycerol (2%)
P.fluorescens	King's B media	PVP K -15 (2%) + Glycerol (2%)

Treatment details

Treatments	***Formulations***	Nutrient solution
T_1	Absolute control	N^-P^-
T_2	*Azospirillum* liquid *formulation*	
T_3	*Bacillus* liquid *formulation*	
T_4	*Acinetobacter* liquid *formulation*	
T_5	*P.fluorescens* liquid *formulation*	
T_6	*Azospirillum* lignite *formulation*	
T_7	*Bacillus* lignite *formulation*	
T_8	*Acinetobacter* lignite *formulation*	
T_9	*P.fluorescens* lignite *formulation*	
T_{10}	Absolute control	N^+P^+(KH_2PO_4)
T_{11}	*Azospirillum* liquid *formulation*	
T_{12}	*Bacillus* liquid *formulation*	
T_{13}	*Acinetobacter* liquid *formulation*	
T_{14}	*P.fluorescens* liquid *formulation*	
T_{15}	*Azospirillum* lignite *formulation*	
T_{16}	*Bacillus* lignite *formulation*	
T_{17}	*Acinetobacter* lignite *formulation*	
T_{18}	*P.fluorescens* lignite *formulation*	
T_{19}	Absolute control	N^+P^+(Rock phosphate)
T_{20}	*Azospirillum* liquid *formulation*	
T_{21}	*Bacillus* liquid *formulation*	
T_{22}	*Acinetobacter* liquid *formulation*	
T_{23}	*P.fluorescens* liquid *formulation*	
T_{24}	*Azospirillum* lignite *formulation*	
T_{25}	*Bacillus* lignite *formulation*	
T_{26}	*Acinetobacter* lignite *formulation*	
T_{27}	*P.fluorescens* lignite *formulation*	

Test plant and planting

Aerobic rice seeds of variety MAS-26 were surface sterilized with 2% sodium hypochlorite solution and washed with sterile distilled water five times to remove the traces of chlorine and then soaked in sterile water for 2 days. After 48 hours, 10 seeds were sown at five cm depth in every single pot. Then the recommended doses of 1.35g of carrier based *formulation* and 1 ml of liquid *formulation* were applied according to the treatment imposed. The pots were watered regularly to maintain the field capacity seedlings were thinned out and five seedlings per pot were maintained.

Observations

Nitrogen content in plant samples

N concentration in shoot and root of plants was estimated by Micro- Kjeldhal method as given by Jackson (1973) [5].

Total N content: The total nitrogen content was calculated by the addition of N content of shoot and root

Statistical analysis

Statistical analysis was done by using two factor randomized complete block design and means were separated by Duncan's Multiple Range Test (DMRT) (Little and Hills, 1978) [6].

Experimental Results

Green house experiment

A greenhouse experiment was conducted to know the effect of thus developed liquid inoculant *formulation* along with lignite based *formulations* in the presence and absence of nutrients and at two forms of available and unavailable forms of phosphorous (KH_2PO_4 and North Carolina Rock Phosphate (NCRP) on aerobic rice variety MAS-26 as test plant

Shoot nitrogen concentration of aerobic rice differed significantly due to inoculation of beneficial microorganisms in different inoculant *formulation* and P source. Highest shoot nitrogen concentration was seen in plants added with *Acinetobacter* in liquid form followed by *Azospirillum* in liquid and least in uninoculated plants. Among different source of P, plants receiving P as KH_2PO_4 were found to have significantly higher shoot N concentration compared to rock phosphate and lowest in nutrient control (Table 1).

Significant difference among treatments in case of shoot nitrogen concentration at all the three sources of P was seen. When P was supplied as rock phosphate, both *Acinetobacter* and *Bacillus* resulted in higher shoot N concentration inoculated as liquid and lignite *formulation* respectively followed by *Azospirillum* in lignite *formulation* and least in uninoculated treatment. When P was supplied as KH_2PO_4 highest shoot Nitrogen concentration was observed in plants inoculated with *Azospirillum*, *Bacillus* and *Acinetobacter* through liquid *formulation* followed by *P. fluorescens* in liquid *formulation* and least in uninoculated plants (Table 1)

Root nitrogen concentration of aerobic rice was differed significantly due to inoculation of beneficial bacteria in different inoculant *formulations* and P sources. Significantly higher root N was observed in plants treated with *P. fluorescens* through liquid inoculant followed by liquid *formulation* of *Bacillus* and least in uninoculated treatment. Among different sources of P treatment receiving P as KH_2PO_4 resulted in higher root nitrogen concentration compared to rock phosphate (Table 2).

Among the treatments receiving P as rock phosphate, highest root N concentration was found in *P. fluorescens* inoculated plants followed by *Acinetobacter* and least in *Azospirillum* inoculated plants. When P source was KH_2PO_4 higher root N concentration was observed in plants treated with *P. fluorescens* through liquid *formulation* and all other treatments showing results on par with uninoculated control under P limiting condition, higher N concentration was observed in plants inoculated with *P. fluorescens* in liquid *formulations* and least in uninoculated control (Table 2).

No significant difference in shoot N content of aerobic rice was observed due to the inoculation of beneficial bacteria in inoculant *formulation* irrespective of the three sources of P. However P main effect differed significantly with highest shoot N content in plants supplied with P as KH_2PO_4 followed by rock phosphate and lowest in nutrient control (Table 3). Amutha *et al.*, 2009 [1] proved that the inoculation of *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Serratia* has resulted in increased number of tillers, height, plant dry weight, N content and number of productive tillers.

Magda *et al.* (2003) studied the effect of phosphate dissolving bacteria *Bacillus megaterium* and Azolla under two different sources of chemical P fertilizer (as superphosphate or rock phosphate) on rice. They reported that application of biofertilizers under chemical Phosphate fertilizer was more effective than chemical P-fertilizer only. P- fertilizer superphosphate (SP) superorder rock phosphate fertilizer in terms of rice growth and yield.

Root N content of aerobic rice did not differ significantly due to inoculation of beneficial bacteria in different inoculant *formulation* irrespective of P source used. However there was significant difference among treatment main effects with highest root N content in plants inoculated liquid *P. fluorescens* followed by lignite *P. fluorescens* and least in uninoculated plants. Among three P sources highest root N was observed when P was supplied as KH_2PO_4 followed by rock phosphate and least in P limiting treatment (Table 4)

Total N content of aerobic rice differed significantly due to inoculation of beneficial microorganisms under available P source, but not under P limiting and rock phosphate treated. Highest total N was observed in plants inoculated with *Azospirillum* and *P. fluorescens* as liquid *formulation* followed by *Bacillus* liquid (Table 5)

Among treatment main effect significantly higher N content was observed in plants treated with *P. fluorescens* in liquid *formulation* followed by *P. fluorescens* in lignite *formulation* and least in uninoculated plants. Among P main effects, higher N content was observed in plants supplied with KH_2PO_4 followed by rock phosphate and least in control (Table 5)

Table 2: Shoot nitrogen concentration (%) of aerobic rice (var. MAS-26) as influenced by different inoculation *formulations* and P source.

Treatments	Shoot nitrogen concentration (%)			
	Source of phosphorus			Treatment main effect
	Nutrient control	KH_2PO_4	Rock phosphate	
Control	2.12d	2.68d	2.47e	2.42g
Azospirillum liquid	2.34ab	3.21a	2.68c	2.75ab
Bacillus liquid	2.24c	3.24a	2.63cd	2.70abc
Acinetobacter liquid	2.24c	3.07a	2.96a	2.75a
P. fluorescens liquid	2.37a	3.05b	2.67c	2.70bc
Azospirillum lignite	2.35a	2.55e	2.81b	2.57e
Bacillus lignite	2.23c	2.91c	2.90a	2.68c
Acinetobacter lignite	2.12d	2.85c	2.56d	2.51f
P. fluorescens lignite	2.26bc	3.02b	2.61cd	2.63d
Phosphorus main effect	2.25c	2.95a	2.70b	

Table 3: Root nitrogen concentration (%) of aerobic rice (var. MAS-26) as influenced by different inoculation *formulations* and P source.

Treatments	Root nitrogen concentration (%)			
	Source of phosphorus			Treatment main effect
	Nutrient control	KH_2PO_4	Rock phosphate	
Control	0.68^{g}	1.45^{b}	1.63^{bc}	1.26^{e}
Azospirillum liquid	1.74^{ab}	1.56^{b}	1.52^{d}	1.61^{b}
Bacillus liquid	1.51^{de}	1.54^{b}	1.71^{ab}	1.58^{b}
Acinetobacter liquid	1.65^{bc}	0.85^{c}	1.75^{a}	1.42^{d}
P. fluorescens liquid	1.81^{a}	1.68^{a}	1.76^{a}	1.75^{a}
Azospirillum lignite	1.49^{def}	1.50^{b}	1.68^{ab}	1.56^{b}
Bacillus lignite	1.38^{f}	1.50^{b}	1.22^{e}	1.37^{d}
Acinetobacter lignite	1.41^{ef}	1.52^{b}	1.53^{cd}	1.48^{c}
P. fluorescens lignite	1.56^{cd}	1.50^{b}	1.71^{ab}	1.59^{b}
Phosphorus main effect	1.47^{b}	1.46^{b}	1.61^{a}	

Table 4: Shoot nitrogen content (mg/plant) of aerobic rice (var. MAS-26) as influenced by different inoculation *formulations* and P source.

Treatments	Shoot nitrogen content (mg/plant)			
	Source of phosphorus			Treatment main effect
	Nutrient control	KH_2PO_4	Rock phosphate	
Control	16.32	190.47	88.03	98.27
Azospirillum liquid	21.06	369.73	40.72	143.83
Bacillus liquid	21.12	363.57	61.32	148.67
Acinetobacter liquid	21.95	321.17	61.40	134.84
P. fluorescens liquid	32.10	363.06	60.35	151.83
Azospirillum lignite	21.29	294.90	60.41	125.53
Bacillus lignite	16.53	292.58	62.97	124.02
Acinetobacter lignite	18.73	292.59	50.45	120.59
P. fluorescens lignite	23.99	359.23	56.71	146.64
	NS	NS	NS	NS
Phosphorus main effect	21.45[c]	316.37[a]	60.26[b]	

Table 5: Root nitrogen content (mg/plant) of aerobic rice (var. MAS-26) as influenced by different inoculation *formulations* and P source.

Treatments	Root nitrogen content (mg/plant)			
	Source of phosphorus			Treatment main effect
	Nutrient control	KH_2PO_4	Rock phosphate	
Control	3.95	33.40	8.49	15.28[d]
Azospirillum liquid	14.28	49.40	12.59	25.42[bc]
Bacillus liquid	10.36	44.42	15.72	23.50[bc]
Acinetobacter liquid	11.98	24.10	17.42	17.84[cd]
P. fluorescens liquid	21.52	53.25	32.60	35.79[a]
Azospirillum lignite	11.02	34.55	13.42	19.66[cd]
Bacillus lignite	8.77	33.59	11.61	17.99[cd]
Acinetobacter lignite	10.19	31.19	13.51	18.29[cd]
P. fluorescens lignite	16.13	43.39	23.03	27.52[b]
	NS	NS	NS	
Phosphorus main effect	12.02[c]	38.59[a]	16.49[b]	

Table 6: Total nitrogen content (mg/plant) of aerobic rice (var. MAS-26) as influenced by different inoculation *formulations* and P source.

Treatments	Total nitrogen content (mg/plant)			
	Source of phosphorus			Treatment main effect
	Nutrient control	KH_2PO_4	Rock phosphate	
Control	20.28	223.88[d]	96.53	113.56[c]
Azospirillum liquid	35.34	419.20[a]	53.32	169.29[ab]
Bacillus liquid	31.49	407.99[ab]	77.04	172.17[ab]
Acinetobacter liquid	33.94	345.27[abc]	78.84	152.68[abc]
P. fluorescens liquid	53.62	416.31[a]	93.04	187.65[a]
Azospirillum lignite	32.31	329.45[bc]	73.83	145.20[abc]
Bacillus lignite	25.30	326.17[c]	74.58	142.02[abc]
Acinetobacter lignite	28.92	323.78[c]	63.97	138.89[bc]
P. fluorescens lignite	40.12	402.60[abc]	79.75	174.15[ab]
	NS		NS	
Phosphorus main effect	33.48[c]	354.96[a]	76.77[b]	

Conclusion

Concluded from the present research results that the liquid *formulations* of beneficial organisms have performed better and also they are at par with that of lignite based *formulations* in performing nitrogen fixation, P solubilization and PGPS production for the benefit of aerobic rice and thus improve the growth parameters and yield quality.

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