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Research Article

Efficacy of co-inoculation of the *rhizobium* and *pseudomonas* in combination with chemical fertilizer on the productivity of rice under legume-based cropping system

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Abstract
The main production restriction in Nepal is a shortage of nitrogen and phosphorus-based fertilizers. In addition, the excessive use of chemical fertilizers to increase rice production alters the soils' physicochemical and biological qualities. The effective application of biofertilizers obtained from beneficial microorganisms is on demand for increasing agriculture production as well as maintaining soil health sustainably. The experiment was conducted in a completely randomized block design (RCBD) with five treatments and four replications at Grain Legumes Research Program (GLRP), Khajura, Banke in order to find out the response of co-inoculation of *Rhizobium* and *Pseudomonas* in combination with different dose of recommended chemical fertilizers. The application of the co-inoculated *Rhizobium* and *Pseudomonas* at the dose of 800 g/ha along with the recommended N:P_2O_5:K_2O dose of 100:60:30 kg/ha was found to be best effective among all treatment combinations. The maximum panicle length, biological yield, grain yield and thousand grains weight were 27.75 cm, 6.55 t/ha, 6.083 t/ha' and 27.67 g, respectively. In this study, co-inoculation of *Rhizobium*, *Pseudomonas* and a full dose of prescribed chemical fertilizers improved panicle length, biological yield, grain yield and thousand grains weight considerably more than others.

Keywords: Biofertilizer, *Pseudomonas*, *Rhizobium*, rice, yield

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INTRODUCTION
The excessive use of chemical fertilizers for increasing rice production causes alterations in physico-chemical and biological properties of the soil. In such condition, the role of adequate population of beneficial microorganisms is essential in maintaining the soil health through

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organic matter decomposition, toxin removal and cycling of C, N, P and S. So, there is recent increased interest in the application of the single and combined application of plant growth promoting rhizobacteria (PGPR) in the improvement of the productivity of rice. The rice crop which contributes as staple food to more than 50% of the world's population, under monoculture and excessive fertilization becomes unfavourable for soil health as it releases up to 46% of N_2O in the environment (Winston *et al.*, 2020).

In Nepal, the major production constraint is lacking enough nitrogen and phosphorus-based fertilizers. In such case, the role of nitrogen fixing, and phosphorus solubilizing bacteria is crucial. *Rhizobium* play significant role in nitrogen fixation through symbiotic relationship with nodules in legumes (Ohara, 2001). While phosphorus being most essential nutrient next to nitrogen plays role in crop growth, N_2 fixation and nodulation (Abel *et al*., 2002).

Though phosphorus is abundant in soil, it's not readily available to crops. Specially, in case of mostly acidic soil just like in Nepal, the phosphorus is locked up in the form of iron and aluminium salt which are in insoluble phosphate form. That's where the role of phosphate solubilizing bacteria lies, which can solubilize and transform insoluble phosphate into soluble form in the soil and make them available to the crops. *Pseudomonas* is one of them.

The inoculation of the soil microbes has proven to increase nutrients uptake capacity and water use efficiency. Among the plant growth promoting rhizobacteria, *Rhizobium* and *Pseudomonas* are the commonly utilized, whose efficacy depends on their survival in the soil, crop compatibility, their interaction ability, and overall environmental factors (Martinez *et al.,* 2010). Their effects are direct in biofertilization through organic matter decomposition, stimulation of root growth and plant stress control while indirect in reducing the impact of diseases through production of phytohormones and improvement of nutrients availability (Egamberdieva *et al*., 2014).

Sarma & Saikia (2014) reported that *Pseudomonas aeruginosa* strain has improved the growth of *Vigna radiata* (mung beans) plants under drought conditions. Ahmad *et al.*, (2013) and Naveed *et al.*, (2014) reported that the stomatal conductance (water vapor exiting through the stomata leaf) of plant leaf was higher in PGPR inoculated plants than non-PGPR inoculated ones under drought conditions. The finding from both studies proves that PGPR-inoculated plants tend to improve the water-use efficiency of plants.

The deficiencies of several nutrients like nitrogen, phosphorus, sulfur, iron, zinc, boron, and molybdenum have been reported to reduce nodulation in legumes (FAO, 1982). And such deficiencies are prevalent more in acidic soils like Nepal, whereas the combined inoculation of *Rhizobium* with mineral fertilizer has been reported to increase the nodulation and yield in soybean, lentil, and chickpea (Bhattarai & Maskey, 1987). To find out the best combination of PGPRs with mineral-based fertilizers for the increment of rice grain yield, this experiment was conducted. The specific objective of this study was to evaluate the effect of co-inoculation of *Rhizobium* and *Pseudomonas* along with a recommended dose of chemical fertilizer for increasing rice productivity under a legume-based cropping system.

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MATERIALS AND METHODS

Experimental site

The experiment was conducted at the research field of the Grain Legumes Research Program (GLRP), Khajura, Banke, Nepal (28° 06" 45' N latitude, 81° 35" 58' E longitude, and 182 masl) from July 2018 to November 2020. The soil type of experimental plots was found to be sandy loam.

Experimental design and treatments

The field experiment was conducted in GLRP, Khajura, Banke, Lumbini Province during the summer season of three consecutive years, 2018, 2019 and 2020. The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications and five treatments. The total area of experimental plot was 240 m^2 with each plot of 12 m^2 (4 m × 3 m). The treatment detailed was given as below:

T1: Control
T2: Inoculation with *Rhizobium* and *Pseudomonas* (800 g/ha)
T3: Fertilization with full dose of recommended $N:P_2O_5:K_2O$ (100:60:30 kg/ha)
T4: Inoculation with *Rhizobium* and *Pseudomonas* (800 g/ha) + full dose of recommended $N:P_2O_5:K_2O$ (100:60:30 kg/ha)
T5: Inoculation with *Rhizobium* and *Pseudomonas* (800 g/ha) + half dose of recommended $N:P_2O_5:K_2O$ (100:60:30 kg/ha)

Climatic condition of the study area

During the study period of June to November the study area received 1144 mm (2018), 1737.6 mm (2019) and 58.7 mm (2020) rainfall, where the November was the coolest month. The mean minimum temperature, maximum temperature, rainfall and relative humidity of the study area are presented in Table 1.

Table 1. Average temperatures, rainfall and relative humidity during study periods of the years, 2018, 2019 and 2020

	2018				2019				2020		
Month	Min Tem (ºC)	Max Tem (ºC)	Rainfall (mm)	Relative humidity (%)	Min Tem (ºC)	Max Tem (ºC)	Rainfall (mm)	Relative humidity (%)	Min Tem (ºC)	Max Tem (ºC)	Rainfall (mm)
June	25.9	34.8	580.9	87.3	26.0	35.1	590.9	77.5	26.5	35.2	15.6
July	26.6	32.8	392.8	85.7	26.5	33.5	534.9	91.9	28.3	33.6	16.8
August	26.3	33.5	170.4	84.6	26.9	34.8	193.7	84.7	29.3	34.1	15.6
September	22.6	33.1	0	80.2	25.6	33.1	418.1	88.4	28.1	34.2	10.7
October	13.9	30.4	0	93.3	20.6	31.4	0	89.9	20.7	31.5	0
November	10.5	25.7	0	87.3	15.5	28.8	0	50.3	15.3	25.9	0

Field operations

The rice variety Ramdhan was transplanted in the same plot of row to row and plant to plant spacing of 20 cm × 20 cm during the 2nd week of July. In rice, fertilizers were applied @ 100:60:30 kg $N:P_2O_5:K_2O$ per ha. The full dose of P_2O_5 and K_2O, and half dose of N were applied at the time of transplanting and other remaining dose of N was applied at 30 days after transplanting, and the remaining cultural practices like weeding and irrigation were adopted as per needed. Initially planted mungbean was harvested and the biomass was incorporated into the same plot.

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Data collection and statistical analysis

All the data on yield and yield attributing parameters like plant height, tillers per meter square, panicle length, biological yield, grain yield and thousand grains weight were analyzed statistically by using MS Excel and GenStat at probability level $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of bio and chemical fertilizers on the agronomic character of rice (2018)

Table 2 shows the impact of chemical fertilizers, biofertilizer, and mixture of biofertilizer and chemical fertilizers on the agronomic features of rice plants during the study year of 2018. The treatment with the prescribed dose of $N:P_2O_5:K_2O$ performed significantly better than all other treatments for most agronomic characteristics measured. With treatment T3 (the recommended $N:P_2O_5:K_2O$ dose), the average plant height (29.25 cm), average panicle length (29.25 cm), thousand grain weight (21.75 g) and tiller per meter square (273) were the highest among the others. Mixture of recommended dose of $N:P_2O_5:K_2O$ and biofertilizer (*Pseudomonas* and *Rhizobium*), treatment T4 had the maximum biological yield and grain yield of 6.75 t/ha and 6.2 t/ha, respectively (Table 2). The control treatment performed poorly in every measure evaluated.

Table 2. Treatment effects on yield and yield attributing parameters of rice during 2018

Treatments	PH (cm)	TPM	PL (cm)	BY (t/ha)	GY (t/ha)	TGW (g)
T1	27.25b	251.50b	27.25b	4.50c	5.12d	20b
T2	27.75ab	259.7ab	27.75ab	5.57b	5.3cd	20b
T3	29.25a	273.0a	29.25a	5.95b	5.72b	21.75a
T4	26.75b	256.2b	26.75b	6.75a	6.2a	20.75b
T5	26.50b	254.5b	26.50b	5.50b	5.53bc	20.25b
SE	0.84	6.55	0.84	0.25	0.13	0.41
LSD $_{(0.05)}$	1.83	14.27	1.83	0.55	0.28	0.90
CV (%)	4.33	3.58	4.33	6.34	3.23	2.84

PH= Plant height, TPM= Tillers per meter square, PL= Panicle length, BY= Biological yield, GY= Grain yield, TGW= Thousand grains weight, CV= coefficient of variation, SEM= Standard Error of Mean, SE= Standard Error, LSD= Least Significant Difference.

There was a significant effect of chemical fertilizer, biofertilizer, and the mixture of biofertilizer and chemical fertilizer, on the agronomic properties of rice plants. Our experiment treatment with a combination of chemical fertilizers and biofertilizers (*Rhizobium* and *Pseudomonas*) yielded the tallest plants. *Rhizobium* species are known to produce phytohormones such as Indole acetic acid (IAA), and gibberellins, along with nitrogen fixation, which may have contributed to plant height increase (Nadarajah, 2017). *Pseudomonas* has been discovered to be an excellent phosphate solubilizer with biocontrol abilities (Beneduzi *et al.*, 2012). The use of biofertilizers is known to increase macronutrient and micronutrient absorption (Nadarajah, 2017). Nguyen *et al.*, (2022) reported the importance of the co-inoculation of specific microbes in soil quality maintenance and plant growth. Biofertilizer inoculation has been shown to improve plant N uptake and enhance several physiological aspects of plant performance. One theory is that the biofertilizer stimulates root growth, increasing the uptake of indigenous N from the soil. Second, greater root development may reduce N fertilizer losses, and biological N fixation may be a third approach (Sharma *et al.*, 2010).

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Effect of bio and chemical fertilizers on the agronomic character of rice (2019)

In the study year 2019, the treatment including a combination of recommended $N:P_2O_5:K_2O$ doses and biofertilizer (*Pseudomonas* and *Rhizobium*), treatment T4 beat all other treatments in all agronomic aspects except for tiller per meter square which are shown in Table 3. The average plant height, panicle length, biological yield, grain yield, and thousand grain weight were 24.75cm, 29.25cm, 7.78 t/ha, 7.20 t/ha, and 37 g respectively. It was followed by the recommended $N:P_2O_5:K_2O$ dose (T3), with an average panicle length of 27 cm, biological production of 6.78 t/ha, grain yield of 6.28 t/ha, and thousand grain weights of 35 g. The control treatment performed poorly in all research parameters except for number of tiller per meter square (326.5).

Table 3. Treatment effects on yield and yield attributing parameters of rice during 2019

Treatments	PH (cm)	TPM	PL (cm)	BY (t/ha)	GY (t/ha)	TGW (g)
T1	24.50	326.50	21.75c	5.62c	5.15d	21.0c
T2	23.25	289.25	22.5c	6.22bc	5.65cd	22.0c
T3	24.25	285.25	27b	6.78b	6.28d	35.0a
T4	24.75	276.25	29.25a	7.78a	7.20a	37.0a
T5	23.00	316.00	23.25c	6.47b	5.88bc	29.5b
SE	0.82	44.49	0.69	0.38	0.27	1.49
LSD $_{(0.05)}$	ns	ns	1.50	0.83	0.6	3.24
CV (%)	4.87	21.70	3.96	8.28	6.56	7.29

PH= Plant height, TPM= Tillers per meter square, PL= Panicle length, BY= Biological yield, GY= Grain yield, TGW= Thousand grains weight, CV= coefficient of variation, SEM= Standard Error of Mean, SE= Standard Error, LSD= Least Significant Difference.

The use of chemical fertilizers and biofertilizers increased the number of grains per panicle, biological yield, and 1000-grain weight of rice. This could be attributed to an increase in nutritional availability. According to Maurya *et al.,* (2021), increasing the N level resulted in a considerable increase in grain yield. Singh *et al*., (2015) reported that biofertilizer inoculation improved rice grain yield. This is also supported by Biswas *et al.*, (2000) who concluded that the rice straw, as well as grain yield, increased due to increased nitrogen uptake by rhizobial inoculation. The increase in yield caused by biofertilizer inoculates may not be attributable entirely to N fixation or phosphate solubilization, but also to various additional causes such as the release of growth-stimulating compounds, the control of plant pathogens, and the proliferation of beneficial organisms (Mathews *et al.*, 2006). The effect of biofertilizer in conjunction with inorganic fertilizer on rice yield, when grown alone or in combination with other crops such as soybean and Vigna radiata, is well documented (Sawar 2005; Wu *et al.,* 2005). Maintaining appropriate N supply from soil and fertilizer at key growth phases is a critical factor for yield. The capacity of microorganisms to maintain nitrogen supply throughout the vegetative and reproductive growth of the plants can be attributed to the better production of a mixture of chemical fertilizer and biofertilizer (Asif *et al*., 1999; Kropff *et al*., 1993).

Effect of bio and chemical fertilizers on agronomic character of rice (2020)

During the study year 2020, the mixture of recommended doses of $N:P_2O_5:K_2O$ and biofertilizers (T4) had the maximum average plant height (102.25 cm), average panicle length (27.25 cm), grain yield (4.85 t/ha) and thousand grains weight (5.25 g) which is shown in Table 4. The study parameter biological yield was maximum (6.80 t/ha) with recommended dose of $N:P_2O_5:K_2O$ (T3) followed by mixture of biofertilizer and recommended dose of

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$N:P_2O_5:K_2O$ (T4) at 5.12 t/ha. When compared to all other treatments, the control treatment performed poorly.

The use of biofertilizers in conjunction with chemical fertilizers resulted in a significant increase in biological yield. The administration of the recommended dose of $N:P_2O_5:K_2O$ in combination with *Pseudomonas* and *Rhizobium* resulted in higher biological yield, while the control resulted in the lowest yield. The increased biological output could be related to the amount of nitrogen accessible through biofertilizers along with $N:P_2O_5:K_2O$ applied. Nitrogen is known to enhance tillering, improve leaf length and width, which increases dry matter and is responsible for an increase in biological yield (Khnda and Dixit, 1995). Gopalswamy *et al.,* (1989) also reported that soil application of biofertilizers boosted rice biological yield.

The thousand grains weight varied significantly between the controls, $N:P_2O_5:K_2O$ fertilizer, and $N:P_2O_5:K_2O$ plus biofertilizer. $N:P_2O_5:K_2O$ plus biofertilizer produced the greatest thousand grains weight, followed by recommended $N:P_2O_5:K_2O$ fertilizer alone. A higher thousand grains weight of seeds were observed as fertility levels increased (Venkateswarlu & Singh, 1980).

Table 4. Treatment effects on yield and yield attributing parameters of rice during 2020

Treatments	PH (cm)	TPM	PL (cm)	BY (t/ha)	GY (t/ha)	TGW (g)
T1	97.25a	279.50	18.75d	3.95cd	3.33b	20.25c
T2	94.75ab	271.25	21.0c	4.53bc	3.88b	22.0b
T3	98.75a	277.00	23.0b	6.80a	4.70a	24.0a
T4	102.25a	267.00	27.25a	5.12b	4.85a	25.25a
T5	88.75b	249.75	23.25b	3.62d	3.58b	21.75bc
SE	3.74	18.91	0.74	0.38	0.32	0.74
$LSD_{(0.05)}$	8.15	ns	1.62	0.84	0.70	1.62
CV (%)	5.50	9.95	4.65	11.80	11.31	4.65

PH= Plant height, TPM= Tillers per meter square, PL= Panicle length, BY= Biological yield, GY= Grain yield, TGW= Thousand grains weight, CV= coefficient of variation, SEM= Standard Error of Mean, SE= Standard Error, LSD= Least Significant Difference.

Combined mean separated values of all study parameter of all three study years

There was no significant difference in parameters: average plant height and numbers of tillers per meter square among the different treatment combinations however there was year wise variation in plant height and tillers per meter square. The grand mean of average plant height in first, second and third year was 90.95 cm, 23.95 cm, 96.35 cm, respectively. The parameters like panicle length, biological yield, grain yield and thousand grain weight are significantly different among all treatment combinations in all three years. The maximum panicle length, 27.75 cm was found in the combination of *Rhizobium*, *Pseudomonas* and full dose of $N:P_2O_5:K_2O$ followed by 26.42 cm in the application of recommended dose of $N:P_2O_5:K_2O$ only. Similar results were obtained in biological yield i.e., maximum biological yield, 6.55 t/ha was found in the combination of *Rhizobium*, *Pseudomonas* and full dose of $N:P_2O_5:K_2O$ followed by 6.26 t/ha in the application of recommended dose of $N:P_2O_5:K_2O$ only. Similarly, the grain yield (6.083 t/ha) as well as thousand grains weight (27.67 g) was highest in the combination of *Rhizobium*, *Pseudomonas* and full dose of $N:P_2O_5:K_2O$.

The application of the combination of *Rhizobium*, *Pseudomonas* and full dose of $N:P_2O_5:K_2O$ was found significantly higher in all parameters under consideration as compared to all

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treatment combinations. Next the full recommended dose of $N:P_2O_5:K_2O$ fertilizers was found to have significant effect over panicle length, biological yield and thousand grain weights. However, the co-inoculation of *Rhizobium* and *Pseudomonas* only and also in combination with half dose of $N:P_2O_5:K_2O$ were also found to be better responsive than the control condition.

The results showed that utilizing biofertilizer can reduce the amount of recommended inorganic fertilizer, with benefits on growth and production comparable to using recommended fertilizer alone. Now, that the ecosystem is suffering from enormous inorganic fertilizer overloads that are damaging to soil health and sustainable productivity. This study and other previous research suggest that biofertilizer, as an ecologically friendly resource, provides a feasible alternative not only for maintaining high yield but also for safeguarding and conserving the environment. Furthermore, a continuing search for biofertilizers and environmentally acceptable crop production system should be undertaken.

Table 5. Effect of co-inoculation of rhizobium and pseudomonas with chemical fertilizers in yield and yield parameters of rice

SN	Treatments	PH (cm)	TPM	PL (cm)	BY (t/ha)	GY (t/ha)	TGW (g)
1	T1	71.25a	285.8a	22.58c	4.69c	4.53c	20.42c
2	T2	70.50a	273.4a	23.75ab	5.44b	4.94c	21.33c
3	T3	71.58a	278.4a	26.42a	6.26a	5.56b	26.92a
4	T4	71.92a	266.5a	27.75a	6.5a	6.083a	27.67a
5	T5	66.83a	273.4a	24.33b	5.2ab	4.992c	23.83b
GM	Year 1	90.95	259	27.50	5.65	5.575	20.55
	Year 2	23.95	298.6	24.75	6.57	6.03	28.90
	Year 3	96.35	268.9	22.65	4.66	4.065	22.65
F test	Treatment	0.097	0.88	<.001	<.001	<.001	<.001
	Year	<.001	0.025	<.001	<.001	<.001	<.001
	Treatment x Year Interaction	0.208	0.858	<.001	0.004	0.18	<.001
SEM		0.450	1.428	13.29	0.364	0.154	0.1182
$LSD_{(0.05)}$		1.284	4.075	37.92	1.039	0.4396	0.337
CV (%)		1.3	7.0	16.7	5.1	9.5	7.8

PH= Plant height, TPM= Tillers per meter square, PL= Panicle length, BY= Biological yield, GY= Grain yield, TGW= Thousand grains weight, CV= coefficient of variation, SEM= Standard Error of Mean, SE= Standard Error, LSD= Least Significant Difference.

CONCLUSION

The panicle length, biological yield, grain yield and thousand grain weight increased significantly with the co-inoculation of *Rhizobium* and *Pseudomonas* along with full dose of chemical fertilizers in the experiment. Neither the application of full recommended dose of chemical fertilizers nor the combination of half dose chemical fertilizers with co-inoculation of *Rhizobium* and *Pseudomonas* gave better results in terms of grain yield. So, the combination of *Rhizobium*, *Pseudomonas* and full dose of chemical fertilizers was found to be superior among all treatments in the experiment.

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Authors' Contributions

All authors listed have made a substantial, direct and intellectual contribution to the experimentation, data recording, and analysis and manuscript preparation.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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