



# Effect of Rhizobium Bacteria Inoculation Rate on Yield and Yield Components of Field Pea (*Pisum sativum* L.) at Awi Zone, Ethiopia

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## RESEARCH ARTICLE

### Abstract

Field peas (*Pisum sativum* L.) is economically a significant crop in Ethiopia. The yield of field peas in our country has fell due to poor management practices of Bio fertilizer rates usage. Three rates of bio fertilizer (0, 250 and 500g ha<sup>-1</sup>) were laid out in Randomized Complete Block Design with three replications. Number of nodules per plant, Pod numbers per plant, plant height, and effective nodule number of productive tillers, total biomass yield, grain yield, and straw yield were recorded and significantly affected by bio-fertilizer rate application. The determined or the highest grain yield (2671kg ha<sup>-1</sup>) was recorded from 250 g ha<sup>-1</sup> bio-fertilizer rate. Almost 250g ha<sup>-1</sup> bio fertilizer rates were superior for the research area. Nevertheless, more investigation has to be done under different locations and seasons to come up with practical recommendations correlated to the current study.

**Keywords:** bio-fertilizer, field pea, inoculation, nodule, *Pisum sativum*, *Rhizobium* bacteria


Received: 18 July 2023

Accepted: 10 January 2024

Published: 15 May 2024

DOI:

10.15835/buasvmcn-agr.2023.0006

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## INTRODUCTION

The global human population expected to be reach 9.8 billion people in 2050 United Nations (Slogrove et al., 2020). Africa's most densely populated regions is Ethiopia's highland areas, with low crop productivity due to limited land size. To feed this quickly growing population, agriculture must trust on scientific methods and inputs (Bodirsky et al., 2014). Regardless of that grain legumes have significant importance; their productivity is very little (Allito et al., 2015). Due to the declining in soil fertility and reduced N<sub>2</sub> fixation this low productivity in grain legumes is common in Ethiopia. Through inoculation of adaptable effective rhizobia, low productivity of grain legumes can be enhanced (Kanasa et al., 2014). There is no doubt that specificity happens between rhizobia strain and the legume variety, and compatibility between them, the strain and variety is essential for positive nodulation and nitrogen fixation even though using rhizobia in inoculating legumes can achieve substantial increases in growth and yield productivity and improve soil fertility (Korir et al., 2017). Rhizobia, a large number of bacterial species, can fix atmospheric N due to a symbiotic relationship with legume plants. In a symbiotic relationship, both the plant and bacteria contribute to each other and benefit as a result of their association (Zafar et al., 2013). Different leguminous crops require specific rhizobium species for the formation of effective nodules and nitrogen fixation. The seed of legume is required to be inoculated with the proper rhizobia specie for new establishments of bacteria around roots of plant. Integrated phosphorus supply and plant growth

promoting rhizo-bacteria on growth, nodulation, yield, and nutrient uptake in *Pisum sativum* (Ndlovu et al., 2017).

The bacteria are applied directly to the seed after the seed is coated with a sticking agent, this is more important to the bacteria are as easily close to the seed at the time of germination (Zafar et al., 2013). Localized invasion of the plant by the bacteria first takes place around the root hair followed by main roots, which shows as soon as infection with a curling response and inside these nodules that N<sub>2</sub> fixation occurs, nodules appear as small swellings on the roots of legume plants (Zafar et al., 2013).

A low level of Bio-fertilizer usage and lack of effective indigenous soil populations of rhizobia strains has a limited potential yield of field peas. In low-input cropping systems of Ethiopia, chemical fertilizers are rarely used in the production of field peas and other pulse crops; instead, these crops are used as a restorer of soil fertility for the following cereal crops (De Varennes and Goss MJ, 2007). The potential biological fertilizers would play a key role in the productivity and sustainability of soil and also protect the environment as eco-friendly and cost-effective inputs for the farmers. However, the improvement of field pea production requires the selection of effective rhizobia strains (Wosenu and Argaw, 2017). Despite the above background information, little information is documented about the role of combined fertilizer and the interactive effect of Blended fertilizer and Rhizobium in influencing nitrogen fixation, growth, and productivity of field peas. Hence, this study aimed to evaluate the combined effect of Rhizobium inoculation and Blended fertilizers application on the growth and yield components of field peas. The productivity of field peas, both national and regional productivity, 22.77 qt ha<sup>-1</sup> and 21.70 qt ha<sup>-1</sup>, respectively, remained low compared to its attainable yield. According to the Central Statistical Agency in the 2020/22 Ethiopian main cropping season, the productivity of field peas in the country was 25.8 qt ha<sup>-1</sup>, which is too low compared to its potential yield of 52 qt ha<sup>-1</sup> of worldwide (Ndlovu et al., 2017).

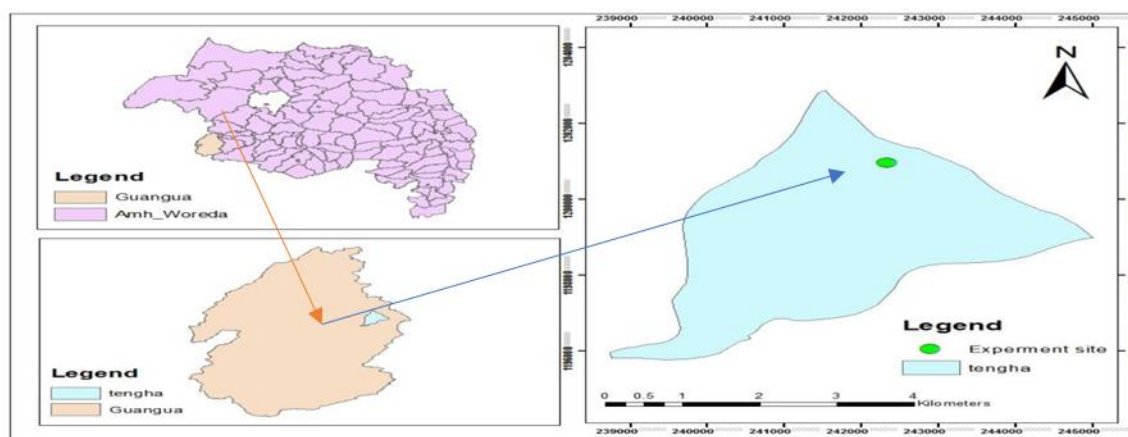
Sustainable crop production can be achieved by integrating high and low-cost inputs such as bio fertilizers and fertilizer. However, the yield in the country in general and in the study area, in particular, is still low, which is below the world average where field pea yield is 17.00 qt ha<sup>-1</sup>. The low productivity is associated with poor agronomic practices mainly inappropriate Bio fertilizer rates together with other factors including soil fertility degradation, soil erosion, inappropriate weeding practices, and the occurrence of different pests and diseases. (Belachew et al., 2022)

So, this study was important to increase the productivity of field peas by optimizing their Rhizobium Inoculation rate in the study area.

## MATERIALS AND METHODS

### Description of the study area

The study was conducted in 2020 main cropping season on the field of farmer's at *Tengeha* Kebele in Guangua district, Awi Zone, Amhara National Regional State. *Guangua Woreda* is located at 201 km far from Bahir Dar, the capital city of the Amhara Region to South western direction and 525 km from Addis Ababa to North western direction. The experimental site is located 25km away from south east of the district town *Chagni* at latitude and longitude of 10° 49' 14" N and 36° 38' 40" E, respectively. The altitude of the research site is 2051 masl.



**Figure 1.** Map of experimental study area

To characterize the soil of the experimental site or plot, soil samples were taken from 12 representative plots of the experimental field on top 0-20 cm depth before seed sowing and mixed thoroughly to have one composite soil sample. The composite soil sample was further air-dried and crushed to pass through 2mm sieve. Laboratory analysis was made by Soil Chemistry and Water Quality institution of Amhara Design and Supervision Works

Enterprise to determine important physical and chemical properties including texture, soil pH, available P, organic carbon content, cation exchange capacity (CEC) and total N using their standard methods and procedures. Texture was measured by a hydrometer method (Day, 1965). Soil organic carbon was estimated through wet digestion method while Kjeldhal procedure was used for total nitrogen (Dori et al., 2022). Soil organic carbon was determined based on the Walkley-Black chromic acid wet oxidation method (Jackson, 2005). Available phosphorous (ppm) was extracted by Olsen method (Ohno and Zibilske, 1991) and the phosphorous concentration of the solution measured by a spectrophotometer and Exchangeable acidity was estimated by saturating the soil samples with 1M KCl solution but titrated with 0.02 M NaOH (Olsen, 1954).

The soil laboratory analysis results showed that the texture of the experimental soil is clay with the composition of 30% sand, 23% silt and 45% clay (Table 1). The organic carbon content of the soil was 1.3% and the textural class was clay, which is categorized as high level (Mamo et al., 2002).

**Table 1.** Some important physio-chemical properties of the experimental soils before sowing

Property of soil	Soil analysis	
	Value	Rating
Clay (%)	45	
Sand (%)	30	
Silt (%)	23	
pH	5.5	Strongly acidic
Soil textural class	Clay	
Organic C (%)	1.3	Low
Total N (%)	0.88	Low
Available P (ppm)	7	Very low
CEC	21	Medium

### Experimental materials used for the study

Field pea of the Welki variety was used as a test crop. The variety was released in the year 2008 by Holetta Agricultural Research Centre (HARC). It can grow in 1920 to 2750 m.a.s.l. altitude area with the ecological requirement of 700 to 1200 mm annual rainfall. Field pea rhizobium strain 17 (EAL 17) was collected from Menagesha Biotechnology Institute of Addis Ababa was used as treatment.

### Experimental treatments, design, and procedures

Three Bio-fertilizer rates (0, 250, and 500 g ha<sup>-1</sup>) were laid out in a randomized complete block design (RCBD) and replicated three times. The predetermined rate of Bio fertilizer was applied during crop sowing. A Uniform rate of urea (20 kg/ha) was applied as the starter. The gross plot size was 2.5 m x 3 m (7.5 m<sup>2</sup>) while the net plot size was determined by excluding one outer row and 0.25 m length from both sides of each plot as border effects, thus the net plot size was 2 m x 2.2 m (4.4 m<sup>2</sup>) area. The spaces between blocks and plots were 1 m and 0.5 m, respectively. The crop was planted in 6 rows on each plot with inter and intra-row spacing of 40 cm and 10 cm, respectively and at 8 cm depth on July 21, 2020.

### Data to collection

Number of nodules per plant (NNP), effectiveness of the nodules (EN), nodulation rating (NR), plant height (PH), number of leaves per plant (NLP), number of productive tillers (NPT), number of pods per plant (NPP), number of seeds per pod (NSP), total biomass yield (TBY kg ha<sup>-1</sup>), seed yield (SY), A hundred seed weight (HSW), straw yield (SY), harvest index (HI %): - It was calculated as a ratio of the dry weight of the grain to the dry weight of the total aboveground biomass yield (%), multiplied by 100 (Yoshida, 1972).

$$HI (\%) = X 100 \frac{GY (kg ha^{-1})}{TBY(kg ha^{-1})}$$

where, HI= harvest index,

$$\text{GY (kg ha}^{-1}\text{) at 12.5\% moisture base} = \text{Yield obtained (kg ha}^{-1}\text{)} \times \frac{100 - \%M_{Ci}}{100 - 12.5 \text{ or } M_{Cf}}$$

M<sub>ci</sub> = initial grain moisture content

M<sub>Cf</sub> = final weight

W<sub>f</sub> = final weight was collected.

### Data analysis

The data composed from the different experiment at different growth stages were subjected to statistical analysis (ANOVA) as per the experimental design using SAS (Statistical Analysis Software) version 9.4. When the ANOVA results showed significant differences between treatments, mean separation was carried out using the least significant difference (LSD) test. Interpretations were made following the procedure described by (Arif et al., 2006).

## RESULTS AND DISCUSSIONS

### Effects of bio-fertilizer application on vegetative growth parameters

Rhizobium inoculation rate was a very highly significant effect ( $P < 0.001$ ) on the number of pods per plant. The highest number of pods per plant (121.9) were recorded from the Rhizobium inoculation rate of 500g ha<sup>-1</sup>, while the lowest number of total nodules (55.1) were produced from plots without rhizobium inoculation.

Rhizobium inoculation resulted in an increased number of pods per plant compared to un-inoculated treatment which could be because inoculated bacteria strain had good nodulation-inducing capacity over the native soil Rhizobium population, low native Rhizobium population in the soil, less competitive native Rhizobium against the inoculated. In line with this result, a significant increase in nodule number in common bean cultivars when inoculated with Rhizobium leguminosarum strain (Mehrpouyan, 2011).

**Table 2.** Effects of bio-fertilizer application on vegetative growth parameters influenced by Rhizobium rate on field pea in 2020 main cropping season

Treatment BF (g ha <sup>-1</sup> )	NPP	ENN	NR	PH	NLPP
0	55.1 <sup>c</sup>	28.8 <sup>c</sup>	55.1 <sup>bc</sup>	118.7 <sup>b</sup>	67.9 <sup>b</sup>
250	113.4 <sup>b</sup>	70.4 <sup>a</sup>	103.7 <sup>ab</sup>	129.0 <sup>a</sup>	76.9 <sup>a</sup>
500	121.9 <sup>a</sup>	62.3 <sup>b</sup>	113.6 <sup>a</sup>	133.9 <sup>a</sup>	77.7 <sup>a</sup>
CV (%)	11.4	15.6	7.1	6.8	11.8
SE ±	3.2	3.8	2.9	2.2	2.2

Note: FT=fertilizer rate, BF=bio fertilizer rate, NPP= Number of Pods per Plant, NNP=number of nodules per plant, EN=effective nodule number, NR=nodulation rating, PH=plant height, and NLPP=number of leaves per plant

The analysis of variance indicated that effective nodules were very highly significantly ( $P < 0.001$ ) influenced by the Rhizobium rate on the Faba bean.

The highest number of effective nodules (70) were recorded from the Rhizobium inoculation rate of 250g ha<sup>-1</sup>. The increase in the number of nodules with inoculation might be due to the interaction the effect of seed inoculation with an appropriate strain of Rhizobium increased root nodulation activities in field peas. Several studies have reported that the application of Rhizobium inoculant influenced nodulation N fixation of legume crops. Researchers reported that the application of Rhizobium inoculation had a positive effect on the nodulation of faba bean (Khalid et al., 2015).

Also, the numbers of effective nodules (62 and 70) were observed from the 250g ha<sup>-1</sup> and 500g ha<sup>-1</sup> Rhizobium rates respectively (Panday et al., 2011).

Researchers have demonstrated enormous potential for developing effective N-fixing inoculants to enhance N fertility in grain legume production. Many researchers agree that inoculation of Faba bean by proper Rhizobium strain results in a higher number of effective nodules per plant than, that of un-inoculated one. Other researchers also reported that the inoculation of Faba bean with effective Rhizobium strains had significantly increased the nodulation and N<sub>2</sub> fixation rates relative to the indigenous Rhizobium population ( Sh Sadak et al., 2015). Similarly, reported that the inoculated treatments had significantly higher effective nodule numbers per plant over un-inoculated treatments (control) on field peas (Gopinath et al., 2009).

The analysis of variance showed that the bio-fertilizer rate of 500g ha<sup>-1</sup> was highly significant (P<0.001) effect on the nodulation rating, hence the highest (113.6) nodulation rating was recorded from the treatment of (500kg<sup>-1</sup>) rhizobium inoculant influenced by Rhizobium rate on field pea. Since nodulation assessment is determined by crop type and spp. similarly, reported that the nitrogen fixation capacity of legume crops is enhanced by the host spp., bacteria, and climate conditions.

Analysis of variance showed that Bio-fertilizer rate inoculation was a very highly significant (P<0.01) effect on plant height. The highest (133.9 cm) and the lowest plant height (118.7cm) of field peas was observed at 500g ha<sup>-1</sup> and 0g ha<sup>-1</sup> Bio-fertilizer respectively. This result was in line with the role of Sulfur which constituent of several plant bio-chemicals which regulate plant growth. In addition to this, have reported increased dry matter production for the application of Blended fertilizers (Dabessa et al., 2023).

The significant increase in plant height in response to Rhizobium inoculation might be ascribed to the increased availability of nitrogen in the soil for uptake by plant roots, which may have sufficiently enhanced vegetative growth through increasing cell division and elongation. In line with this result, reported that increasing the N level from 0 kg ha<sup>-1</sup> to 25 kg ha<sup>-1</sup> increased the plant height of faba bean at both *Hirna* and *Haramaya*. The promotion effect of high N in bio-fertilizer level supply on plant height may be due to better development of the root system and nutrient absorption. In contrast, reported that phosphorus rate had no significant effect on plant height in field pea (*Vicia faba* L.) crop. The increment of plant height due to Rhizobium inoculation might be due to an adequate amount of nitrogen fixed by the bacteria which promoted the vegetative growth of the plants. Consistent with this result, he reported that Rhizobium inoculation in cowpea significantly improved measured plant height at four, six, and eight weeks after planting in both greenhouse and field experiments relative to the control treatment. Likewise, reported that combined application of NPSB fertilizer with Rhizobium inoculation provided the highest plant height

The present research result showed that the main effects of Rhizobium rate significantly (P<0.05) affect the number of leaves per plant in field peas (Agegnehu, 2009). Inoculated plants produced more leaves as compared to controlled treatments. Similarly, the study of climbing bean varieties inoculated with Rhizobium showed an increased number of leaves per plant after six weeks of planting (Miwanda et al., 2014). Application of Blended fertilizer along Rhizobium inoculation resulted in a relatively higher number of leaves per plant as compared to applying without Rhizobium inoculation (Arif et al., 2006). Similarly, the study revealed that the number of leaves per plant increased by 20% 4 weeks after planting relative to control in the greenhouse experiment (Gadissa et al., 2022).

### Effects of bio-fertilizer application on yield parameters

The analysis of variance indicated that the number of productive tillers was very highly significantly (P<0.001) influenced by the Bio fertilizer rates. The highest numbers of productive tillers (2.4) were recorded from 250g ha<sup>-1</sup> Rhizobium; while the lowest numbers of productive tillers (1.9) were recorded from control treatment with Rhizobium 0g ha<sup>-1</sup>.

Similarly, reported the highest number of productive tillers per plant of field pea (5.7) at 250 g ha<sup>-1</sup> bio fertilizer rate. Likewise, reported the maximum number of productive tillers per plant (7) due to the application of recommended rate of bio fertilizer rate of (250g ha<sup>-1</sup>)

Rhizobium inoculation rate was very highly significant (P<0.001) on the number of pods per plant. Among the treatments, the highest number of pods per plant (13) were recorded from treatment received with 250g ha<sup>-1</sup> of Rhizobium; while the lowest number of pods per plant was recorded from control plots. This might be due to the optimum availability and utilization of bio-fertilizer and other important nutrients for reproductive development. In addition, it may be due to the positive influence of nitrogen, phosphorus and inoculant with an application of an optimum amount of mineral bio-fertilizer on pod formation of legumes. Also, the application of nitrogen increases panicles or heads in cereals and the number of pods in legumes (Bahru, 2019). In line with this result, reported that the application of phosphorus increases nutrients which stimulated the plants to produce more pods per plant as phosphorus strongly encourages flowering and podding (Fatima et al., 2008).

**Table 3.** Effects of bio-fertilizer application on yield parameters influenced by Rhizobium rate on field pea in 2020 main cropping season

Treatments BF (g ha <sup>-1</sup> )	NPT	NPP	NSPP	HSW
0	1.9 <sup>b</sup>	10.7 <sup>c</sup>	3.1 <sup>b</sup>	62 <sup>a</sup>
250	2.4 <sup>a</sup>	13.1 <sup>a</sup>	4.0 <sup>a</sup>	62.5 <sup>a</sup>
500	2.1 <sup>ab</sup>	11.9 <sup>b</sup>	3.6 <sup>a</sup>	62.5 <sup>a</sup>
CV (%)	11.8	11.4	21.9	7.1
SE +	0.1	0.4	0.2	1.2

Note: BF=bio fertilizer rate, NPT=number of the productive tiller, NPP= number of pods per plant, NSPP= Number of seeds per plant, HSW=hundred seed weight

The analysis of variance indicated that the number of seeds per plant of field peas pods were very highly significantly ( $P < 0.001$ ) influenced by the Rhizobium inoculation rate. Significantly highest number of seeds per pod (4.0) were recorded from treatment received from Rhizobium inoculation rate  $250 \text{ g ha}^{-1}$ , while the lowest number of seeds per pod (3.1) were from control plots as well as inoculation alone.

The increment of seeds per pod with increasing bio-fertilizer application up to the optimum level might be an adequate supply of nutrients in bio-fertilizer for nodule formation, protein synthesis, fruiting, and seed formation. Sufficient availability of these nutrients enables the plant to acquire a higher number of pods per plant and seeds per pod, by influencing photosynthetic activity and its proper partitioning. On another hand, the availability of Bio-fertilizers improves the formation of the pollen tube and seed formation (Agegnehu, 2009). In line with this result, also reported the highest number of seeds per pod (5.85) at an applied rhizobium rate of  $250 \text{ g ha}^{-1}$ . Similarly, reported a relatively highest number of seeds per pod with the application of  $250 \text{ g ha}^{-1}$  of rhizobium bacteria (Dori et al., 2022).

The analysis of variance showed that total biomass yield was significantly affected by Rhizobium inoculation rate and the highest total biomass yield ( $7076 \text{ kg ha}^{-1}$ ) was observed treatment received from Rhizobium inoculation rate of  $500 \text{ kg ha}^{-1}$  while the lowest total biomass yield ( $6393 \text{ kg ha}^{-1}$ ) was observed from control treatment of  $0 \text{ kg ha}^{-1}$  rate. This is in agreement with found that high biomass yield is obtained from treatments that receive the highest rate of bio-fertilizer compared with plots that receive the lowest rate of bio-fertilizer or control plots. Similarly, reported that the highest ( $16867.7 \text{ kg ha}^{-1}$ ) average biomass of yield of maize was obtained with the application of bio-fertilizer and also and reported that the application of bio-fertilizer increases biomass yield than a lower rate of bio-fertilizer rate.

The present research result showed that the highest grain yield ( $2671 \text{ kg ha}^{-1}$ ) was obtained from treatment received  $250 \text{ g ha}^{-1}$  Rhizobium inoculation rate, while the lowest grain ( $2025 \text{ kg ha}^{-1}$ ) was obtained from controlled treatment which is  $0 \text{ kg ha}^{-1}$ . The observed yield improvements with rhizobium bacteria inoculation might be due to the increased N as a result of atmospheric nitrogen fixation from effective strain application. In line with this result, reported that a mixture of Rhizobium strains with phosphorus recorded higher seed yield of soybean over inoculant without phosphorus, and other researchers also reported that inoculation along with phosphorus fertilizer had a significant effect on nodulation, shoot dry matter, and grain yield on Faba bean. Similarly, reported that, the highest grain yield ( $2547 \text{ kg ha}^{-1}$ ) of field pea from the application of  $250 \text{ g ha}^{-1}$  bio-fertilizer  $\text{ha}^{-1}$  (Dhabessa et al., 2021).

**Table 4.** Effects of bio-fertilizer application on yield parameters influenced by Rhizobium rate on field pea in 2020 main cropping season

Treatments BF ( $\text{g ha}^{-1}$ )	TBY	GY	SY	HI
0	6393 <sup>b</sup>	2025 <sup>c</sup>	4036 <sup>a</sup>	32 <sup>b</sup>
250	6370 <sup>ab</sup>	2671 <sup>a</sup>	4080 <sup>a</sup>	40 <sup>a</sup>
500	7076 <sup>a</sup>	2425 <sup>b</sup>	4358 <sup>a</sup>	34 <sup>b</sup>
CV (%)	12.7	12.8	19.1	14
SE $\pm$	253.4	111.1	197.4	1.5

Note: BF=bio fertilizer rate, TBY=total biomass yield, GY=grain Yield, SY= straw yield, and HI= harvest Index

In line with this result, reported that Rhizobium inoculation significantly improved the yield and yield components of legumes such as the number of pods per plant, number of seeds per pod, number of seeds per plant, 100 seed weight, and seed yield relative to control (De Varennes and Goss MJ, 2009). Similarly, reported that inoculation significantly increased grain yield in field peas (De Souza et al., 2021).

The result of the analysis of variance showed that straw yield was not significantly affected by the Rhizobium inoculation rate.

Analysis of variance showed that Rhizobium inoculation rate was very highly significantly ( $P < 0.001$ ) affect the harvest index of field peas. The increased harvest index of field peas with inoculation of Rhizobium bacteria rate might be because Rhizobium inoculation increased the number of effective nodules per plant and increased the hundred seed weight of field pea (Day, 1965). Similarly, reported that grain increased with the inoculation increased the nodules number per plant and gave the highest harvest index and hundred grains weight of chickpea (Dabessa et al., 2023).

## CONCLUSIONS

Low soil fertility status and reduced biological nitrogen fixation are some of the major constraints limiting Faba bean yield in the study area. Ensuring Rhizobium inoculation rates to the crop may result in higher seed yield. Limited research has been done on the effect of Rhizobium inoculation rates on yield and yield components of field

peas (*Pisum sativum L.*). Therefore, a field experiment was conducted to evaluate the effect of Rhizobium inoculation rates on the yield and yield components of field pea production. Three levels of Rhizobium inoculation rates (0, 250, and 500g ha<sup>-1</sup>), were tested in factorial combination in three replications in a Randomized Complete Block Design. The results of the study showed that a high yield (2671kg ha<sup>-1</sup>) was obtained when the field pea was sown from 250g ha<sup>-1</sup> Bio fertilizer. As Bio fertilizer rates increased from 0 to 500g ha<sup>-1</sup> all yield and yield-related components were increased. From the present study, it is possible to conclude that 250g ha<sup>-1</sup> Bio fertilizer performed better and gave a higher grain yield (2671 kg ha<sup>-1</sup>) and had the highest grain yield advantage over the remaining Bio fertilizer rates. However, to make a reliable and acceptable recommendation it is better to repeat this experiment across locations and over seasons.

**Author Contributions:** The author (Birhanu Gebeyehu) was performed all data collected; designed the analysis as well wrote the paper.

**Funding Source:** The authors received no specific funding for this work.

### Acknowledgments

I would like to thank UASVM Cluj-Napoca guided me to submit and edit my work and the publication of the article.

### Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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