

## Article

# Effectiveness of *Rhizobium tropici* sp. Strain UD5 Peat Biofertilizer Inoculant on Growth, Yield, and Nitrogen Concentration of Common Bean

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**Abstract:** Common bean (*Phaseolus vulgaris* L.) ranks among the most produced and consumed legume crops and contains essential macro- and micronutrients. Grain yield of the food crop is markedly decreased by poor management, especially a lack of additional essential nutrient elements through the application of fertilizers. In addition to the application of fertilizers, scholarly research and crop farmers have shown that the use of biofertilizer inoculants improves the yield of legume crops. The objective of this research study was to assess the effectiveness of peat-based *Rhizobium tropici* sp. UD5 on the growth, yield, and nitrogen concentration of common bean. The peat inoculant contained  $6.5 \times 10^9$  viable cells/g. The experiment was conducted in two climatic zones, as described by the Koppen–Gieger climatic classification system. Treatments involved the peat-based inoculant *Rhizobium tropici* (T0 = 0 g without inoculation, T1 = 250 g of peat inoculant of strain UD5 for 50 kg seeds, T2 = 500 g of inoculant of strain UD5, and T3 = 200 g of comparative peat inoculant). The results indicated that common-bean-inoculated formulation of *R. tropici* sp. strain UD5 increased the following parameters compared to the controls: plant height (T1 = 18.22%, T2 = 20.41%, and T3 = 19.93% for bioclimatic zone 1; T1 = 16.78%, T2 = 20.71%, and T3 = 19.93% for bioclimatic zone 2), root length (T1 = 13.26%, T2 = 21.28%, and T3 = 19.38% for zone 1; T1 = 15.06%, T2 = 23.70%, and T3 = 19.20% for zone 2), number of nodules (T1 = 1162.57%, T2 = 1166.36%, and T3 = 1180.30% for zone 1; T1 = 1575%, T2 = 1616.5%, and T3 = 1608.25% for zone 2), size of nodules (T1 = 224.07%, T2 = 224.07%, and T3 = 208.33% for zone 1; T1 = 166.4%, T2 = 180%, and T3 = 140% for zone 2), and yield (T1 = 40.49%, T2 = 47.10%, and T3 = 45.45% for zone 1; T1 = 62.16%, T2 = 54.05%, and T3 = 58.55% for zone 2). *R. tropici* sp. UD5 peat inoculant formulation also increased the nitrogen concentration in leaves compared to the control (T1 = 3.75%, T2 = 1.12%, and T3 = 8.72%) in both bioclimatic zones. The findings of this study provide significant information on the positive effect of *R. tropici* UD5 strain peat inoculant application in the improvement of plant growth, development, and yield through the formation of nodules.

**Keywords:** rhizobium; biofertilizer; growth; inoculant formulation; nitrogen content; peat; yield



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## 1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a popular, widely cultivated and consumed food legume across the world [1]. About 50% of grain pulse products consumed by humans are produced from common bean [2]. The seeds are the most profitable of the organs of common bean [3]. The seeds are widely used to develop a range of food by-products that exhibit enhanced nutritional properties and boast a prolonged storage life of up to two years if moisture content is lower than 10% at 8 °C [1]. Therefore, the production and consumption of common bean makes a significant contribution to human nutrition

and they are considered a good source of protein, carbohydrates, vitamins, and essential nutrient elements [4].

The proteins contained in common bean are equal or superior in quality to those in soybean in terms of emulsifying properties, high hydrophobicity, and foaming [5]. *Phaseolus* species have adapted to a range of different bioclimate conditions and play an important role in addressing the challenges of food insecurity across the world [6].

Among food crops, *Phaseolus* species are the most widely cultivated and their grain yield ranks among the highest producers. For example, in 2020, 27.5 million metric tons of common bean grain was harvested from 34.8 million hectares [7]. Asia contributes the largest tonnage of common bean with 43% of global production, followed by America with 29% (North, Central, and South America), and Africa with 26%, while Europe and Oceania contribute approximately 2% [4]. Countries including Myanmar, India, Brazil, China, Tanzania, Uganda, USA, Mexico, Kenya, and Burundi are ranked as the 10 largest producers of common bean with an average seed yield of 1557 kg/ha. Together, they contribute about 70.8% of common bean worldwide, making 20.5 million tons [8].

Like most cultivated food crops, in order to improve the yield of common bean, there is a need for the addition of agricultural inputs, including inorganic fertilizers, organic fertilizers, and/or biofertilizers. In general, biofertilizers are categorized as either liquid or peat-based, and the most used biofertilizer in Africa and South America, among other continents, is *Rhizobium* bacteria. Their application, alone and at recommended rates of use, alleviates the negative effects of chemical fertilizers on the natural environment and, thus, explains their wide usage, especially by farmers that cultivate leguminous crops [6]. Chianu et al. [9] reported that low yields associated with grain legumes are largely caused by low soil fertility and decreased biological N<sub>2</sub> fixation due to biological, ecological, and environmental factors. Through the process of biological nitrogen fixation, microorganisms convert atmospheric dinitrogen into forms that are usable or absorbed by plants [10]. The N contributed through this symbiotic interaction has had positive effects on the quality of legumes and soils [11].

While the N contributed through BNF plays a significant role in performance enhancement, the BNF process ought to be enhanced through inoculation with rhizobia [12]. The N nutrient element is the most limiting in croplands and hampers the yield of common bean in the world [13]. Therefore, inoculation of legumes with *Rhizobium*-based biofertilizers not only enhances the BNF process but also reduces the usage of chemical fertilizers [14] linked to the contamination of water resources. The latter explains why inoculation with biofertilizers is considered an important aspect of natural resource management in crop production. Scholarly studies have shown that improving BNF through the use of rhizobial inoculants reduces costs associated with the production of legumes [15]. While studies conducted on other continents have proven the effectiveness of biofertilizers in common beans [16], currently, there is little published information on whether rhizobial inoculation enhances the performance of common bean grown under South African conditions. Also, there is a dearth of published studies on whether the rhizobial strain UD5 (widely credited for enhancing legume growth, yield, and quality [17]) improves the performance of leguminous crops grown in South Africa.

The objectives of this research were to assess the effectiveness of peat-based *Rhizobium* sp. strain UD5 on the growth, yield, and N concentration of common bean grown in two climatic zones in South Africa.

## 2. Materials and Methods

### 2.1. Preparation of Peat Inoculant

*Rhizobium tropici* sp. UD5 strain was obtained from the Agricultural Research Council, Plant Health, Pretoria (South Africa). In preparing the peat formulation of *R. tropici* sp., the *R. tropici* sp. UD5 strain culture was grown on yeast mannitol broth as described by [18]. A total of 10 mL inoculum was transferred to 750 mL yeast extract mannitol broth and shaken at 150 r.p.m. at 30 °C for 3–5 days until a concentration of  $6.5 \times 10^9$  cells mL<sup>-1</sup>

was obtained. A mixture of formulation additives was aseptically added together, and then 60 mL of mannitol broth yeast containing both *R. tropici* sp. UD5 strain and additives was homogeneously mixed with 250 g sterile peat. The resulting formulation produced standardized values, indicating an analysis of reagents (g) produced 1 kg of the formulation. *R. tropici* sp. UD5 strain peat inoculant comprised 6.5 billion CFU g<sup>-1</sup>. Chemicals used were obtained from Sigma-Aldrich company in Johannesburg, South Africa.

## 2.2. Experiment Locations

Experiments were performed in two climatic zones, as described by the Koppen–Gieger climate classification [19]. Of the two study locations, one was in the Gauteng province, Pretoria. The location is categorized under a humid subtropical climate, defined as C-wa (Gauteng, South Africa), and positioned at 25°48'30" S latitude and 28°44'26" E. Sandy clay loam soil was used in the trial.

The second site of the experiment was in the North West province of South Africa and is classified as an arid climate characterized by BWwh. It is located in the geographical co-ordinates of 26°47'16" S latitude and 26°53'59" E longitude with sandy loam soil. The rainfall of the area is 670 mm.

## 2.3. Analysis of Soil

In order to assess physical and chemical properties of the soil, soil samples were taken randomly across each location at a depth of 0–40 cm according to the Soil Classification Working group (1991). The experimental sites had Hutton soil [20] form with a clay content of 36% at 0–40 cm soil depth similar to that reported by Gatabazi et al. [18]. Neither the soil nor plants were supplied with fertilizer at both locations. Weather data for both experiments were similar to those reported by Gatabazi et al. [18].

## 2.4. Experimental Design and Treatments

The experimental plots were arranged in a randomized split plot design and each plot consisted of four treatments, replicated six times. The inter-row and intra-row spacing used were 0.9 m and 0.075 m, respectively, giving a plant population density of 148,148 per hectare, making 67.5 m<sup>2</sup>. Treatments included *R. tropici* (T0 = 0 g without inoculation, T1 = 250 g of peat inoculant of strain UD5 for 50 kg seeds, T2 = 500 g of inoculant of strain UD5, and T3 = 200 g of comparative peat inoculant for 50 kg seeds). Inoculant was applied onto 50 kg of seeds of common bean. A planter was used to sow after the sites had been ploughed and harrowed to a depth of 20 cm and separated into experimental plots.

## 2.5. Collection of Data

### Determination of Growth, Yield, and Quality Parameters

Final emergence rates were recorded when 80% of common bean plants had emerged in each of the experimental plots. Growth parameters that were measured included the number of root nodules, height of plants, and the length of roots, all determined during the flowering growth stage, on 20 non-border plants selected randomly in each row. Each plant was carefully dug and root nodules on each root counted. Nodule size was determined by measuring the vertical length of the cylindrical nodule using a caliper. The height and length of each plant were determined by measuring using a 30 cm ruler. During the flowering growth stage, top-canopy leaves of each of the selected plants were collected, put into paper sample bags, and oven-dried at 65°C to constant weight. The fine powder (1 mm size) was used to determine nitrogen concentration through using the Kjeldahl method, as described by [21]. Fresh roots of each of the 20 plant stands were cut and used to measure length.

Yield was determined in 20 plants, selected from the non-border rows of each experimental plot. Yield was calculated and expressed in tons per hectare (t/ha).

## 2.6. Statistical Analysis

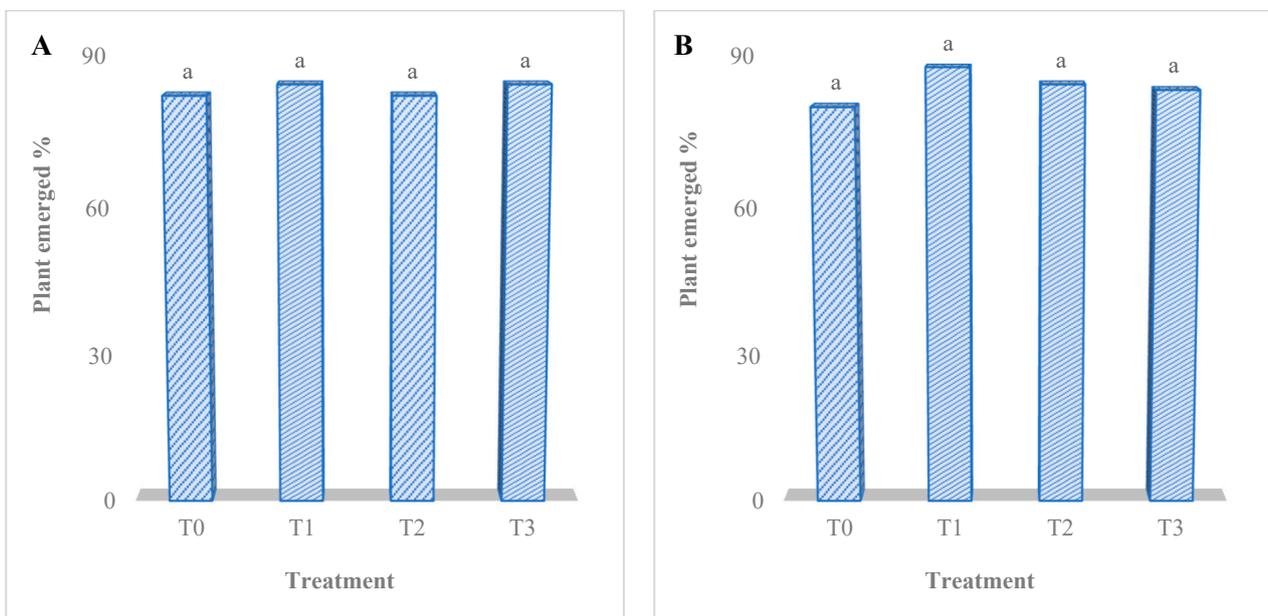
Data was analyzed using the Statistical Analysis Software (SAS) Version 4.2.1. Where significant, mean values were separated through using the Fisher's least significant difference (LSD) at 5% level of significance.

## 3. Results

### 3.1. Growth, Yield, and N Cocentration

#### 3.1.1. Plant Emergence

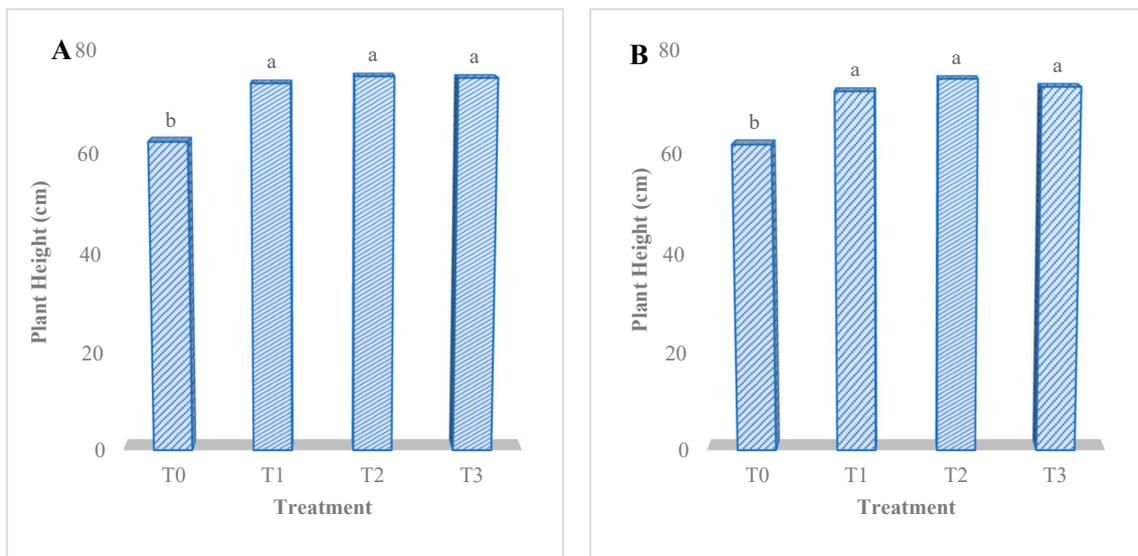
The percentage emergence of plants grown under both climatic zones, namely A and B, under different levels of inoculation with peat formulation are presented in Figure 1. Of the plants that emerged at both study locations, there were no significant differences between the treatments. At climatic zone A, mean values ranged from 82.22% in T2 to 84.44% in T4. For plants at climatic Zone B, emergence percentage ranged from 80% in T0 to 87.77% in T1. In a comparison between both climatic zones, the results on percentage emergence also showed no significant differences.



**Figure 1.** Emergence of dry bean under biofertilizer inoculant. Treatments sharing the same letters are not significantly different; (Lsd Zone A) = 6.61 and (Lsd Zone B) = 8.01.

#### 3.1.2. Plant Height

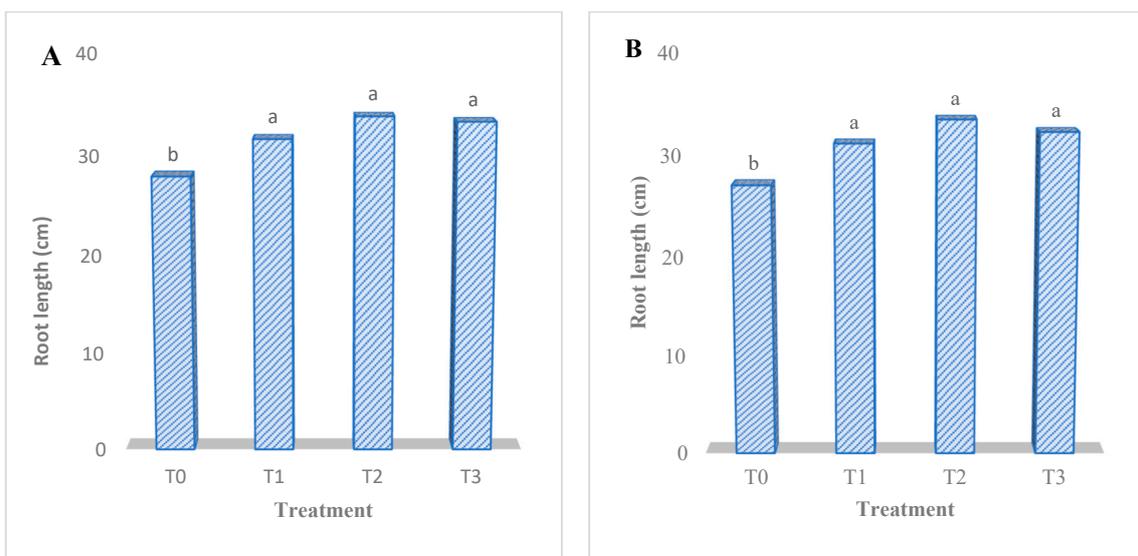
The results revealed significant differences between the height of the plants' grown at each of the selected climatic zones. For example, the plants inoculated with 250 g, 500 g, and 200 g inoculant at both locations were taller compared to those of the control (Figure 2). Compared to the control treatment, *R.-tropici*-sp-inoculated common bean plants significantly increased as follows: T1 = 18.22%, T2 = 20.41%, and T3 = 19.93%, as well as T1 = 16.78%, T2 = 20.71%, and T3 = 19.93%, respectively, for climatic zone 1 and 2. The results reveal that plant height was similar between rhizobial-inoculated and uninoculated common bean.



**Figure 2.** Dry bean plant height under biofertilizer inoculation. Treatments sharing the same letters are not significantly different; (Lsd Zone A) = 3.42 and (Lsd Zone B) = 3.2.

### 3.1.3. Root Length

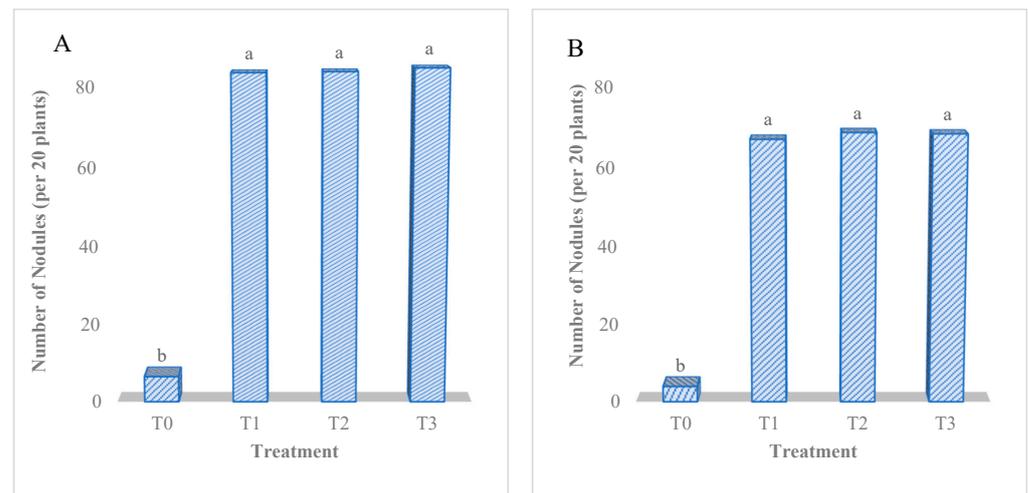
Common bean plants grown at climatic zones A and B and treated with 250 g, 500 g, and 200 g of the peat-based *R. tropici* inoculant revealed significantly higher ( $p < 0.01$ ) root length compared to the control treatment 0 g (T0) (Figure 3). Of the treatments, plants inoculated with 500 g of *R. tropici* sp. exhibited the highest root length in both climatic zones A and B. The increases in root length associated with inoculation with *R. tropici* sp. were as follows: T1 = 13.26%, T2 = 21.28%, and T3 = 19.38% and T1 = 15.06%, T2 = 23.70%, and T3 = 19.20% for zones 1 and 2, respectively. In comparison with both climatic zones A and B, the results revealed a similar length of roots of the plants grown between the climatic zones. All applied treatments with inoculants showed the same significant differences in both climatic zone A and B. The untreated peat inoculants also showed the same results in both climatic zones A and B.



**Figure 3.** Dry bean root length under biofertilizer inoculation. Treatments sharing the same letters are not significantly different; (Lsd Zone A) = 2.77 and (Lsd Zone B) = 2.72.

### 3.1.4. Number of Nodules

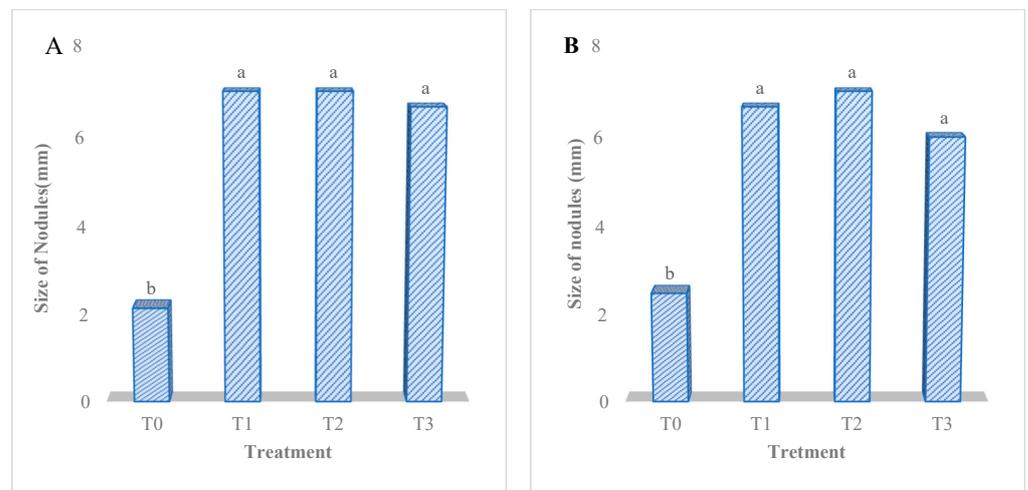
At climatic zones A and B, inoculating with *R. tropici* at the rate of 250 g, 500 g, and 200 g markedly increased the number of nodules by 12.8-fold between T0 and T3 at zone 1, as well as 17-fold between T0 and T2 at zone B (Figure 4). Common bean plants inoculated with *R. tropici* sp. revealed an increased number of nodules, as follows: T1 = 1162.57%, T2 = 1166.36%, and T3 = 1180.30% and T1 = 1575%, T2 = 1616.5%, and T3 = 1608.25%, respectively, for zone 1 and zone 2.



**Figure 4.** Dry bean nodule number under biofertilizer inoculation. Treatments sharing a letter are not significantly different; (Lsd Zone **A**) = 33.24 and (Lsd Zone **B**) = 13.08.

### 3.1.5. Size of Nodules

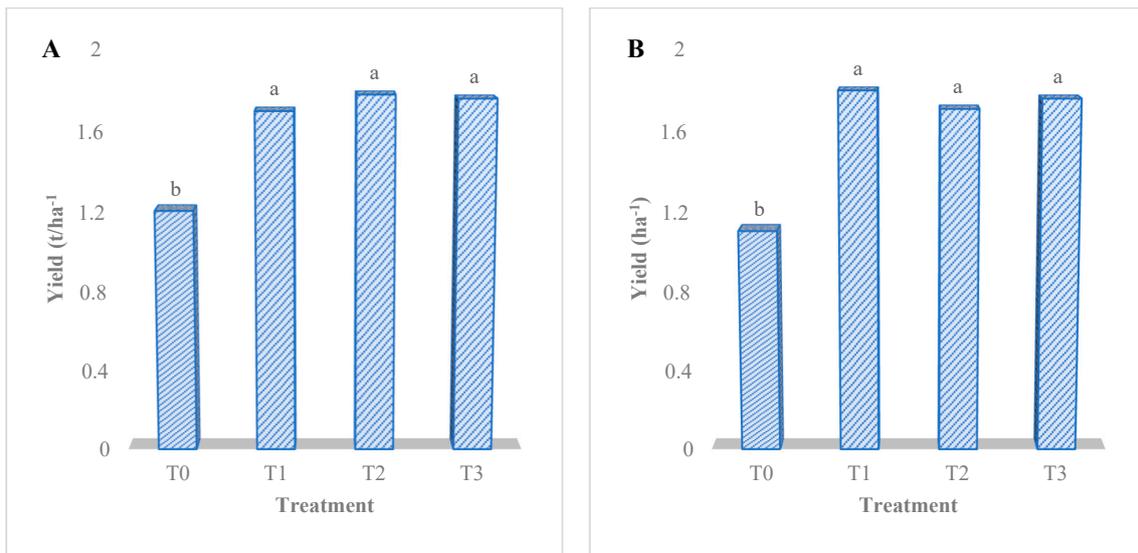
In a trend similar to that shown for number of root nodules, the size of the root nodules was also increased in T1, T2, and T3 compared to T0 at both climatic zones. Increases in the size of root nodules were associated with inoculation with *R. tropici* at 250 g, 500 g, and 200 g compared to the control (Figure 5). Increases in the size of root nodules were as follows: T1 = 224.07%, T2 = 224.07%, and T3 = 208.33% and T1 = 166.4%, T2 = 180%, and T3 = 140%, respectively, for zone 1 and 2. The results on the size of root nodules showed a similar trend between the climatic zones.



**Figure 5.** Dry bean size of nodules under biofertilizer inoculation. Treatments sharing the same letters are not significant; (Lsd Zone **A**) = 3.12 and (Lsd Zone **B**) = 1.86.

### 3.1.6. Grain Yield

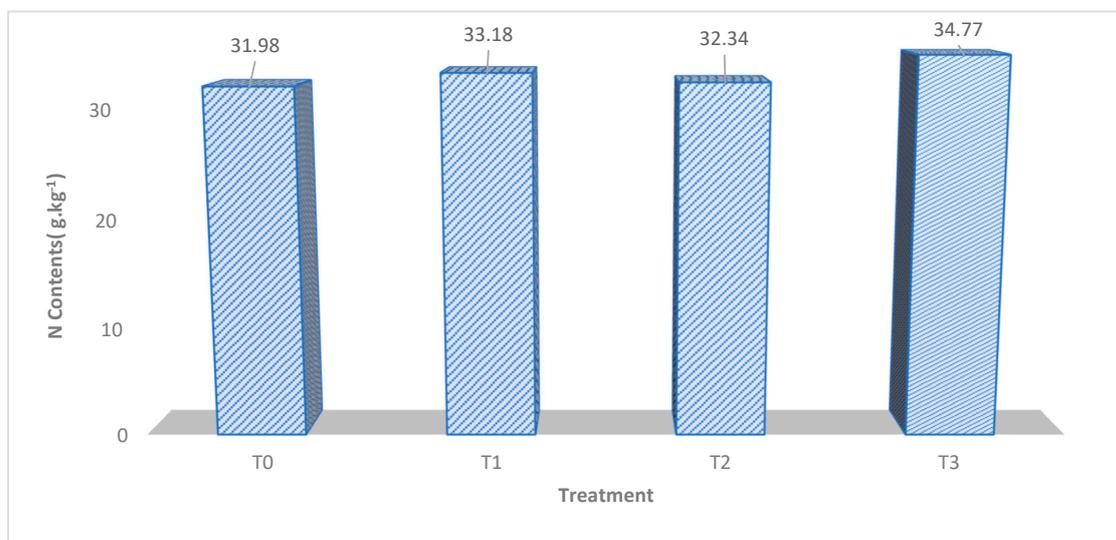
Grain yield determined on common bean grown at both climatic zones was significantly higher when inoculated with *R. tropici* sp. UD5 (250 g, 500 g, and 200 g) compared to the control (T0). For example, the grain yield of plants inoculated with *R. tropici* UD5 and denoted as T1, T2, and T3 was similar; however, each of these treatments was higher than T0 (Figure 6). Increased percentages were as follows: T1 = 40.49%, T2 = 47.10%, and T3 = 45.45% and T1 = 62.16%, T2 = 54.05%, and T3 = 58.55%, respectively, for zone 1 and 2. The study showed no differences in grain yield of plants established between both climatic zones, irrespective of treatment.



**Figure 6.** Dry bean yield under biofertilizer inoculation. Treatments sharing a letter are not significantly different; (Lsd Zone A) = 0.13 and (Lsd Zone B) = 0.1.

### 3.1.7. Leaf N Concentration

The concentration of N determined in leaves of T1, T2, and T3 was numerically higher than that in T0 (Figure 7). *R. tropici* showed increased percentages as follows: T1 = 3.75%, T2 = 1.12%, and T3 = 8.72%.



**Figure 7.** Dry bean nitrogen uptake under biofertilizer inoculation.

#### 4. Discussion

In this study, common bean was grown in two climatic zones in South Africa and inoculated with *R. tropici* sp. Results revealed that inoculating with 250 g, 500 g, and 200 g of the peat-based rhizobial inoculant had a significant effect on the legume's growth and yield (Figures 2–4 and 6). Also, the results revealed that leaf N concentration was increased by supplying 250 g, 500 g, and 200 g of the peat-based rhizobial inoculant compared to that of uninoculated plants (T0) (Figure 7). The markedly increased growth and yield-related parameters of rhizobial-inoculated common bean is a trend supported by other studies [22] where another rhizobial inoculant, *leguminosarum* *bv. viciae* sp., had a similar effect on common bean. In addition to improved aboveground legume parameters, scholarly studies have also shown enhanced belowground legume parameters, including increased number, size, and weight of root nodules. For example, seed inoculation with *Rhizobium* stimulates nodule formation [13,14].

For this study, inoculating with the peat-based rhizobial inoculant at 250 g, 200 g, and 500 g significantly increased the number and size of root nodules of the common bean cultivar compared to those of the uninoculated treatment T0 when grown under both bioclimatic zones A and B (Figures 4 and 5).

As shown by Anteneh and Ayele [22], *Rhizobium* inoculation of common bean significantly increases the growth and yield and the approach reduces the need for application of high rates of inorganic N, especially beyond 20 kg/ha. In another study conducted in North America, Minnesota, inoculation of seeds of common bean with a rhizobial inoculant revealed significantly increased seed yield [14]. Lastly, the performance of common bean inoculated with rhizobial inoculants has shown improved performances even when planted under different conditions in the African continent. For example, rhizobial inoculation of common bean established in Ethiopia showed increased nodule formation as well as dry weights of shoots and roots [23].

Apart from improving nodulation, *Rhizobium* inoculation significantly increased the yield of the common bean cultivar planted in both bioclimatic zones (Figure 6). The results showing an increase in grain yield agree with those shown in previous research conducted on other grain legumes. For example, when soybean and lucerne were inoculated with *Bradyrhizobium japonicum* and *Sinorhizobium meliloti*, they demonstrated greater number and weight of root nodules as well as increased grain yield [18]. Similarly, an earlier study conducted in the Limpopo province of South Africa revealed that *Rhizobium* inoculation of common bean significantly improved the grain yield by approximately 16.15 and 27.50% compared to the control [24]. In another study, *Rhizobium* inoculation increased the nodulation of bean cultivar BRS Cometa and the dry biomass when supplied at 232 and 221 mL ha<sup>-1</sup>, respectively. Inoculating with 257 mL ha<sup>-1</sup> of the liquid-based inoculant in topdressing during the V4 stage and on seeds, the latter approach showed markedly greater yield of common bean established without the addition of inorganic nitrogen fertilizer [25].

While an increase in the formation of root nodules of rhizobial-inoculated legumes can be attributed to various factors, the effectiveness and/or competitiveness of the rhizobial inoculant ought to be considered as an important factor [26].

The results on the concentration of N in leaves of the common bean (Figure 7) agree with those reported in Ethiopia on soybean [13]. However, the increase in the concentration of N in leaves of the inoculated treatments (T1 = 250 g, T2 = 500 g, and T3 = 200 g) were minimal compared to that on soybean [18]. Although not assessed in this study, the results on leaf N could imply that the selected cultivar of common bean could have been less efficient in fixing N through BNF, compared to that shown on soybean and lucerne [18,27].

The increase in the concentration of leaf N observed in this study can be attributed to enhanced nodule formation of inoculated treatments, a trend also shown in other studies conducted in soybean [18] and lucerne [28]. The increase in the number and size of nodules of the common bean could have a direct effect on the process of BNF. Htwe et al. [29] showed that an increase in the growth and nodulation of legumes, including soybean and mung beans, has a direct effect on their biological nitrogen fixation. In fact,

inoculation of soybean with *B. japonicum* increases biological nitrogen fixation and nitrogen uptake [29]. Our results are substantiated by those of Buetow et al. [30,31], who found that Rhizobium inoculation of common bean improved the number of root nodules compared to uninoculated plants. A study conducted in Iran indicated that inoculating seeds of common bean with *Rhizobium phaseoli* resulted in an improvement in plant productivity and quality. Therefore, inoculation of common bean can lead to improved grain yield and reduce the adverse environmental effects and reduces the need to apply high rates of especially nitrogen fertilizer [32]. Rhizobia strain inoculation increased nodulation, plant growth, and grain yield in all cultivars used in the study [33]. In contrast, a study conducted in Zimbabwe demonstrated that improved common bean varieties that are currently on the market did not respond to rhizobia inoculum currently marketed in Zimbabwe. Further investigation of wide varieties needs to be investigated for a balanced conclusion [34].

Solaiman et al. [35] reported that rhizobial inoculation of chickpea enhanced nodulation, growth, and nitrogen fixation. The study also revealed significant and positive correlations between the number and dry weight of nodules, N content, and N uptake by shoots of chickpea. Another study on common bean reported increased nodule number and dry matter yield for most varieties used. The same study demonstrated markedly increased grain yield and dry matter yield, as well as a significant correlation for grain yield and dry matter yield [36].

A study conducted on the influence of Rhizobium inoculation on the growth and yield of groundnut (*Arachis hypogaea*) cultivars showed that Rhizobium inoculation significantly enhanced the growth and yield parameters [37]. Khaitov et al. [38] demonstrated beneficial effect of Rhizobium inoculation on growth and yield of chickpea (*Cicer arietinum* L.) grown in saline soils. Their results demonstrate that Rhizobium inoculation significantly increased the growth and yield of chickpea. They added that the effect of Rhizobium inoculant significantly stimulates higher nodulation efficiency and can, therefore, be considered as a biofertilizer for enhancing the productivity of chickpea.

Research conducted by Ali et al. [39] on the effect of Rhizobium inoculation to different varieties of garden pea (*Pisum sativum* L.) showed increased nodule number, nodule weight, root weight, shoot, stover yield, and seed yield compared to non-inoculated plants. Our results are supported by findings of other studies, including those conducted by Khaitov et al. [38] and Ali et al. [39]. A study conducted by [40] demonstrates that Rhizobium inoculation increases the uptake of nitrogen from soil and stimulates aboveground productivity. The effect of Rhizobium inoculants on growth, yield, and nitrogen concentration has been studied by different researchers; all reported the significant effect of seed inoculant on plant growth and yield [41]; Gamini et al. [42] and Zhang et al. [43]. The underlying mechanism in the increase in growth and yield can be attributed to the synthesis of phytohormones such as auxin as secondary metabolites in Rhizobium-inoculated plants [44].

Findings from our study regarding *Rhizobium tropici* sp. strain UD5 demonstrate that the growth, nodule number, size, yield, and nitrogen concentration were significantly higher compared to the control. It also highlighted that *Rhizobium tropici* T1 = 250 g of peat inoculant of strain UD5 for 50 kg seeds, T2 = 500 g of inoculant of strain UD5, and T3 = 200 g of comparative peat inoculant were optimum inoculants for common bean.

## 5. Conclusions

Results of this study show that inoculation with a peat-based formulation of *R. tropici* improves the growth and yield-related parameters of common bean grown in both climatic zones. Specifically, the enhanced performance was as the result of inoculation with 250 g, 500 g, and 200 g inoculant. In particular, the increased number of root nodules of *R.-tropici*-inoculated plants also showed an increase in the growth and yield, which improved the content of N in the dry bean. The results shown in this study indicate that inoculating common bean with the peat-based rhizobial inoculant has beneficial effects on common bean. Therefore, it can improve its production under South African conditions. It can also be said that inoculating other related legumes with the peat-based rhizobial inoculant

would improve their growth, yield, and grain quality and, therefore, reduce the need for the addition of higher rates of inorganic N fertilizers. In addition, the enhanced growth, yield, and nitrogen shown using the rhizobial inoculant ought to be accompanied by sound crop management practices. Based on the global reduction in soil fertility due to agriculture practices and climate-related pressure, future research in common bean could also focus on the improvement of the understanding of soil fertility conditions, mainly on nutrient availability, including micronutrient fertilizer applications in combination with *Rhizobium* inoculation to improve low-fertility soils and the crop yield. Secondly, the investigation of secondary metabolites as a mechanism in the improvement of plant growth and yield should be investigated further to determine the selection markers for a breeding program in abiotic stress conditions.

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