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# **Research Article**

# Biofertilizer Reduces the Dependency on Chemical Fertilizer on Wheat

**Production** 

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# Introduction

The current global demand for nitrogeneous, phosphatic and potassic fertilizer are 111.59 million mt, 49 million mt and 40.23 million mt respectively and the supply is in positive balance with total world capacity of fertilizer production 264 million mt for agricultural use (FAO, 2022). The Indo-Gangetic Plains in Bangladesh, India, Pakistan and Nepal comprise an area of 13.5 million ha of wheat, feeding population of about a billion. The region comprises of about 30% of global synthetic fertilizer consumption, and

only 32% of farmer use organic fertilizers (Velayudhan et al., 2021).

Profitability from wheat production is declining primarily due to higher prices and non-availability of synthetic chemical fertilizer in time in Nepal. Out of tendered 5,20,000 mt of chemical fertilizers in 2019/20, 298,000 mt of fertilizer has been imported which shows severe fertilizer crisis is in Nepal (MoF, 2021). At present, there is no any chemical fertilizer industry in Nepal (Shrestha, 2010). In 2021 AD, record highest sum of US \$ 117.9 million was allocated for procurement of chemical fertilizer by

# **Abstract**

Scarce availability of chemical fertilizers threatens sustainability of wheat production in Nepal. Combined application of chemical and biofertilizer can reduce chemical fertilizer and enhance the yield under limited fertilizer availability. A field experiment was carried out to assess the combined application of chemical and biofertilizer on the growth and yield of spring wheat at Rampur, Chitwan from November 2020 to April 2021. The experiment was laid out in strip plot design with four nitrogen and P<sub>2</sub>O<sub>5</sub> levels (0, 50, 75 and 100% of recommended dose) in vertical plots and three biofertilizer application methods (not applied, seed applied and soil applied) in horizontal plots with three replications. The research results revealed significant interaction between nitrogen and P2O5 levels and biofertilizer application methods. The highest grain yield (4624.48 kg ha<sup>-1</sup>) of wheat was obtained with 100% levels of N and P2O5 with soil applied biofertilizer at par with 75% levels of N and P<sub>2</sub>O<sub>5</sub> with soil applied biofertilizer (4457.54 kg ha<sup>-1</sup>). The total nitrogen uptake was higher in biofertilizer applied wheat as compared with no application of biofertilizer although statistically not significant. The yield increment in 100% and 75% N and P2O5 levels with soil applied biofertilizer over same level N and P<sub>2</sub>O<sub>5</sub> with no biofertilizer application were 10.96% and 29.60% respectively. The higher gross return, net return and B:C ratio were obtained with 75% and 100% N and P<sub>2</sub>O<sub>5</sub> levels with soil and seed applied biofertilizers. The result indicates that 25% recommended dose of N and P2O5 could be reduced by soil application of biofertilizer without compromising the grain yield of wheat.

government and the budget is still deficit by US \$ 39.31 million (Prasain, 2022). Regulating inorganic chemical fertilizer price by subsidy on price or transportation cannot stimulate fertilizer use in Nepal since price response and returns to inorganic chemical fertilizer is relatively low in hills and mountains (Takeshima *et al.*, 2016). This presents huge potentiality of alternative fertilizers sources like biofertilizers to mitigate these dual problems of synthetic fertilizer unavailability and low returns to price on synthetic fertilizer.

Therefore, the present investigation was carried out with following objectives:

#### General objective

• To reduce the amount of chemical fertilizer for sustained wheat production in Nepal

# Specific objective

 To evaluate different levels of nitrogen, phosphorus, biofertilizer application and their interaction on growth, yield attributes, yield and economics of wheat

# Methodology

# Experiment site

The experiment was conducted in research block of Agronomy farm of Agriculture and Forestry University (AFU) Rampur, Chitwan from November 2020 to April 2021. The research site lies in the subtropical humid zone of Nepal at an altitude of 288 meters above sea level. Geographical coordinate of the research site is  $27^{0}38$  North latitude and  $84^{0}19$  East longitudes. The experimental plot was under rice-wheat cropping system for past three years. The texture of the soil of research site was sandy loam. The soil was acidic in nature with low in organic matter (2.35%), available phosphorus (20.9 kg ha<sup>-1</sup>) and available potassium (87.10 kg ha<sup>-1</sup>) and medium in total nitrogen (0.15%).

#### **Experiment Details**

The experiment was laid out in strip plot design with three replications with three Biofertilizer treatment as horizontal factor (1. B0: No biofertilizer, 2. BSE: Biofertilizer applied through seed treatment and 3. BSO: Biofertilizer through soil) and four levels of Nitrogen and Phosphorus as vertical factor (1. NP0: 0% Nitrogen and Phosphorus, 2. NP50: 50% recommended dose of Nitrogen and Phosphorus, 3. NP75: 75 % recommended dose of Nitrogen and Phosphorus and 4. NP100: 100% recommended dose of Nitrogen and Phosphorus). Recommended dose of fertilizer for wheat 120:50:50 N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O was used through Urea, Single Super Phosphate and Muriate of Potash respectively. Full dose of phosphorus and potassium was applied as basal while nitrogen fertilizer was applied in 3 splits with 50% nitrogen fertilizer applied at basal and remaining 50% was applied at two splits during Crown Root Initiation (CRI) stage and before heading respectively.

The field was ploughed two times right before sowing of wheat and weeds were removed manually. Fine tilth of the soil was made one day before sowing at the time of final land preparation. Seeds of variety Gautam were sown on 25<sup>th</sup> of November at the rate of 120 kg ha<sup>-1</sup>. Spacing of 25 cm between rows and continuous planting within rows were maintained. 2, 4-D ethyl ester was applied for control of weed at the rate of 0.5 kg a.i. ha<sup>-1</sup> at 25 days after sowing. Two irrigations were given during the entire crop growth at Crown Root Initiation (CRI) stage and flowering stage. Cholopyrifos (50% EC) and Cypermethrin (5% EC) were used at the rate of 3 ml per litre of water for pest control 50 days after sowing.

The mean maximum temperature of the experiment ranged from 10°C to 34°C, with April recording the highest and November the lowest values. Relative humidity ranged from 59% in April to 96% in November. The average minimum temperature ranged from 7°C (November) to 15 °C (April). Only 34 mm of rain was recorded in the month of April.

Liquid biofertilizer prepared in glycerine consisting of *Azotobacter* sp., *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* was procured from Om Krishi Udhyog Private Limited Khairehani -1, Chitwan on 15<sup>th</sup> November. The procured biofertilizer had *Azotobacter* (CFU count  $5\times10^2$  ml<sup>-1</sup> minimum), *Bacillus amyloliquefaciens* (CFU count  $2\times10^4$  ml<sup>-1</sup> minimum) and *Pseudomonas fluorescens* (CFU count  $2\times10^4$  ml<sup>-1</sup> minimum).

A 10 % jaggery solution was prepared in water by boiling. The 10% jaggery solution was used as sticker for liquid biofertilizer inoculants to seed. The contents were cooled and liquid based biofertilizer inoculant was mixed in 10% jaggery solution. The slurry thus prepared was coated uniformly over the wheat seeds spread on a cemented floor such that a thin layer was formed around the wheat seed. The inoculated seeds were dried in shade for 30 minutes before sowing into the plots. Biofertilizer seed treatment was applied at the rate of 100 ml for 5 kg seed.

A 10% jaggery solution was prepared by boiling in water. After the cooling of the content, Biofertilizer was mixed in 10% jaggery solution. The solution was left undisturbed for few days. After appearance of foams in the solution due to Bacillus, the solution was stirred 2-3 times per day. After 15 days the slurry was ready to be incorporated in vermicompost. The slurry thus prepared was mixed along with vermicompost (1000 ml liquid inoculum per 25 kg vermicompost). The mixture prepared was stored for 1 week under sterile condition for inoculum multiplication and 50% moisture was maintained during entire period. The mixture was used for soil application in rows by thoroughly mixing with the soil inside treated plot (600 g per plot). Irrigation was provided immediately after mixing with soil as the plots were dry. Equal amount of vermicompost (600 g per plot) without biofertilizer was added to untreated and

seed treated plot to provide uniform condition as described by Khandare *et al.* (2020).

The complete recorded data were subjected to analysis of variance and Duncan's Multiple Range Test (DMRT) for mean comparison at probability level <0.05 from the reference of Gomez and Gomez (1984). Considering the software programs, Microsoft word 2016 was used for word processing, MS excel for table, statistical analysis was carried out by using R Studio. For estimating total nitrogen content of straw and grain samples were digested with concentrated sulphuric acid and digestion mixture in micro Kjeldahl's assembly.

#### **Results and Discussions**

# Yield attributing characters

Effective tillers m<sup>-2</sup>, sterility percentage, thousand grain weight and number of grains per spike were statistically non-significant as affected by biofertilizer application methods (Table 1). Although these yield attributing parameters were statistically non-significant, there was slight improvement in biofertilizer applied plots either soil or seed applied than without biofertilizer application.

Sterility percentage and thousand grain weight was statistically non-significant for different nitrogen and phosphorus levels but with higher levels of nitrogen and phosphorus sterility percentage was relatively lower (Table

2). Effective tillers m<sup>-2</sup> and number of grains spike<sup>-1</sup> were significantly influenced by different levels of nitrogen and phosphorus with statistically higher values with increasing nitrogen and phosphorus levels. Kushare *et al.* (2009) stated that because of increasing dry matter accumulation with each increasing level of nitrogen and phosphorus, partitioning of dry matter into reproductive parts resulted in significantly higher number of spikes m<sup>-2</sup> and number of grains spike<sup>-1</sup>. Similar results were also obtained by El-Gizawy (2009); El-Razek and El-Sheashtawy (2013) and Shende *et al.* (2020).

# Number of effective tillers per square meter

There was significant interaction between levels of nitrogen and  $P_2O_5$  and biofertilizer application for effective tillers per square meter (Table 2). With 75% and 100% nitrogen and  $P_2O_5$  level, effective tiller per  $m^2$  didn't vary among biofertilizer application methods. At 50% nitrogen and  $P_2O_5$  level, soil applied biofertilizer had significantly higher effective tillers per  $m^2$  (277.33) followed by no application (244) and seed applied biofertilizer (216.67). While at 0% nitrogen and  $P_2O_5$  level, seed applied biofertilizer had highest effective tillers per  $m^2$  (205.67) statistically at par with no biofertilizer application (191) but significantly higher than soil applied biofertilizer (166). This result was in line with Al-Shamma and Al shahwany (2014); Hussain *et al.* (2016) and Taha *et al.* (2016).

**Table 1**: Yield attributes of Wheat as influenced by biofertilizer application methods and different levels of nitrogen and phosphorus at Rampur, Chitwan in 2020-21

Treatments	Effective	Thousand grain weight	Number of	Sterilitypercentage
	tillers per m <sup>2</sup>		grains spike <sup>-1</sup>	
Horizontal Factor: Biofertilizer a	pplication methods			
Biofertilizer not applied	251.25	47.82	38.25	37.31
Biofertilizer seed applied	250.00	48.27	38.92	35.75
Biofertilizer soil applied	261.25	49.11	39.75	35.62
LSD (0.05)	NS	NS	NS	NS
SEm±	6.31	0.61	0.62	0.90
CV (%)	8.59	4.38	5.51	8.59
Vertical Factor: NP levels				
NP0	187.56 <sup>c</sup>	49.61	35.22°	37.23
NP50	246.00 <sup>b</sup>	49.34	38.11 <sup>b</sup>	37.97
NP75	289.78 <sup>a</sup>	47.98	37.67 <sup>b</sup>	35.21
NP100	294.89 <sup>a</sup>	46.66	44.89 <sup>a</sup>	34.50
LSD (0.05)	19.44	NS	2.13	NS
SEm±	5.62	0.76	0.62	1.02
CV (%)	6.62	4.72	4.74	8.48
Grand Mean	254.56	48.40	38.97	36.23

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance.

# Number of grains per spike

Interaction between nitrogen and  $P_2O_5$  levels and biofertilizer application methods was significant in case of

number of grains per spike (Table 3). At 0% nitrogen and  $P_2O_5$  level, no biofertilizer plots had significantly higher number of grains per spike (36.67) than soil applied

biofertilizer (35) but statistically at par with seed applied biofertilizer (34). Biofertilizer application had statistically similar number of grains per spike with 50% and 75% nitrogen and  $P_2O_5$  level. At 100% nitrogen and  $P_2O_5$  level, soil applied biofertilizer had significantly higher number of grains per spike (46.33) than no biofertilizer application (43.33) but at par with seed applied biofertilizer (45). This result was in conformity with Cisse *et al.* (2019); Hussain *et al.* (2016) and Mahato and Kafle (2018).

# Grain yield, Straw yield and Harvest index

#### Grain yield

Grain yield was not influenced by different biofertilizer application methods but were relatively higher for biofertilizer application (Table 4). Grain yield in soil applied biofertilizer and seed applied biofertilizer were 8.45% and 4.67% higher than without biofertilizer application respectively. Taha *et al.* (2016) stated that biofertilizers enhances biological activity of soil with better

mobilization of nutrient from fertilizer Biofertilizers also regulate dynamics of organic matter decomposition and the availability of plant nutrients along with increased fixation of nitrogen. Relatively higher yield in soil applied biofertilizer might be due to effect of carrier based biofertilizer effectively increasing growth and maintaining osmotic balance with increment in antioxidant enzymes activity and yield parameters (Hassan and Bano, 2016). Grain yield was significantly influenced by main effect of different levels of nitrogen and P<sub>2</sub>O<sub>5</sub> (Table 4). Higher grain yield at higher levels might be due to cumulative effect of yield attributes like effective tiller m<sup>-2</sup>, number of grains spike-1, spike length, spike weight and grain weight spike<sup>-1</sup>. Mohanta et al. (2020) reported that when levels of nitrogen and P2O5 were increased from control, effective tillers per m<sup>-2</sup> and number of grains per spike also increased in ascending order. Kushare et al. (2009) reported that recommended dose of fertilizer was more efficient and high yielding.

**Table 2**: Effective tillers per square meter of wheat as influenced by interaction between biofertilizer application methods and nitrogen and P<sub>2</sub>O<sub>5</sub> levels at Rampur, Chitwan, 2020-2021

		Effective tillers per square meter	
NP levels		Biofertilizer application methods	
-	Biofertilizer not applied	Biofertilizer seed applied	Biofertilizer soil applied
NP0	191 <sup>cd</sup>	205.67°	166.00 <sup>d</sup>
NP50	244 <sup>b</sup>	216.67°	277.33ª
NP75	283.67 <sup>a</sup>	$282.00^{a}$	303.67 <sup>a</sup>
NP100	$289.00^{a}$	297.67 <sup>a</sup>	$298.00^{a}$
LSD (0.05)		26.25	
SEm (±)		8.52	
CV (%)		5.80	

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance. NPO, No application of N and P<sub>2</sub>O<sub>5</sub>; NP50, 50% of recommended dose of N and P<sub>2</sub>O<sub>5</sub>; NP75, 75% of recommended dose of N and P<sub>2</sub>O<sub>5</sub>; NP100, 100% of recommended dose of N and P<sub>2</sub>O<sub>5</sub>.

**Table 3**: Number of grains per spike of wheat as influenced by interaction between biofertilizer application methods and nitrogen and P<sub>2</sub>O<sub>5</sub> levels at Rampur, Chitwan, 2020-2021

		Number of grains per spike		
NP levels	Biofertilizer application methods			
	Biofertilizer not applied	Biofertilizer seed applied	Biofertilizer soil applied	
NP0	36.67 <sup>cd</sup>	34.00 <sup>e</sup>	35.00 <sup>de</sup>	
NP50	$38.00^{\circ}$	37.67 <sup>c</sup>	38.67°	
NP75	$35.00^{de}$	39.00°	39.00°	
NP100	43.33 <sup>b</sup>	$45.00^{ab}$	46.33 <sup>a</sup>	
LSD (0.05)		2.42		
SEm (±)		0.79		
CV (%)		3.49		

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance. NP0, No application of N and  $P_2O_5$ ; NP50, 50% of recommended dose of N and  $P_2O_5$ ; NP75, 75% of recommended dose of N and  $P_2O_5$ ; NP100, 100% of recommended dose of N and  $P_2O_5$ .

**Table 4**: Grain yield (kg ha<sup>-1</sup>), straw yield (kg ha<sup>-1</sup>) and harvest index (%) of wheat as influenced by biofertilizer application methods and different levels of nitrogen and phosphorus at Rampur, Chitwan in 2020-21

Treatments	Grain Yield (kg ha <sup>-1</sup> )	Straw yield (kg ha <sup>-1</sup> )	Harvest Index (%)
Horizontal factor: Biofertilizer application methods	(kg IIa )	(Kg IIa )	muex (70)
Biofertilizer not applied	3416.47	5429.67	35.59
Biofertilizer seed applied	3651.92	5551.72	36.59
Biofertilizer soil applied	3720.06	5681.00	36.23
LSD (0.05)	NS	NS	NS
SEm±	123.64	131.96	0.62
CV (%)	11.91	8.23	5.97
Vertical Factor: NP levels			
NP0	2299.85 <sup>d</sup>	3842.37°	$34.45^{b}$
NP50	3715.40°	5356.22 <sup>b</sup>	37.90a
NP75	4030.45 <sup>b</sup>	6326.11 <sup>a</sup>	$35.86^{ab}$
NP100	4338.90a	6692.44 <sup>a</sup>	36.33ab
LSD (0.05)	236.31	0.46	2.22
SEm±	68.32	133.86	0.64
CV (%)	5.70	7.23	5.31
Grand Mean	3596.15	5554.32	36.14

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance.

**Table 5**: Grain yield of wheat as influenced by interaction between biofertilizer application methods and different levels of nitrogen and phosphorus at Rampur, Chitwan in 2020-21

phosphorus at Rampar, Chiewan n	1 2020 21		
	Grain yield (kg ha <sup>-1</sup> )		
Biofertilizer application methods			
Biofertilizer not applied	Biofertilizer seed applied	Biofertilizer soil applied	
2302.84 <sup>d</sup>	2442.83 <sup>d</sup>	2153.87 <sup>d</sup>	
3755.46°	3746.41°	3644.34°	
3439.62°	4194.20 <sup>b</sup>	4457.54 <sup>ab</sup>	
4167.96 <sup>b</sup>	4224.25 <sup>b</sup>	4624.48 <sup>a</sup>	
	373.96		
	121.25		
	5.84		
	Biofertilizer not applied 2302.84 <sup>d</sup> 3755.46 <sup>c</sup> 3439.62 <sup>c</sup>	Biofertilizer application methods	

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance. NP0, No application of N and  $P_2O_5$ ; NP50, 50% of recommended dose of N and  $P_2O_5$ ; NP75, 75% of recommended dose of N and  $P_2O_5$ ; NP100, 100% of recommended dose of N and  $P_2O_5$ .

Grain yield was also significantly influenced by interaction effect between biofertilizer application methods and different levels of nitrogen and P<sub>2</sub>O<sub>5</sub> (Table 5). The highest grain yield (4624.48 Kg ha<sup>-1</sup>) was obtained for 100% nitrogen and P<sub>2</sub>O<sub>5</sub> level with soil applied biofertilizer which was statistically at par with 75% nitrogen and P<sub>2</sub>O<sub>5</sub> level and soil applied biofertilizer (4457.54 Kg ha<sup>-1</sup>). 75% nitrogen and P<sub>2</sub>O<sub>5</sub> level with biofertilizer soil applied was statistically at par with 100% nitrogen and P<sub>2</sub>O<sub>5</sub> level without biofertilizer application. This might be due to different root stimulating hormones like auxin, gibberellins, cytokinin etc, mechanisms of N2 fixation, ammonia excretion, phosphate solubilization by inoculated microorganisms and their reaction (Narula et al. 2011; Sood et al. 2019). Furthermore, microorganism is supplemented by vermicompost as carrier in enhancing biological properties of soil to increase nutrient mobilization from applied nutrients (Kumar et al., 2014; Rady et al., 2016). This result was also in agreement with those obtained by Abdel - Lateef, (2018); Cisse et al. (2019) and Kumar et al. (2014).

# Straw yield

Straw yield was significantly influenced by different levels of nitrogen and  $P_2O_5$  (Table 3). Straw yield was found higher when nitrogen and  $P_2O_5$  rate was increased from 0% to 100% of recommended dose. Higher straw yield at higher levels of nitrogen and  $P_2O_5$  might be due to higher dry matter production (El-Gizawy, 2009).

#### Harvest index

Harvest index was significantly influenced by different levels of nitrogen and  $P_2O_5$  (Table 3). Harvest index was also found higher when nitrogen and  $P_2O_5$  rate was increased from 0% to 100% of recommended dose. Higher harvest index might be due to higher grain yield due to well utilization of nitrogen and  $P_2O_5$  in metabolism and meristematic activity (El-Gizawy, 2009).

# Nitrogen Uptake in Grain and Straw

The main effect of biofertilizer application was statistically non-significant for nitrogen uptake in grain and straw as well as total nitrogen uptake (Table 6). Patra and Singh (2018) also found non-significant effect of biofertilizer application on nitrogen uptake as compared to no inoculation. Despite not being significant, nitrogen uptake

in grain for soil applied biofertilizer and seed applied biofertilizer were 16.4% and 9.74% higher than biofertilizer not applied plot respectively. Similarly, nitrogen uptake in straw were 13.92% and 9.13% higher than biofertilizer not applied treatment. Abbasi and Yousra (2012) found that bacterial strains like *Pseudomonas, Azospirrillium* and *Agrobacterium* produces plant growth regulators like indole acetic acid and increases root length, area and biomass which enhances nutrient uptake. Bangash *et al.* (2021); El-Zeky (2019) and Mohanta *et al.* (2020) also found higher nitrogen uptake in grain and straw of wheat due to biofertilizers *Azotobacter, Pseudomonas* and *Bacillus*.

Grain nitrogen uptake, straw nitrogen uptake and total nitrogen uptake were significantly influenced by different levels of nitrogen and  $P_2O_5$ . Total nitrogen uptake in 100% nitrogen and phosphorus level were 14.20%, 29.10% and 162.15% higher than 75%, 50% and 0% nitrogen and phosphorus level respectively while grain nitrogen uptake in 100% nitrogen and phosphorus were 14.31%, 27.09% and 140.09% higher than 75%, 50% and 0% of nitrogen and phosphorus uptake respectively. Similar results were also obtained by Mohanta *et al.* (2020); El-Gizawy (2009) and Harfe (2017).

#### **Economics**

Cost of cultivation increased as levels of nitrogen and  $P_2O_5$  increased (Table 7). This might be due to increased nitrogen and  $P_2O_5$  fertilizer cost for purchasing additional amount. Gross returns were significantly higher in 100% nitrogen and  $P_2O_5$  level followed by 75%, 50% and 0% of recommended dose of nitrogen and  $P_2O_5$ . Net returns were statistically at par for 100% and 75% of nitrogen and  $P_2O_5$  followed by 50% and 0% of nitrogen and  $P_2O_5$  level. Higher

gross and net returns at higher level of nitrogen and  $P_2O_5$  is due to more grain and straw yield which fetched more financial returns than at lower levels of fertilizer (Abdel - Lateef, 2018; Behera and Rautaray, 2010). 100%, 75% and 50% of recommended dose of nitrogen and  $P_2O_5$  had statistically similar B:C ratio than no nitrogen and  $P_2O_5$  treatment.

B:C ratio, Net returns and Gross returns were significantly influenced by interaction effects between biofertilizer application methods and different levels of nitrogen and  $P_2O_5$  (Table 8 and 9). Significantly higher gross returns (184.81 thousand ha<sup>-1</sup>), net returns (111.92 thousand ha<sup>-1</sup>) and B:C ratio (2.54) were obtained from interaction of 75% nitrogen and  $P_2O_5$  level with soil applied biofertilizer (Figure 8, 9 and 10). It might be due to higher grain and straw yield along with reduced nitrogen and phosphatic fertilizer requirement. Similar results were also obtained by Kushare *et al.* (2009); Pannu *et al.* (2022) and Singh *et al.* (2017).

B:C ratio was also found higher for 50% nitrogen and P<sub>2</sub>O<sub>5</sub> level with biofertilizer application. Behera and Rautaray (2010) stated that combined application of biofertilizers and 50% of the recommended dose of fertilizer was not as effective as 100% recommended dose of fertilizer but poor farmers with low purchasing power may choose combined use of biofertilizers along with lower fertilizer rates for obtaining higher net returns. Narula *et al.*, 2005) reported net saving of 25-30 kg nitrogen by using biofertilizer in wheat. Hussain *et al.* (2016) also reported saving of 30% fertilizer in wheat if *Pseudomonas* and *Bacillus spp.* is combined with lower levels of chemical fertilizer.

**Table 6**: Nitrogen uptake in grain and straw of wheat as influenced by biofertilizer application methods and different levels of nitrogen and phosphorus at Rampur, Chitwan in 2020-21

Treatments	Grain nitrogen	Straw nitrogen	Total nitrogen
	uptake (kg ha <sup>-1</sup> )	uptake (kg ha <sup>-1</sup> )	uptake (kg ha <sup>-1</sup> )
Horizontal factor: B	Biofertilizer application methods		
Biofertilizer not applied	55.52	21.90	77.42
Biofertilizer seed applied	60.93	23.90	84.83
Biofertilizer Soil applied	64.63	24.95	89.59
LSD (0.05)	NS	NS	NS
SEm±	2.70	1.20	3.71
CV (%)	15.47	17.66	15.33
Vertical Factor: NP levels			
NP0	$32.67^{d}$	9.58 <sup>d</sup>	42.25 <sup>d</sup>
NP50	61.72°	24.27°	85.79 <sup>c</sup>
NP75	68.62 <sup>b</sup>	28.37 <sup>b</sup>	96.98 <sup>b</sup>
NP100	78.44 <sup>a</sup>	32.32a	110.76 <sup>a</sup>
LSD (0.05)	5.42	3.14	5.12
SEm±	0.17	0.91	1.48
CV (%)	7.80	11.53	5.28
Grand Mean	60.36	23.58	83.94

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance.

**Table 7**: Profitability of wheat as influenced by biofertilizer application methods and different levels of nitrogen and phosphorus at Rampur, Chitwan in 2020-21

Tuestanoute	Cost of cultivation	Gross returns	Net returns	B:C
Treatments	(NRs. 000 ha <sup>-1</sup> )	(NRs. 000 ha <sup>-1</sup> )	(NRs.000 ha <sup>-1</sup> )	ratio
Horizontal factor: Biofertilizer app	olication methods			
Biofertilizer not applied	62.61	142.75	80.58	2.28
Biofertilizer seed applied	64.40	151.67	88.19	2.37
Biofertilizer soil applied	68.49	142.74	85.99	2.21
LSD (0.05)		NS	NS	NS
SEm±		5.147	5.147	0.09
CV (%)		11.91	20.10	13.67
Vertical Factor: NP levels				
NP0	51.52	95.84 <sup>d</sup>	44.31°	1.86 <sup>b</sup>
NP50	63.24	153.97°	90.73 <sup>b</sup>	2.44a
NP75	69.11	167.54 <sup>b</sup>	$98.44^{ab}$	$2.42^{a}$
NP100	76.20	181.18 <sup>a</sup>	106.21a	$2.42^{a}$
LSD (0.05)		10.225	10.225	0.17
SEm±		2.955	2.955	0.05
CV (%)		5.92	10.44	6.78
Grand Mean		149.63	84.92	2.28

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance.

Table 8. Gross income of wheat (NRs. 000 ha<sup>-1</sup>) at harvest as influenced by interaction between biofertilizer application methods and different levels of nitrogen and phosphorus at Rampur, Chitwan in 2020-21

		Gross income (NRs. 00	00 ha <sup>-1</sup> )
NP levels	Biofertilizer application methods		
	Biofertilizer not applied	Biofertilizer seed applied	Biofertilizer soil applied
NP0	95.96 <sup>d</sup>	101.63 <sup>d</sup>	89.92 <sup>d</sup>
NP50	155.64°	154.99°	151.29°
NP75	143.42°	174.40 <sup>b</sup>	184.81 <sup>ab</sup>
NP100	175.97 <sup>b</sup>	175.65 <sup>b</sup>	191.92 <sup>a</sup>
LSD(0.05)		15.18	
SEm (±)		4.92	
CV (%)		5.70	

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance. NPO, No application of N and P<sub>2</sub>O<sub>5</sub>; NP50, 50% of recommended dose of N and P<sub>2</sub>O<sub>5</sub>; NP75, 75% of recommended dose of N and P<sub>2</sub>O<sub>5</sub>; NP100, 100% of recommended dose of N and P<sub>2</sub>O<sub>5</sub>.

Table 9. Net returns ratio of wheat as influenced by interaction between biofertilizer application methods and nitrogen and  $P_2O_5$  levels at Rampur, Chitwan, 2020-2021

	Net returns (NRs. 000 ha <sup>-1</sup> )			
NP levels	Biofertilizer application methods			
_	Biofertilizer not	Biofertilizer seed	Biofertilizer soil applied	
	applied	applied		
NP0	46.99 <sup>ef</sup>	51.34 <sup>e</sup>	34.63 <sup>f</sup>	
NP50	94.94 <sup>bc</sup>	92.98b <sup>c</sup>	84.27 <sup>cd</sup>	
NP75	$76.86^{d}$	106.52ab	111.93 <sup>a</sup>	
NP100	103.54 <sup>ab</sup>	101.90 <sup>ab</sup>	113.18 <sup>a</sup>	
LSD(0.05)		15.18		
SEm (±)		4.93		
CV (%)		10.05		

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance. NP0, No application of N and  $P_2O_5$ ; NP50, 50% of recommended dose of N and  $P_2O_5$ ; NP75, 75% of recommended dose of N and  $P_2O_5$ ; NP100, 100% of recommended dose of N and  $P_2O_5$ .

**Table 10**: Benefit cost ratio of wheat as influenced by interaction between biofertilizer application methods and nitrogen and P<sub>2</sub>O<sub>5</sub> levels at Rampur, Chitwan, 2020-2021

		B:C ratio	
NP levels	Biofertilizer application methods		
	Biofertilizer not applied	Biofertilizer seed applied	Biofertilizer soil applied
NP0	1.96°	2.02°	1.63 <sup>d</sup>
NP50	$2.56^{a}$	$2.50^{a}$	$2.26^{\mathrm{abc}}$
NP75	2.15 <sup>bc</sup>	2.57 <sup>a</sup>	2.54ª
NP100	2.43 <sup>ab</sup>	$2.38^{ab}$	2.44 <sup>ab</sup>
LSD(0.05)		0.28	
SEm (±)		0.09	
CV (%)		7.03	

Note: Treatments means in columns followed by common letter (s) are not significantly different from each other based on DMRT at 5% level of significance. NP0, No application of N and  $P_2O_5$ ; NP50, 50% of recommended dose of N and  $P_2O_5$ ; NP75, 75% of recommended dose of N and  $P_2O_5$ ; NP100, 100% of recommended dose of N and  $P_2O_5$ .

#### Conclusion

Chemical fertilizer like nitrogen and  $P_2O_5$  application can be reduced by 25% (30 kg ha<sup>-1</sup> and 12.5 kg ha<sup>-1</sup>  $P_2O_5$ ) without compromising yield by combining biofertilizer with 75% Nitrogen and  $P_2O_5$  as compared with 100% Nitrogen and  $P_2O_5$  application. The benefit cost ratio can be increased by combining 75% N and  $P_2O_5$  application with biofertilizer as compared with 100% N and  $P_2O_5$  application without biofertilizer.

## **Authors' Contribution**

Rabin Khadka & Shrawan Kumar Sah\_designed the research plan and analysed the data; Rabin Khadka, Anil Balchhaudi, Amrit Aryal\_performed experimental works & collected the required data and prepared the manuscript. Final form of manuscript was approved by all authors.

#### **Conflict of Interest**

The authors declare that there is no conflict of interest with present publication.

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