



Effect of Rhizobium Inoculation, NPS Fertilizer and Vermicompost on Nodulation and Yield of Soybean (*Glycine max* (L. Merrill) at Bako, Western Ethiopia

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Abstract: In Ethiopia, acidity-related soil fertility problems are the main production constraints, reducing productivity of major crops grown in the country. The experiment was carried out to determine influence of Blended NPS fertilizer, Seed Inoculation with Rhizobium Bacteria and Vermicompost (VC) application on soybean nodulation and yield of soybean. Factorial combinations of Rhizobium (uninoculated, inoculated), three VC levels (0, 1 and 2 t ha⁻¹) and three NPS levels (50%, 75% and 100% of 100 kg NPS ha⁻¹) were laid out in Randomized Complete Block Design (RCBD) with three replications. The results showed that the highest number of nodule per plant (32.0) and number of effective nodule per plant (31.4) were recorded at the combination of 2 tons VC ha⁻¹ and 75 kg NPS ha⁻¹ while the highest aboveground biomass (8953 kg ha⁻¹) was recorded at the combination of 2 tons VC ha⁻¹ with 100 kg NPS ha⁻¹. Likewise, the combination VC 2 tons ha⁻¹ with Rhizobium inoculation (TAL-379) gave the highest number of effective nodules per plant (26.3). On the other hand, three factors interaction of Rhizobium inoculation, VC and NPS rates significantly influenced the number of primary branches (NPB), number of pod per plant, seed yield and harvest index where the highest number of pods per plant (87.6), maximum seed yield (4180 kg ha⁻¹) and maximum harvest index (47%) were recorded from the plots treated with 100 kg NPS ha⁻¹ + 2 t VC ha⁻¹ inoculated with Rhizobium TAL-379 strain. Thus, considering the importance of integrated nutrient management in climate mitigation and adaptation; combined application of 2 t VC ha⁻¹ and 75 kg NPS ha⁻¹ inoculated with Rhizobium strain TAL-379 had resulted in better and optimum yield of 3870 kg ha⁻¹ and is tentatively recommended for use.

Keywords: Biomass, Nodulation, NPS fertilizer, Rhizobium TAL-379, Vermicompost

1. Introduction

Soybean [*Glycine max* (L.) Merrill], family of Fabaceae originated in Eastern Asia, probably in North and Central China [1]. Soybean is a promising pulse crop proposed for alleviation of acute shortage of protein since it contains about 38 - 42% and oil worldwide and a source of unsaturated fatty acid, minerals (Ca and P) and vitamins A, B, C and D [2-4]. Soybean is used for preparation of different kinds of soybean foods, animal feed, soy milk, raw material for the processing factories like tasty soya; Fafa food factory. Since it is a

legume crop, soybean is used in climate mitigation and adaptation through N₂ fixation, as crop diversification and reducing amount inorganic N fertilizer [5].

In Ethiopia, soybean could be grown between 1300 and 1800 m altitude with annual rain fall of 900 - 1300 mm an average annual temperature between 20 - 25°C and soil pH of 5.5 [6]. The major areas currently growing soybean in the country are situated in the western and south western parts of the country. The area of 38,072.70 ha of land was covered by

soybean which produced 86467.87 tons with the productivity of 2.27 t ha^{-1} which is low as compared to world average 2.6 t ha^{-1} [7, 8].

Despite numerous benefits of soybean, the grain yield per unit area is low in Ethiopia, which is an average of $2.27 \text{ ton per hectare}$ as compared to the potential of the crop [7]. The low yield of the crop is associated with poor soil fertility, periodic moisture stress, diseases and insect-pests, weeds and poor crop management practices [9]. Soil acidity and low fertility status of most of the cultivated tropical soils has been identified as a major factor causing low crop yield [10]. For instance, about 41% of potential arable land of Ethiopian is acidic [11]. Likewise, substantial increase intensity of rainfall accompanied by leaching of macro-nutrients could lead to increased soil erosion and acidity problems that cause widespread of crop production in constraints in western Ethiopia [12, 13]. Soil acidity causes the deficiency of phosphorous, potassium, molybdenum and sulfur nutrient that are contributing factors for low yield of soybean. Nutrient depleted soils are characterized by low soil organic matter, unavailable phosphorus and total nitrogen especially in south and western of Ethiopia [14, 13]. As results, reduced availability of Nitrogen (N) and Phosphorus (P) in predominantly acidic soils is responsible for reduced soybean performance through reduced photosynthesis and early root development, low microbial activity and poor nitrogen fixation, leading to low yields [15]. Likewise, in acidic soil, P is a major limiting nutrients due to fixation by clays dominated with Al and iron (Fe) hydroxides [16] and more than 8% of P fertilizer applied to acidic soil is quickly transformed into the form of P that is not available to plant [17]. Aluminium and manganese toxicity, as well as calcium and phosphorus deficiency in acid soils, inhibit Rhizobium growth and root infection resulting in symbiotic failure, limiting Rhizobium survival and persistence in soil and reducing nodulation causes nutrient imbalance [18, 19]. Low soil nitrogen, phosphorus and sulfur are among the major factors limiting production and productivity of Legumes crops [20]. In low pH soil, nodule formation has been reported to be reduced by $> 90\%$ and nodule dry weight by $> 50\%$ in soybean and other nodule forming legumes due to unavailability of nutrients [21].

Accordingly soybean productivity around the study areas is not more than 2 t ha^{-1} which is below the national average productivity (2.6 t ha^{-1}). Therefore, some studies have been conducted to sustain soybean productivity on acidic soil using integrated inorganic fertilizers and Rhizobium inoculation [20]. Soybean Boshe variety yield increased by 100-200% using combined application of organic and inorganic fertilizers as compared to the control on acidic soil of Bako, Western Ethiopia [22]. Inorganic fertilizers are one of the expensive inputs in agriculture which not viable option for small holder farmers because of most of them lack financial resource [23] and continued use of inorganic fertilizers also increased the acidity of the soils and emission of greenhouse gasses [24]. Thus, to reduce the amount of

mineral fertilizers required there is a need to adopt integrated nutrient management technologies which combine organic fertilizers with small amounts of mineral fertilizers and build up soil conditions to enhance soil microbial activity, biological N fixation and soybean yields.

Moreover, integrated nutrient management is considered to be a key cultural practice to avert the problem of crop production by enhancing soil fertility and has advantage in climate change mitigation [22, 24]. Likewise, integrated nutrient management maximize carbon sequestration from air in soil organic matter, sustainably increase yields and improves soil health resulting in more CO_2 capture from the atmosphere [24] and build up soil carbon stocks, hence mitigating CO_2 and N_2O emission from soil [25]. A soil acidity problem in Ethiopia is amended by applying agricultural lime stone. In western Ethiopia, significant results have been obtained on acidic soil by combined application of lime, inorganic fertilizers with Rhizobium inoculation [8]. However, there is limited information at study area regarding the effects of combined application of newly introduced blended NPS fertilizer, vermicompost and Rhizobium inoculation on soybean nodulation and productivity. The specific objectives of the study were to determine effects of integrated application of Rhizobium inoculation, vermicompost, and inorganic NPS on nodulation and grain yield of soybean at western, Oromia Ethiopia.

2. Materials and Methods

2.1. Description of the Experimental Site

The experiment was carried out at Bako Agricultural Research Center (BARC) which is located in Oromia Regional State, West Shoa Zone, at about 250 km away from the capital city Addis Ababa on the way to Nekemte town about 8 km from Bako town at altitude of 1650 meter above sea level. The Center is located at $09^\circ 6' 00'' \text{ N}$ latitude and $37^\circ 09' 00'' \text{ E}$ longitudes. The area has a warm humid climate with annual mean minimum and maximum temperature of 13.9 and 29.8°C respectively. It receives annual rainfall of 1237mm mainly from May to October with maximum precipitation in the month of June to August (Figure 2).

The predominant soil type of the area is Nitisols which is characteristically reddish brown with a pH that falls in range of very strongly acidic to very acidic. Being located to tepid to cool sub humid mid high lands (SH2), the area is a mixed farming zone, encompassed by Gibe River and abundant natural vegetation. The area is also known for its mixed crop livestock farming system in which cultivation of maize (*Zea mays* L.), hot pepper (*Capsicum annum* L.), soybean (*Glycine max* L. Merril.), common bean (*Phaseolus vulgaris* L.), Niger seed (*Guzotia abyssinica* L.), mango (*Mangifera indica* L.), tomato (*Lycopersicum esculentum*) and sugarcane (*Saccharum officinarum* L.) are major cropping activities that are practiced.

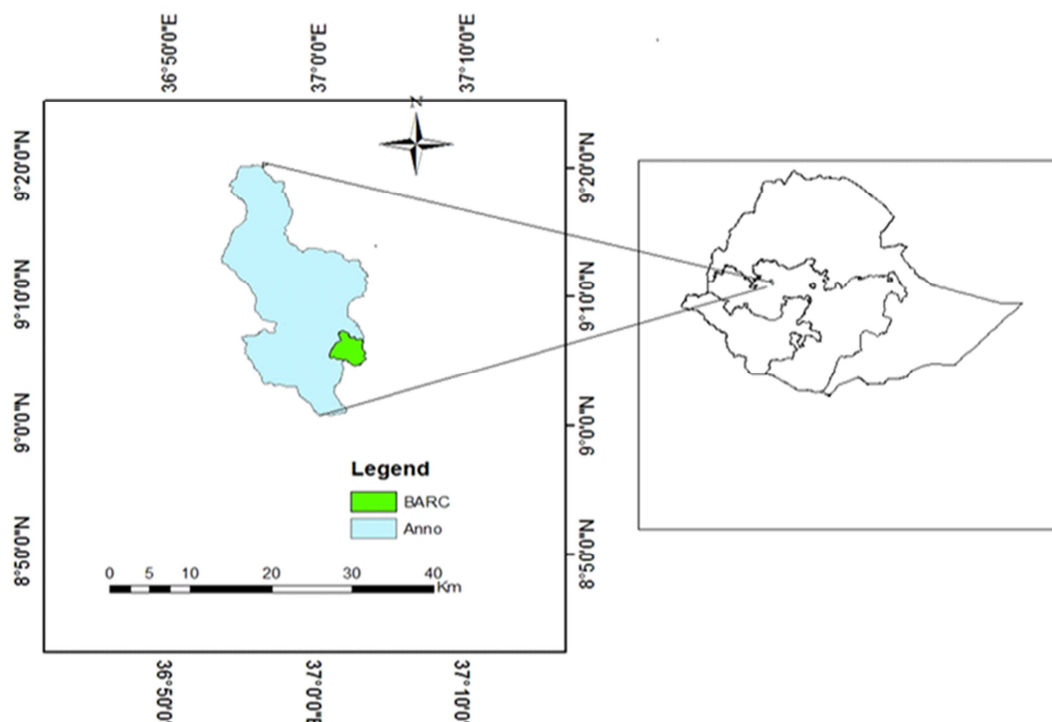


Figure 1. Map of the study area, Bako Agricultural Research Center.

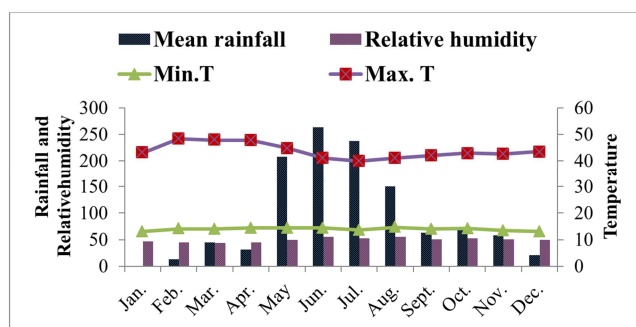


Figure 2. Monthly total rainfall (mm), relative humidity, means minimum and maximum temperature of experimental station in 2018.

2.2. Description of Experimental Materials

Crop: Improved soybean variety (Boshe) was used as a test crop. The variety was released in 2009 by Bako Agricultural Research Center (BARC). It is early maturity group (100-110) having intermediate growth habit and a yield potential of 2.2-3 t ha⁻¹ at research station and disease resistance [26]. It needs greater than 500 mm rainfall and grows at altitude of 1300-1900 meter above sea level. The variety needs about 54-57 days to flower and 100-110 days to reach maturity. Blended fertilizer NPS which contains about 19% N, 38% P₂O₅ and 7% S was used as source of nitrogen, phosphorus and sulfur [27]. It was purchased from local market (farmers' cooperative). Vermicompost prepared from raw materials like soybean straw, wood ash and animal manure. Decomposition process was facilitated by earthworms (Red wigglers). Carrier based Bradyrhizobium strain TAL-739 was obtained from Menagasha Biotechnology Private Limited Company,

Addis Ababa, Ethiopia.

2.3. Soil Sampling and Analysis

Representative soil samples before planting were taking by using an auger at a depth of 0-20 cm randomly in zigzag pattern from the whole experimental field prior to planting from 12 spots. Finally, composite sample was prepared for analysis to determine physico-chemical properties of the soil of experimental site. A 1 kg of composite sample was prepared and put into polyethylene labeled and was taken to Bako Agricultural Research Center Soil Laboratory. The soil sample was air dried ground and sieved with 2 mm sieve for analysis of texture, pH, available phosphorus, sulfur, cation exchange capacity (CEC), for the analysis of total N and OC sub soil sample was ground to the size of 0.5 mm. Organic carbon content was determined by volumetric methods as described by FAO guide to laboratory establishment for plant nutrient analysis using 1.0 g of the prepared soil sample [28, 29]. Soil texture was determined by Bouyoucos hydrometer method [30]. The pH of the soil was measured in water at soil to water ratio of 1:2.5 with potentiometric pH meters and glass electrode attached to digital pH meter [31].

Total nitrogen was determined using Kjeldhal method [32]. Available soil phosphorus was extracted by the Bray II procedure and determined calorimetrically [33]. To determine the cation exchangeable capacity (CEC) soil was leached with 1M ammonium acetate and be washed with ethanol. The adsorbed ammonium was replaced by sodium (Na). Then, CEC was determined titrimetrically by distillation followed by titration as described by of that was displaced by Na [34]. Available sulfur (S) was measured using turbidimetric

method [35]. Particle size distribution was determined by the hydrometer method (differential settling within a water column) according to FAO [29] using particles less than 2mm diameter. The procedure measured percentage of sand (0.05-2.0mm diameter), silt (0.002-0.05mm diameter), and clay (<0.002mm diameter) fractions in the soils [36].

Table 1. Selected soil physico-chemical properties of the experimental site before planting.

Soil properties	Value	Rating	Reference
1. Particle size distribution			
Clay (%)	69		
Silt (%)	13		
Sand (%)	18		
Soil textural class	Clay Soil		[36]
2. Chemical Properties			
pH (1:2.5 H ₂ O) Suspension	5.16	Strongly acidic	[37]
Organic carbon (%)	1.83	Medium	[36]
Total nitrogen (%)	0.16	Low	[36]
Available phosphorus (mg kg ⁻¹ soil)	9.2	Low	[38]
Available S (mg kg ⁻¹ soil)	20.5	medium	[35]
CEC cmol (+) kg ⁻¹	20.39	Medium	[39]

2.4. Vermicompost Sampling and Analysis

The known earth worm species red wigglers (*Eisenia fetida*) using soybean (crop residues) and cow manure (wastes) were used for vermiculture preparation. The dried plant residues were chopped and then mixed with cow manure (in ratio of 7 parts of cow manure and 3 parts of waste, dry weight basis). The mixture allowed moisturizing for two weeks and earthworms were added to the prepared bedding materials on the box. The moisture content was maintained at 70% by regular addition of water using water cans. After two months cast (vermicompost yield) were harvested and five sub-samples were taken from the prepared cast to make one composite sample and then air dried, ground and sieved. Then, it was subjected to the analysis of pH, OM, N, P and S following standard procedures. The C: N ratio was estimated from OC and N contents of the vermicompost.

Table 2. Selected chemical properties of vermicompost.

pH	TN (%)	OM (%)	Av. P (mg/kg)	Av. S (mg/kg)	C:N
7.42	0.34	6.72	222.3	160.2	11.47

pH: Power of hydrogen, Av. P: Available phosphorus, OM: Organic Matter, TN: Total nitrogen, Av. S: Available sulfur

2.5. Treatments and Experimental Design

The experiment consists of factorial combinations of three rate of NPS [50%, 75%, and 100% of recommended dose of fertilizer (RDF) of 100 kg NPS ha⁻¹] based on national blanket recommendation for soybean in the study area. Three levels of vermicompost rate (0, 1 and 2 t ha⁻¹ and two Rhizobium strain (TAL-379) i.e., inoculated and uninoculated. The treatments were arranged as 3×3×2 in factorial combinations in RCBD with three replications. The gross plot was comprised of seven rows of 3 m length (7 × 0.4 m × 3 m=8.4 m²) and the two outer most rows (one row

from each side) of each plot was considered as border rows. One destructive row from each plot of the similar direction was used for recording data on total number of nodules per plant, effective and non-effective and aboveground dry biomass. Other Agronomic data were collected from the central four rows (4 × 0.4 m × 3 m=4.8 m²).

Table 3. Details of the treatment combinations.

Treatment	Treatment Descriptions
T1	50 kg NPS ha ⁻¹ + uninoculated + 0 t vermicompost ha ⁻¹
T2	50 kg NPS ha ⁻¹ + inoculated + 0 t vermicompost ha ⁻¹
T3	50 kg NPS ha ⁻¹ + uninoculated + 1 t vermicompost ha ⁻¹
T4	50 kg NPS ha ⁻¹ + inoculated + 1 t vermicompost ha ⁻¹
T5	50 kg NPS ha ⁻¹ + uninoculated + 2 t vermicompost ha ⁻¹
T6	50 kg NPS ha ⁻¹ + inoculated + 2 t vermicompost ha ⁻¹
T7	75 kg NPS ha ⁻¹ + uninoculated + 0 t vermicompost ha ⁻¹
T8	75 kg NPS ha ⁻¹ + inoculated + 0 t vermicompost ha ⁻¹
T9	75 kg NPS ha ⁻¹ + uninoculated + 1 t vermicompost ha ⁻¹
T10	75 kg NPS ha ⁻¹ + inoculated + 1 t vermicompost ha ⁻¹
T11	75 kg NPS ha ⁻¹ + uninoculated + 2 t vermicompost ha ⁻¹
T12	75 kg NPS ha ⁻¹ + inoculated + 2 t vermicompost ha ⁻¹
T13	100 kg NPS ha ⁻¹ + uninoculated + 0 t vermicompost ha ⁻¹
T14	100 kg NPS ha ⁻¹ + inoculated + 0 t vermicompost ha ⁻¹
T15	100 kg NPS ha ⁻¹ + uninoculated + 1 t vermicompost ha ⁻¹
T16	100 kg NPS ha ⁻¹ + inoculated + 1 t vermicompost ha ⁻¹
T17	100 kg NPS ha ⁻¹ + uninoculated + 2 t vermicompost ha ⁻¹
T18	100 kg NPS ha ⁻¹ + inoculated + 2 t vermicompost ha ⁻¹

2.6. Experimental Procedure and Field Managements

The experimental land was ploughed by tractor, disked and harrowed. Fine seedbeds were prepared and leveled manually and rows were made across each plot. The seeds were planted at spacing of 40 cm and 10 cm between rows and within rows respectively. The spacing between plot and block were 0.6 m and 1.2m respectively.

Based on blanket recommended dose of NPS fertilizer (100 kg NPS ha⁻¹) [27] around area, preparation of fertilizer per plots were done for 100%, 75% and 50% respectively. Also vermicompost was prepared by calculating per plots based on its respective rates and applications were done at planting time by drilling into prepared rows accordingly. After application of the fertilizer, it was covered with sparse soil before planting seed to protect burning of seed.

Carrier based inoculant were applied at the rate of 500 g inoculants per 80 kg ha⁻¹ seed of soybean (Manufacturer recommendation). The inoculant was mixed by sugar with the addition of some water in order to facilitate the adhesion of the strain on the seed. To ensure that the applied inoculants stick to the seed, the required quantities of inoculant was suspended in 1:1 ratio in 10% sugar solution. The thick slurry of the inoculant was gently mixed with the dry seeds so that all the seeds were received a thin coating of the inoculants. To maintain the viability of the cells, inoculation was done under the shade and allowed to air dry for 30 minutes and planted at the recommended spacing. Seeds were immediately covered with soil after sown to avoid death of cells due to the sun's radiation. A plot with un-inoculated seeds was planted first to avoid contamination. Two seeds were planted per hill and then were thinned to one plant after

seedling establishment. Rouging of lately emerging weeds and off type plants was done to avoid interference with the soybean cultivar. All other management practices were done as per recommendations. Harvesting was done on November 5, 2018. After harvesting the crop, threshing was done; the yield was recorded and adjusted at 10% grain moisture content.

2.7. Crop Data Collection and Measurements

2.7.1. Nodule and Growth Parameters

Plant height (cm): Plant height was measured at physiological maturity from soil surface to the apex of each plant on five randomly selected plants from the central area. The average height of five plants was taken from each plot at physiological maturity.

Number of primary branches: This was determined by counting of primary branches on the main stem from randomly selected five plants from each net plot at physiological maturity.

Total number of nodules: Bulk roots of five randomly selected plants were carefully exposed at flowering and uprooted for nodulation study. Roots were carefully washed under gently flowing tap water on a screen and nodules were separated and counted.

Effective number of nodules: For effective number of nodules the color on inside of nodule was observed by

cutting with sharp blade and a pink to dark-red color was considered as effective whereas a green colored one was identified as non-effective nodules.

2.7.2. Yield Components and Yield

Number of pods per plant: The number of pods per plant were counted from five randomly selected plants from four middle rows at physiological maturity and expressed as an average of each plant.

Number of seeds per pod: Numbers of seeds per pod was counted from the randomly taken five pods from the net plot and were expressed as an average of five pods.

Hundred seed weight: Randomly sampled 100 seeds were taken from the harvest bulk and their weight was recorded using sensitive balance and adjusted at 10% standard moisture content.

Aboveground biomass yield (kg ha⁻¹): This refers to the dry biomass of the whole aboveground plant at physiological maturity. The total fresh biomass of five randomly selected plants were taken and dried in a forced draft oven dry at 70°C for 48 hours until constant weight was obtained.

Seed yield (kg ha⁻¹): Seed yield was measured from harvested central unit area. Seeds were cleaned following harvesting and threshing, weighed using electronic balance, and adjusted to 10% moisture content using the formula. Then the weight was converted to kg ha⁻¹.

$$\text{Adjusted yield (kg/ ha) at standard moisture} = \frac{\text{yield obtained kg/ha} \times (100 - \text{sample Moistur Content})}{100 - \text{standard moisture content}}$$

Harvest index (HI): This was calculated using the formula below.

$$\text{HI\%} = \frac{\text{Seed yield}}{\text{Total Aboveground Dry biomass yield}} \times 100$$

2.8. Statistical Data Analysis

All collected data were subjected to ANOVA using software [40]. Whenever the effects of the treatments were significant, the means were compared using Fisher's protected Least Significant Difference (LSD) test at 5% level of significance.

3. Results and Discussions

3.1. Nodulation and Growth Parameters

Total nodule per plant: the analysis of variance showed that the total number of nodule per plant was significantly influenced by the main effect of NPS inorganic fertilizer application, vermicompost application and interaction effect of NPS and vermicompost application.

NPS plays a significant role in legume nodulation through its ability to enhance root development and proliferation thereby, affording the Rhizobia more sites for infection and initiation of nodule formation. Number of nodules was significantly higher in vermicompost and increased NPS application rates. The highest number of nodules per plant of (32.0) was recorded at 75 kg NPS ha⁻¹ application, combined

with 2 tons vermicompost ha⁻¹ while the lowest number of nodules per plant (14.0) was recorded in the treatment applied with 50 kg NPS ha⁻¹ combined with 0 ton vermicompost ha⁻¹ (Table 4). The possible reason for the greater number of nodules developed on the root of soybean after vermicompost application and NPS fertilization might be the importance P and S elements that found in NPS used in enhancing nodulation, symbiotic association between rhizobia and host plant and consequently improved N₂ fixation. Likewise, application of vermicompost supply necessary nutrients (N, P, S, K and Fe) important for nodules formation and Rhizobia bacteria are sensitive to soil acidity and require P, adequate soil moisture for their multiplication [41, 42]. In agreement with this result application of 16 t FYM ha⁻¹ + 60 kg S ha⁻¹ on soybean-wheat cropping system produced maximum mean number of nodules per 5 plant (231) across 3 years [43]. In line with this results application of organic and chemical fertilizers (46 kg N ha⁻¹ + 33.3 kg P₂O₅ ha⁻¹ + 8 t FYM ha⁻¹) resulted in significant increase in number of nodules of some food grain legumes like common bean, green bean and lima bean [44]. Furthermore, manure (compost and vermicompost) are known by its ability of improving soil physical properties such as structure which in turn improves soil moisture, microbial activity and makes nutrients available [44]. According to other author application P fertilizer at the rate of 30 kg P₂O₅ ha⁻¹ combined with manure (5 ton ha⁻¹) increased significantly number of nodules per plant (24.6) of soybean in Kenya, at

Embu County [45]. NPS application combined with vermicompost also supplied sufficient S which is very essential in nodule formation. Similarly, S plays many important roles in the growth and development of plants including chlorophyll and nitrogenase formation, promotes nodule formation and enzyme activation [16]. In similar manner application of RDF (20:40:30 kg NPK ha⁻¹) + VC 5.0 t ha⁻¹ on mung bean obtained maximum number of nodules per plant (30.14) [46].

On the other hand, number of nodule per plant at high rate of NPS fertilizer combined with vermicompost application was reduced. This indicates that the efficiency of soybean for nodule formation decreases with high level of N. Moreover, the decrease in number of nodules per plant at highest rates of NPS combined with highest rate of vermicompost might be due to increasing nitrogen application rates and thereby attributed to the negative effect of fertilizer-N on nodule formation and growth at the high rates. This result is in line with the application of high rate of nitrogen (56.58 kg N ha⁻¹), resulted in reduction of nodule number in soybean [47]. Similarly, high levels of inorganic N, especially nitrate N, have been shown to suppress nodulation of legumes [48].

Table 4. Interaction effects of NPS fertilizer rate and vermicompost application on number of nodule per plant of soybean.

NPS rate (kg ha ⁻¹)	Vermicompost t ha ⁻¹		
	0	1	2
50	14.0 ^d	19.5 ^{bc}	20.5 ^b
75	20.1 ^{bc}	22.3 ^b	32.0 ^a
100	20.6 ^b	22.3 ^b	25.7 ^b
LSD (0.05)	4.41		
CV (%)	18.1		

Where, CV: Coefficient of variation, LSD (0.05): Least significant difference at 5% level of significance; Means in columns and rows followed by the same letter are not significantly different at 5% level of significance

Effective nodule per plant: the number of effective nodules per plant was significantly ($p < 0.01$) influenced by the main effect of *Rhizobium*, NPS and Vermicompost rates and interaction effect of Rh \times VC and NPS \times VC. The number of effective nodules per plant was significantly influenced by the interaction effect of *Rhizobium* and vermicompost levels. Consequently, the highest number of effective nodules per plant (26.3) was recorded from application of 2 tons vermicompost ha⁻¹ inoculated with *Rhizobium* while the lowest number of effective nodules per plant (15.9) was recorded in the 0 ton vermicompost ha⁻¹ without inoculation (Table 5). The maximum number of effective nodule per plant was due to the positive role of vermicompost and *Rhizobium* inoculation initial nodule formation and development. This result is in line with fact that legume nodules having dark pink or red centers denoting the presence of leghaemoglobin that is used as an indicator for effectiveness of the nodules and has positive coloration with N₂ fixation [49, 50].

Similarly, the interaction between vermicompost and NPS rate had significant ($p < 0.01$) effect on number of effective nodules per plant. The highest number of effective nodules

per plant (31.4) was recorded at the rate of 75 kg NPS ha⁻¹ combined with 2 tons vermicompost ha⁻¹ whereas the lowest number of effective nodules per plant (13.9) was recorded from treatment with 50 kg NPS ha⁻¹ combined with 0 ton vermicompost ha⁻¹ (Table 5). This result revealed that when NPS levels increased without vermicompost, minimum nodule number was recorded but increased with the application of vermicompost in combination with NPS fertilizer. The possible reason of high number of effective nodules with vermicompost application might be due to the availability of calcium might enhance root development and root nodulation [51, 52]. This might be due to combination of organic and inorganic nutrition provides better soil environment for root growth, nodule formation, availability and absorption of nutrient from soil [46, 42]. Similarly application of 16 t FYM ha⁻¹ + 60 kg S ha⁻¹ on soybean-wheat cropping system produced maximum mean number of effective nodules per 5 plants (325) across 3 years, [43]. In agreement with this result, the highest number of effective nodule per plant (28% of the total nodules) over the control, where inoculation was combined with the application of 30 kg N ha⁻¹ and 35 kg P ha⁻¹ hence underlining the role played by phosphorus in nodule development and the role of starter N in soils which are low in N [53]. Likewise, integrated use of 50% RDF (10:30:20 kg NPK/ha) + 50% vermicompost (2.5 t/ha) obtained 19.21 number of effective nodules per plant in soybean [54]. In addition this result, application of phosphorus plays a vital role in increasing plant tip and root growth, decreasing the time needed for developing nodules to become active (effective) for the benefit to the host legume [55]. The increased number of effective nodules with the application of 75 kg NPS ha⁻¹ combined with 2 t vermicompost ha⁻¹ over the 50 kg NPS ha⁻¹ combined with 2 t vermicompost ha⁻¹ might also be increased sulphur application increasing its availability along with other major nutrients. This result is in line with the reported significant increase in the number of active nodules of soybean with the application of sulphur up to 20 kg ha⁻¹ increased in response to sulphur application which is involved in the formation of nitrogenous enzyme known to promote nitrogen fixation in legumes [16].

Table 5. Interaction effects of NPS fertilizer rate, *Rhizobium* inoculation and vermicompost on number of effective nodule per plant of soybean.

Vermicompost (t ha ⁻¹)	<i>Rhizobium</i>		NPS rate (kg ha ⁻¹)		
	Un-inoculated	Inoculation	50	75	100
0	15.9 ^d	17.6 ^{cd}	13.9 ^d	16.2 ^{cd}	20.2 ^b
1	21.2 ^b	20.6 ^{bc}	19.1 ^{bc}	21.7 ^b	21.9 ^b
2	21.3 ^b	26.3 ^a	20.1 ^b	31.4 ^a	20.7 ^b
LSD (0.05)	3.13		3.83		
CV (%)	15.9				

Where, CV: Coefficient of variation, LSD (0.05): Least significant difference at 5% level of significance; Means in the columns and rows followed by the same letter are not significantly different at 5% level of significance

The main effect of *Rhizobium* inoculation, NPS rate and vermicompost showed significant ($p < 0.01$) effect on plant

height while the interaction effects were not significant. The tallest plant height (70.5 cm) was obtained from plots inoculated with Rhizobium strain (TAL-379) whereas the shortest plant height (66.3 cm) was recorded from the uninoculated plots (Table 6) which was about 5.96% difference in height. The increase of plant height by 5.96% might be due to Rhizobium inoculation was produce higher rate of N₂ fixation by inoculum, which plays a vital role in the vegetative growth of soybean. This result in agreement with inoculation of *Bradyrhizobium japonicum* improved the mean plant height of soybean by 6.5% than the uninoculated soybean [56]. Likewise, the inoculation of soybean seeds with Rhizobium sp. produced higher plant height than non-inoculated plants [57]. Similarly, Rhizobium inoculation increased plant height up to 12% in soybean [58].

Among various NPS levels, the maximum plant height (70.1 cm) was recorded at the rate of 100 kg NPS ha⁻¹ while the lowest plant height (67.8 cm) was recorded at 50 kg NPS ha⁻¹ (Table 6). This positive growth response of soybean at the higher rate of NPS in acidic soil may be related with better availability of P and S for the crop as the rate of NPS application increased. The increase in plant height might also be ascribed to better root formation due to sulfur, which in turn activated higher absorption of N and thereby playing synergistic role of P, N and S on the growth processes of the plant. Moreover, the increase in plant height with the increased NPS application rate indicates maximum vegetative growth of the plants under higher N and S availability and P also, plays a pivotal role in early root proliferation that might increase the nutrient up take of the plant thus resulted in increased vegetative growth and increased cell elongation and their multiplication. In

conformity with this result application phosphorus at rate of 45 kg P₂O₅ ha⁻¹ significantly increase plant height (72.35 cm) by 6.87% over the control treatment (67.38 cm) in soybean [59, 60]. Similarly, plant height was significantly increased up to 160 kg N ha⁻¹ in soybean [61]. Also, the positive interaction effect of P and S on the plant height of soybean [62]. Likewise, application of 30 kg S ha⁻¹ on soybean plants increase plant height by 14% compared with the control [63]. In addition, the tallest plant (132.5 cm) was recorded with the combined application of 92 kg P₂O₅ with 30 kg S ha⁻¹ on faba bean at Sinana, South-Eastern, Ethiopia [64].

Application of vermicompost significantly increased plant height of soybean where the highest plant height (72.0 cm) was recorded with the application at 2 tons vermicompost ha⁻¹ while the lowest plant height (65.6 cm) was recorded from 0 ton vermicompost ha⁻¹ (Table 6). The possible reason for maximum height in vermicompost treatment may be due to organic fertilizer sources fulfilled the macro and micronutrients requirements provided the crop with maximum nutrients in later stages. The result indicated that applying vermicompost to the soil might considerably improve the nutrient availability, particularly micro and macronutrients, since it improves soil pH under which maximum availability of the nutrients may be obtained. In addition, increased plant height could also be due to the growth promoting substances, which are present in vermicompost. Similarly, vermicompost application at rate of 4.50 t ha⁻¹ on soybean growth gave the highest plant height (66.46 cm) but lodging effect was observed at Asosa, western Ethiopia [42]. Likewise, the tallest plant height with 73.93 cm was obtained from use of 10 t/ha vermicompost on soybean at Guilan province, north of Iran [65].

Table 6. The main effect of Rhizobium inoculation, NPS and vermicompost on plant height Plant height (cm).

	NPS rate (kg ha ⁻¹)			Vermicompost (t ha ⁻¹)			Rhizobium inoculation	
	50	75	100	0	1	2	uninoculated	inoculated
Plant height (cm)	67.8 ^b	67.2 ^b	70.1 ^a	65.6 ^b	67.5 ^b	72.0 ^a	66.3 ^b	70.5 ^a
LSD (0.05)	1.99			1.99			1.63	
CV (%)	4.3							

The number of primary branches per plant: the analysis of variance showed significant ($p < 0.01$) main effects of NPS fertilizer, Rhizobium inoculation and vermicompost application on the number of primary branches. On the other hand, the three factor interaction of NPS × VC × Rh had significant ($p < 0.01$) effect on the number of primary branches.

The maximum number of primary branches per plant (9.2) was produced by plants treated with the combined application of 100 kg NPS ha⁻¹ and 1 ton vermicompost ha⁻¹ inoculated with Rhizobium whereas the minimum number of primary branches per plant (5.5) was produced at 50 kg NPS ha⁻¹ combined with 0 ton of vermicompost ha⁻¹ without inoculation (Table 7). The maximum primary branches (9.2) at higher rate of vermicompost and NPS rate combined with Rhizobium inoculation TAL-379 strain might be due to greater availability of macro and micronutrients, form of

organic and inorganic sources which assisted in acceleration of various metabolic processes of N P and k which help in better absorption of nutrients combined with proper distribution. The results of this study is in agreement with combined application of vermicompost at rate of 3.75 t ha⁻¹ with 25% rate of NPK fertilizers lead to an increase in number of primary branches of french bean (*Phaseolus vulgaris*) [66]. Similarly, combined application of vermicompost at 2.5 t ha⁻¹ + RDF (25:50:50 kg NPK ha⁻¹) + copper ore tailing recorded higher number of branches (6.92) in groundnut [67]. Otherwise, the application of recommended doses of N, P and K fertilizers (20:60:20 kg/ha) on soybean along with 20 kg S/ha and 10 tonnes FYM/ha resulted in highest number of branches per plant [68]. Also, the highest number primary branches per plant (10.21) at RDF (20N: 45P₂O₅ kg ha⁻¹ + 5.0 ton ha⁻¹ VC + Rhizobium + PSB in chick pea [69].

Table 7. Interaction effect of NPS, Vermicompost and Rhizobium inoculation on number of primary branches of soybean.

NPS rate (kg ha ⁻¹)	Vermicompost (t ha ⁻¹)	Rhizobium inoculation	
		Un-inoculated	Inoculated
50	0	5.5 ⁱ	6.7 ^{cdefgh}
	1	6.3 ^{efgh}	6.8 ^{cdefg}
	2	6.1 ^{ghi}	6.9 ^{cdef}
75	0	6.2 ^{fghi}	6.9 ^{cdef}
	1	6.0 ^{hi}	7.0 ^{cde}
	2	6.3 ^{efgh}	7.3 ^{bc}
100	0	6.4 ^{defgh}	6.8 ^{cdefg}
	1	7.0 ^{cde}	9.2 ^a
	2	7.1 ^{bcd}	7.8 ^b
LSD (0.05)	0.75		
CV (%)	6.6		

Where, CV: Coefficient of variation, LSD (0.05): Least significant difference at 5% level of significance; Means in the columns and rows followed by the same letter are not significantly different at 5% level of significance.

3.2. Yield Components and Yield

Number of seed per pods: the productive potential of soybean is ultimately determined by the number of pods per plant which is a main yield component. The analysis of variance showed that the main effects of NPS, VC and Rh were significant ($p < 0.01$) on the number of pods produced per plant. Moreover, the three factor interaction of NPS \times Rh \times VC was significant ($p < 0.01$) on the number of pods produced per plants.

Generally, an integrated use of organic, inorganic and bio-fertilizer influence the number of pods per plant. The maximum number of pods per plant (87.6) was produced at the combination of highest rates of the two fertilizers (100 kg NPS ha⁻¹, 2 tons vermicompost ha⁻¹) with inoculation whereas the minimum number of pods per plant (51.53) was recorded from plants supplied with combined 50kg NPS ha⁻¹ with 0 ton vermicompost ha⁻¹ without inoculation (Table 8). This indicates that the synergistic effect of the mineral fertilizers and mineralization of organic manures and bio-fertilizer throughout growing period did not put the plants nutrient stress at any stage resulting in enhancing number of pods per plants.

Moreover, the supply of adequate nutrients through combined application Rhizobium, vermicompost and NPS might have facilitated the production of primary branches, secondary branches and plant height which might in turn have contributed for the production of higher number of pods per plant. And also the increased in number of pods with these levels might be due to various enzymatic activities which controlled flowering and pod formation. This results corroborates with the study that reported the highest number pods per plant (54.57) at RDF (20N: 45P₂O₅ kg ha⁻¹ + 5.0 ton ha⁻¹ VC + Rhizobium + PSB in chick pea [69]. Likewise, combined application of vermicompost + FYM + phosphate + Rhizobium inoculation lead to significant improvement in

number of pods per plant (20) of French bean (*Phaseolus vulgaris* L.) [70].

Table 8. Interaction effects of NPS, Vermicompost and Rhizobium inoculation on number of pods per plant of soybean.

NPS rate (kg ha ⁻¹)	Vermicompost (t ha ⁻¹)	Rhizobium inoculation	
		Un-inoculated	Inoculated
50	0	51.53 ^g	64.10 ^{def}
	1	63.30 ^{ef}	64.93 ^{def}
	2	66.80 ^{cde}	67.90 ^{cd}
75	0	66.80 ^{cde}	62.33 ^f
	1	64.27 ^{def}	64.33 ^{def}
	2	63.90 ^{def}	66.20 ^{cdef}
100	0	64.60 ^{def}	63.13 ^{ef}
	1	67.10 ^{cde}	70.13 ^c
	2	81.20 ^b	87.60 ^a
LSD (0.05)	2.09		
CV (%)	4.7		

Where, CV: Coefficient of variation, LSD (0.05): Least significant difference at 5% level of significance; Means in the columns and rows followed by the same letter are not significantly different at 5% level of significance

Number of seeds per pod: neither the main effect of Rhizobium inoculation, vermicompost and NPS fertilizer application rate nor their interactions were significant on the number of seed per pods. Most probably the number of seed per pod is significantly varied among different genotypes; however, the seed per pod are less affected by external factors like fertilization when a single genotype is considered. This result is in agreement with authors who reported that rhizobial inoculation and phosphorus application did not significantly affect number of seeds per pod of soybean [71, 72]. Also while faba bean treated with rhizobium, lime and phosphorus rate [73]. On the other hand, significant increase in number of soybean seeds per pod reported due to inoculation of soybean with different *Bradyrhizobium* strains [74].

Hundred seeds weight (g): analysis of variance showed that the main effect of NPS fertilizer revealed significant ($p < 0.01$) effect on hundred seeds weight. Significantly the highest hundred seeds weight (13.72 g) was recorded at 100 kg NPS ha⁻¹ (Table 9). The possible reason might be the nutrient use efficiency of soybean was enhanced at optimum level of NPS since grain weight indicates the amount of resource utilized during critical growth periods. And also due to the supply of phosphorus that increase the formation of seed. In line with this finding 100 seed weight was increased with increased phosphorus application rate (4.45 g) on soybean [72]. In line with this result the combined application of P and S further increased test weight of soybean and the highest test weight (94.61 g) was recorded with the treatment combination of 30 kg P ha⁻¹ and 20 kg S ha⁻¹ [75].

Table 9. The main effect of Rhizobium inoculation, NPS and vermicompost application on stand count, number of seeds per pod and hundred seed weight.

	NPS rate (kg ha ⁻¹)			Vermicompost (t ha ⁻¹)			Rhizobium inoculation	
	50	75	100	0	1	2	uninoculated	inoculated
HSW (g)	12.7 ^b	12.4 ^b	13.7 ^a	12.8	13.0	13.2	12.9	13.1
LSD (0.05)	0.49			NS			NS	
CV (%)	5.6							

	NPS rate (kg ha ⁻¹)			Vermicompost (t ha ⁻¹)			Rhizobium inoculation	
	50	75	100	0	1	2	uninoculated	inoculated
SPP	2.6	2.5	2.5	2.6	2.5	2.6	2.6	2.5
LSD (0.05)	NS			NS			NS	
CV (%)	8.7							

Where, CV: Coefficient of variation, LSD (0.05): Least significant difference at 5% level of significance; Means in the columns and rows followed by the same letter are not significantly different at 5% level of significance

Aboveground biomass (kg ha⁻¹): Aboveground biomass was significantly ($p < 0.01$) influenced by the main effects of Rhizobium, NPS and Vermicompost rates and interaction effect of NPS \times VC rates. The maximum aboveground biomass (8953 kg ha⁻¹) was obtained from application of 100 kg NPS ha⁻¹ combined 2 tons vermicompost ha⁻¹, while the lowest aboveground biomass (7059 kg ha⁻¹) was recorded in the treatment supplied with 50 kg NPS ha⁻¹ combined with 0 ton vermicompost ha⁻¹ (Table 10). The increase in the aboveground biomass at the highest rates of NPS and VC might be due to the increase in nutrient availability which increases plant growth and development. Other possible reason for increasing in the aboveground biomass due to NPS and VC might be that nitrogen, sulfur and phosphorus is essential in most metabolic processes like energy generation, nucleic acid synthesis, photosynthesis, respiration, glycolysis, membrane synthesis and integrity, enzymatic activation or inactivation, redox reactions, signaling and carbohydrate metabolism leading to the enhancement of dry biomass yield that happen above the ground [76]. The availability of macro and micro nutrients facilitates photosynthesis and increase biomass. In agreement with these results authors reported that biomass yield of soybean increased significantly with the combined application of phosphorus and sulphur in increasing rate [62, 77]. The increase in aboveground biomass yield of soybean at high rate of vermicompost and maximum rate NPS may be that P and S nutrients found in applied fertilizers were playing a role in metabolism, chlorophyll formation, and photosynthesis activities of the plant which in turn increase the biological yield [16]. Likewise, the maximum above ground biomass of 12500 kg ha⁻¹ recorded at combined application of 92 kg P₂O₅ with 30 kg S ha⁻¹ on faba bean compared with the control treatment at Sinana, South-Eastern, Ethiopia [64].

Table 10. Interaction of NPS and Vermicompost application on aboveground biomass (kg ha⁻¹) of soybean.

NPS rate (kg ha ⁻¹)	Vermicompost (t ha ⁻¹)		
	0	1	2
50	7059 ^g	8287 ^{bcd}	8299 ^{bcd}
75	8002 ^d	8352 ^{bc}	8393 ^b
100	80072 ^{cd}	8379 ^b	8953 ^a
LSD (0.05)	303.6		
CV (%)	3.2		

Where, CV: Coefficient of variation, LSD (0.05): Least significant difference at 5% level of significance, Means in the columns and rows followed by the same letter are not significantly different at 5% level of significance.

Seed yield (kg ha⁻¹): the analysis of variance showed that the main effects of NPS, VC and Rh significantly ($p < 0.01$) influenced seed yield. Also the two factor interaction of NPS \times VC significantly influenced seed yield. Moreover, soybean

seed yield was significantly ($p < 0.01$) influenced by the three factor interaction of NPS \times Rh \times VC.

Thus, the highest seed yield (4180 kg ha⁻¹) was obtained at the highest combined rates (100 kg NPS ha⁻¹ + 2 tons ha⁻¹ vermicompost and Rhizobium inoculation while the lowest seed yield (2364 kg ha⁻¹) was recorded for 50 kg NPS ha⁻¹ without application of vermicompost and under non-inoculation (Table 11). This higher yield from the combined application of mineral fertilizer, vermicompost and Rhizobium inoculation may be attributable to the availability of macro and micro-nutrients and occurrence of different beneficial microorganisms, presence of growth promoting substances. In conformity with this result, the combined use of vesmicompost, chemical fertilizers and bio-fertilizers lead to higher yields on different legumes cropping systems [77]. Likewise, the combination of 75% RDF with vermicompost at the rate of 1 ton ha⁻¹ and phosphate soluble bacteria (PSB) produced significantly higher grain yield (1.92 ton ha⁻¹) of soybean than the other treatments [78]. The application of integrated nutrient management of bio-fertilizer (Bradyrhizobium) at the rate of 1 kg ha⁻¹ with $\frac{1}{2}$ NPKS of the recommended dose produced the best quality soybean with improved growth parameters, such as number of nodules per plant, nodule dry weight per plant, pods per plant, grains per pod, and grain yield [4].

On other hand, increased seed yield at higher rate of vermicompost and NPS rate combined with Rhizobium inoculation may due to increased availability of major nutrients to plant which enhanced early root growth and cell multiplication leading to more absorption of other nutrients from deeper layers of soil ultimately resulting increased seed yield. Application of nutrients through 75% RDF (20 N: 40 P₂O₅: 20 K₂O kg ha⁻¹) + 2.5 t ha⁻¹ vermicompost + rhizobium + Phosphate solubilizing bacteria (PSB) significant improvement gave seed yield (12.34 qt/ha) in green gram in India [79]. Likewise, the application NPS combined with vermicompost supplied S nutrient to soybean which might have increased the availability of nutrient to soybean plant due to improved nutritional environment, which in turn, favorably influenced the energy transformation activation of enzymes, chlorophyll synthesis as well as increased carbohydrate metabolism [80, 81]. Similarly, application of 60 kg P₂O₅ along with 40 kg S ha⁻¹ obtained significantly higher grain yield (2.50 t/ha) of soybean compared to control in a field experiment conducted in a sandy loam soil of Umam (Meghalaya) [77].

Harvest Index: harvest index is very useful in measuring nutrient partitioning in crop plants, which provides an indication of how efficiently the plant utilized acquired nutrients for grain production. The analysis of variance revealed that the main effects of NPS, VC and Rh significantly

($p < 0.01$) influenced harvest index of soybean. On the contrary, the three factor interaction of NPS \times VC \times Rh had significant ($p < 0.01$) effects on the harvest index of soybean.

Application of 100 kg NPS ha⁻¹ and 2 tons vermicompost ha⁻¹ and Rhizobium inoculation with strain TAL-379 showed the maximum harvest index (0.47) while the lowest harvest index (0.38) was measured at the combined application of 50 kg NPS ha⁻¹, 0 ton vermicompost ha⁻¹ without inoculation (Table 11). This may due to increased availability of major nutrients to plant which enhanced early root growth and cell multiplication leading to more absorption of other nutrients from deeper layers of soil finally resulting increased harvest index. In line with this result harvest index of 28.32% was

recorded with application of nutrients through 75% RDF (20 N: 40 P₂O₅: 20 K₂O kg ha⁻¹) + 2.5 t ha⁻¹ vermicompost + rhizobium + Phosphate solubilizing bacteria (PSB) as compared control [79]. This increased in harvest index at the highest rates of NPS and VC with inoculation could be due to the integrated use of organic and inorganic fertilizers which played beneficial role in improving soil pH and organic carbon. The increased in harvest index per plant with the increased of NPS fertilizer rate and vermicompost along with Rhizobium inoculated treatment might be due to availability of P and S that the influence of greater pod and seed setting than above ground biomass yield. Likewise, harvest index was significantly influenced by applied P in soybean [82].

Table 11. Interaction effect of NPS, Rhizobium inoculation and vermicompost application on seed yield and harvest index of soybean.

NPS rate (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)		Harvest index		
	Vermicompost (t ha ⁻¹)	Un-inoculated	Inoculated	Un- inoculated	Inoculated
50	0	2364 ^h	3108 ^g	0.38 ^g	0.42 ^{ef}
	1	3593 ^{cde}	3573 ^{de}	0.43 ^{def}	0.44 ^{bcd}
	2	3577 ^{cde}	3713 ^{bcd}	0.45 ^{abcd}	0.45 ^{abcd}
75	0	3335 ^f	3340 ^{ef}	0.42 ^{ef}	0.41 ^f
	1	3511 ^{def}	3784 ^{bc}	0.45 ^{abcd}	0.45 ^{abcd}
	2	3592 ^{cde}	3870 ^b	0.43 ^{def}	0.46 ^{abc}
100	0	3445 ^{ef}	3590 ^{cde}	0.42 ^{ef}	0.44 ^{bcd}
	1	3608 ^{bcde}	3852 ^b	0.43 ^{def}	0.46 ^{abc}
	2	3908 ^b	4180 ^a	0.45 ^{abcd}	0.47 ^a
LSD (0.05)	209.5			0.02	
CV (%)	4.8			2.8	

Where, CV: Coefficient of variation, LSD (0.05): Least significant difference at 5% level of significance; Means in columns and rows followed by the same letter are not significantly different at 5% level of significance.

4. Summary and Conclusions

In conclusion, the use of integrated plant nutrient management (INM) is essential avert the problem of crop production by enhancing soil fertility and crop productivity and has its own advantages under changing climate. The result of this study indicated that application 100 kg NPS ha⁻¹ + 2 tons vermicompost ha⁻¹ combined with Rhizobium strain (TAL-379) inoculation gave highest seed yield (4180 kg ha⁻¹). Thus, considering the role of integrated nutrient management in climate mitigation and adaptation by reducing synthesized fertilizer; combined application of 2 t VC ha⁻¹ and 75 kg NPS ha⁻¹ inoculated with Rhizobium strain (TAL-379) had resulted in better and optimum yield of 3870 kg ha⁻¹ which is environmentally sound for soil management and is tentatively recommended for use.

Competing Interests

Authors have declared that no competing interests exist.

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