Symbiotic effectiveness of inoculation with *Bradyrhizobium* isolates on Cowpea (*Vigna unguiculata* (L.) Walp) varieties

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Symbiotic effectiveness of inoculation with Bradyrhizobium isolates on Cowpea (Vigna unguiculata (L.) Walp) varieties

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Abstract: Cowpea (Vigna unguiculata (L.) Walp) is one of the widely cultivated crops, contributing for sustainable farming development due to its excellent nitrogen contribution via symbiosis. The response of Bradyrhizobium inoculants depends on the compatibility of the strain and the legume crop. Therefore, the current work aimed to evaluate the symbiotic effectiveness of the Bradyrhizobium isolates with cowpea varieties tested on sand. For these purpose, five Bradyrhizobium isolates (CP-24, CP-10, CP-37, GN-33 and GN-102) and five cowpea varieties (Keti (IT99K-1122), TVU, Black eye bean, White wonderer trailing and Bole) were used. The experiment was arranged using CRD, with three replicates. The result of the experiment showed, significant (P ≤ 0.05) differences for all the measured parameters. White wonderer trailing inoculated with CP-24 exhibited the higher plant height (28.33 cm), whereas, the least plant height recorded when Keti (IT99K-1122) variety was inoculated with GN-102. The highest nodule number per plant (47.67) recorded from CP-24 inoculated Black eye bean variety. The highest nodule dry weight recorded from CP-24 inoculated TVU variety. A pink nodule color and green leaf observed from the cowpea varieties inoculated with Bradyrhizobium isolates. For all the parameters considered, the control treatments showed significantly lower performance compared with the inoculated treatments. Hence, this study proves the need for Bradyrhizobium inoculation to improve the growth, biomass and nodulation performance, and to use these isolates for field production of cowpea in the study area and similar agro-ecologies.

Subjects: Agriculture & Environmental Sciences; Botany; Soil Sciences

Keywords: BNF; isolates effectiveness; legumes; nodulation; symbiosis

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PUBLIC INTEREST STATEMENT

Bringing climate friendly technologies into the farming system is the most important approaches, which can serve for improving productivity and achieve food security during the climate change period. However, the technologies should be confirmed for their suitability for the desired aims and adaptation in the given environment before wider application. Therefore, in this pot experiment, cowpea inoculating Bradyrhizobium isolates was assessed for their effectiveness in nodule formation and growth promotion under sand media and the best-performing strains decided to be used in the field.
1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important legume crops serving several purposes (Timko & Singh, 2008). These days, cowpea is the most extensively produced legume crop in sub-Saharan Africa (Walker, 2016). For instance, only in 2014, more than 12.3 million ha of land allotted for cowpea production in the region (FAOSTATS, 2014). Cowpea performs well and most popular in the semi-arid of the tropics where other food legumes do not perform potentially (Sankie et al., 2012). It is an extremely resilient crop and cultivated under extreme environments (Owolade et al., 2006). Cowpea has a unique ability to fix atmospheric nitrogen (N) under stressed conditions (Antova et al., 2014), with a potential to fix up to 240 kg N ha⁻¹ (Appiah et al., 2015). Thus, the crop can be taken as a strategic choice in the era when mineral nitrogen is becoming a treat to the environment and costly for poor farmers (Abdulkadir et al. 2017).

Cowpea in small family farms under low input agricultural systems, where the farm receives no fertilizer, the production is partly dependent on the biological N fixation (BNF). However, its efficiency depends on the interaction of cowpea varieties and rhizobia strains (Freitas et al., 2012). Being a legume, cowpea covers more than 50% of its N requirement through BNF (Samuel Adjei-Nsiah et al., 2012). The rhizobia capable of fixing atmospheric N to a form usable by plants live in symbiotic association with the roots of cowpea, which is an opportunity for higher N₂ fixation (Appiah et al., 2015). Therefore, there is a need to pre-test the effectiveness of the strains used for inoculation to make the inoculation-based field production of cowpea successful. Hence, the authentication for effectiveness of the *Bradyrhizobium* species isolates of cowpea genotypes for maximum levels of N₂-fixation in the field condition remains a high priority in symbiosis research.

In the present investigation, therefore, symbiotic effectiveness of inoculation with five *Bradyrhizobium* strains on five cowpea varieties were thoroughly examined on sand hypothesizing for improved growth and biomass of cowpea due to *Bradyrhizobium* inoculation and effectiveness of inoculants in forming nodules.

2. Materials and methods

2.1. Sources of the experimental materials

The cowpea varieties, which has been approved to be adapted under Ethiopian field conditions were used for this study, were provided by the Melkasa Agricultural Research Center, Ethiopia [http://www.eiar.gov.et/marc](http://www.eiar.gov.et/marc). Five cowpea varieties Keti (IT99K-1122), TVU, Black eye bean, White wonderer trailing, and Bole were used for the experiment. *Bradyrhizobial* isolates, namely (CP-10, CP-24, CP-37, GN-33 and GN-102), were used as a source of inoculants. These isolates were obtained from N₂ Africa Hawassa University Project, Ethiopia [http://www.hu.edu.et/hu/](http://www.hu.edu.et/hu/).

2.2. The bacterial growth medium

To prepare a growth medium of Yeast-extract Mannitol Agar (YMA): 10 g mannitol, 0.5 g K₂HPO₄, 0.2 g MgSO₄-7H₂O, 0.1 g NaCl, 0.5 g yeast-extract and 15 g agar in 1 l of distilled water containing 25 μg/ml ml⁻¹ Congo Red (CR) dye was used. It was adjusted to 6.8 with 10% (0.1 N) solution of NaOH or HCl by using microprocessor pH meter. It was autoclaved at 121°C and 15 lb/in² for 15 minutes, cooled to 55°C, dispensed into sterile Petri dishes and solidified in a Laminar Hood Flow.

A loop full of the strain suspension was then taken from the preserved vials and streaked on YMA. The streaked medium was incubated at 28°C ± 2 in an incubator and checked daily for colony appearance after 2nd to 13th day of inoculation. The appearance of rhizobial colonies viability checked on the medium. The *Bradyrhizobial* colonies were repeatedly streaked on YMA until a pure isolate was obtained. Then, the isolates were preserved in 50% glycerol.

2.3. Authentication test

Modified Leonard jars were prepared from two plastic cups for growing cowpea plants for authenticating presumptive *Bradyrhizobia*. The upper cups were filled with pre-treated river sand, which
was washed in quarter strength of 98% H₂SO₄ acid solution, to support the seedlings. The pH of the sand was raised to nearly 7.0 by washing with tap water. The second cups were connected to the upper cups by cotton. Cowpea seeds were surface sterilized and allowed to germinate in sterilized Petri dishes containing sterile tissue paper. The pre-germinated cowpea seedlings then aseptically transferred into the jars (one seedling per jar). The treatments were arranged in RCD with three replications, having 35-treatment combination and 105 total pots.

2.4. Seedling management in the jar
Each seedling inoculated with 1 ml of presumptive isolates were taken by micropipette from pure broth cultures at their logarithmic growth phases. The jars randomly arranged and set in the shade, at College of Agriculture, Hawassa University. The seedlings were weekly watered with sterile quarter-strength of Jenson’s modified N-free nutrient solution (1 g CaHPO₄, 0.2 g KH₂PO₄, 0.2 g MgSO₄.7H₂O, 0.2 g NaCl, 1 ml trace elements stock solution, 0.1 g FeCl₃ in 4 l of distilled water) and with sterilized distilled water as it necessitated. Positive and negative controls were included in the test treatment. Seedlings that were not inoculated with the isolates were given with potassium nitrate solution (0.5 g KNO₃ per 1 l of sterilized distilled water) as positive controls. The other categories of the isolate uninoculated, were given only N-free nutrient solution and considered as a negative control.

2.5. Data collection
The preliminary effectiveness test of the rhizobia isolates were qualitatively examined by observing the leaf color and vegetative performance of the seeding at 45 days from establishment. At 45 days of age, the seedlings were uprooted and separated in to shoots (leaves and stems), roots and nodules. The leaf color was recorded as green, light green or yellow to determine the extent at which the seedlings depends on BNF. The nodules were picked and spread on the sieve to wash and drain water from their surface, then after it was counted and expressed as nodule number plant⁻¹. Then, the shoots and nodules were oven-dried at 70°C for 48 hours for determination of shoot and nodule dry weight.

2.6. Statistical data analysis
Data were subjected to analysis of variance (ANOVA) using the SAS software package version 9.4. The least significant difference (LSD) was used to separate treatment means at 5% level of significance.

3. Results and discussion

3.1. Effects of inoculation on cowpea plant height
Inoculations had a significant (P ≤ 0.05) effect on plant height at 45 days from planting (Table 1). The tallest plant (28.33 cm) was recorded when variety White wonderer trailing inoculated with Bradyrhizobium strains CP-24 followed by Keti (IT99K-1122), and Black eye bean varieties when inoculated with the same strain and Bole when inoculated with Bradyrhizobium strains CP-10. Generally, the plant height of all the varieties was improved compared with the least plant height obtained from the control. Argow (2014), reported a similar finding where soybean genotypes plant height affected significantly by inoculation with Bradyrhizobium strains. Egamberdiyeva et al. (2004) also reported an improvement in plant height of legumes due to inoculation with Bradyrhizobium strains.

3.2. Effects of inoculation on nodule number plant⁻¹
Inoculation with Bradyrhizobium strains significantly improved nodulation in cowpea varieties (Table 2). Although nodule formed on roots of all inoculated cowpea, nodulation performance varied in response to the different Bradyrhizobium strains. This finding is strongly supported by the finding reported by Ndungu (2017), where nodulation response of cowpea to inoculation by different Bradyrhizobium strains varies while nodules were formed on roots of all inoculated cowpea plants with none on the control treatment.
Table 1. Mean plant height of five cowpea varieties inoculated with *Bradyrhizobium* strains during 2018

| Treatment | Black eye bean | White wonderer trailing | TVU | Keti (IT99K-1122) | Bole |
|-----------|----------------|-------------------------|-----|-------------------|------|
| CP-24     | 26.33<sup>a,b</sup> | 28.33<sup>a</sup> | 16.33<sup>h</sup> | 27.33<sup>ab</sup> | 20.00<sup>c-j</sup> |
| CP-10     | 21.67<sup>b</sup> | 23.00<sup>a</sup> | 12.33<sup>j</sup> | 18.00<sup>h-m</sup> | 26.67<sup>a-c</sup> |
| CP-37     | 19.00<sup>f</sup> | 25.00<sup>g</sup> | 13.33<sup>f</sup> | 13.67<sup>n</sup> | 26.00<sup>a-a</sup> |
| GN-102    | 19.00<sup>j,k</sup> | 20.67<sup>b-h</sup> | 16.67<sup>j</sup> | 12.67<sup>k-n</sup> | 25.33<sup>f-t</sup> |
| GN-33     | 16.66<sup>h</sup> | 20.67<sup>b-h</sup> | 20.33<sup>f-t</sup> | 18.33<sup>g-m</sup> | 21.33<sup>b-g</sup> |

Note: +ve control- given with solution 0.5 g KNO₃ per 1 liter of sterilized distilled water, -ve control- given only N-free nutrient solution. Means followed by dissimilar letters in the columns and rows are significantly different at p = 0.05.

Table 2. Mean nodule number of five cowpea varieties inoculated with *Bradyrhizobium* strains during 2018

| Treatments | Black eye bean | White wonderer trailing | TVU | Keti (IT99K-1122) | Bole |
|------------|----------------|-------------------------|-----|-------------------|------|
| CP-24      | 47.67<sup>a</sup> | 40.67<sup>c</sup> | 32.33<sup>f</sup> | 29.00<sup>c-f</sup> | 27.00<sup>d-g</sup> |
| CP-10      | 44.33<sup>a</sup> | 37.67<sup>a</sup> | 13.33<sup>i</sup> | 30.67<sup>c-f</sup> | 29.33<sup>c-f</sup> |
| CP-37      | 35.33<sup>e</sup> | 39.00<sup>d</sup> | 11.33<sup>j</sup> | 22.33<sup>e-h</sup> | 29.67<sup>c-f</sup> |
| GN-102     | 34.00<sup>e</sup> | 29.00<sup>b-d</sup> | 14.67<sup>r</sup> | 24.00<sup>e-h</sup> | 40.00<sup>d-d</sup> |
| GN-33      | 27.00<sup>f</sup> | 29.00<sup>c-e</sup> | 20.67<sup>f-t</sup> | 27.67<sup>c-g</sup> | 23.33<sup>d-g</sup> |

Note: +ve control- given with solution 0.5 g KNO₃ per 1 liter of sterilized distilled water, -ve control- given only N-free nutrient solution. Means followed by dissimilar letters in the columns and rows are significantly different at p = 0.05.

Among the varieties, relatively higher nodules were recorded from Black eye bean when inoculated with strain CP-24 followed by Black eye bean and White wonderer trailing varieties inoculated with strains CP-10 and CP-24, and variety Bole when inoculated with strain GN-102, respectively (Table 2). No nodules were recorded from both the +ve and – ve control treatments (Table 3). The finding of Argaw (2014)

Table 3. Leaf and nodule color of *Bradyrhizobium* inoculated cowpea varieties at Hawassa, Ethiopia during 2018

| Leaf color | Treatments | Nodule color |
|------------|------------|--------------|
| Dark green | CP-10      | 15 pink      | CP-10      |
|            | CP-24      | 15 pink      | CP-24      |
| Pale green | CP-37      | 15 pink      | CP-37      |
|            | GN-33      | 15 pink      | GN-33      |
|            | GN-102     | 14 pink      | GN-102     |
|            | 1 White    | 14 pink      | GN-102     |
| +ve control| a          | a            | a          |
| -ve control| a          | a            | a          |

GN: Groundnut, CP: Cowpea, “no nodule formed"
supports the result of the current finding, who indicated the symbiotic effectiveness of varying \textit{Bradyrhizobium} isolates on soybean varieties at different maturity stages. Similarly, Stephen Kyei-Boahen et al. (2017) also reported a significant increase of nodule number in cowpea when inoculated with \textit{Bradyrhizobium} compared with the non-inoculated plants in Nampula and Ruace sites. Beyan et al. (2018) and Melchiore et al. (2011) confirmed the possible and significant effects of inoculation on the performance of nodulating legumes.

### 3.3. Effects of inoculation on cowpea leaf and nodule colors

The leaf and nodule colors can be used as a criterion in evaluating the symbiotic effectiveness of rhizobial inoculants. When comparing the phenotype of plants inoculated with either of the \textit{Bradyrhizobium} strains (Figure 1(a,b,e)) and the negative control (Figure 1(c)) it was, evident that the strains were effective (dark green leaves) and the negative control ineffective (with pale green to yellow leaves) in forming symbiosis with cowpea (Figure 1 and Table 3). In addition, with the color, the plant stand performance also shows a clear difference between the control and inoculated plants, where the pots with the negative control shows week performance with stunted growth of Keti (IT99K-1122) variety of cowpea (Figure 1). This result is supported by the findings of Ndungu (2017), who reported the formation of dark green versus yellowish leaf when cowpea plant was inoculated with \textit{Bradyrhizobium} strains and uninoculated, respectively.

Pink nodule color indicates an effective nodule, while white nodule color categorized as ineffective one. The result indicated that all strains resulted with a pink nodule color except one treatment with CP-37 found to have a white color whereas, the un-inoculated treatment not formed nodule (Table 2). The result of this experiment revealed that, the leaf color of strains CP-10, CP-24 and CP-37 inoculated cowpea varieties showed a dark green leaf and the GN-33 and GN-102 strain inoculated varieties showed pale green leaf color (Table 3). In agreement with the current finding, Degefu et al. (2018), based on their experiment on the authentication and preliminary symbiotic effectiveness test of cowpea, reported that, isolates effective in fixing atmospheric N found to show deep green and green leaf and deep red and pink nodule color.

### 3.4. Effects of inoculation on cowpea nodule fresh and dry weight

Inoculation with \textit{Bradyrhizobium} strains significantly affected the nodule fresh and dry weight of all the tested cowpea varieties (Tables 4 and 5). Significantly, higher fresh weight of nodule was obtained from the variety White wonderer trailing when inoculated with strains CP-10 and CP-37 as well as from the Black eye bean and Bole varieties when inoculated with strains CP-24 and CP-10, respectively. However, both the positive and negative controls did not formed nodule.

Figure 1. Photographs showing 6 weeks old Keti (IT99K-1122), varieties inoculated with CP10, CP37, and CP24 (a, b & e) and Keti (IT99K-1122) with positive control (d) and Keti (IT99K-1122) with negative control (c) treatments, Hawassa, Ethiopia, during 2018.
Table 4. Mean nodule fresh weight of five cowpea varieties inoculated with *Bradyrhizobium* during 2018

| Treatments | Black eye bean | White wonderer trailing | TVU | Keti (IT99K-1122) | Bole |
|------------|----------------|-------------------------|-----|------------------|------|
| CP-24      | 1.11<sup>a,c</sup> | 0.73<sup>c</sup>-<sup>g</sup> | 0.18<sup>h</sup> | 0.79<sup>c</sup>-<sup>f</sup> | 0.17<sup>e</sup> |
| CP-10      | 1.03<sup>b,d</sup> | 1.54<sup>a</sup> | 0.37<sup>e</sup>-<sup>f</sup> | 0.82<sup>c</sup>-<sup>f</sup> | 1.07<sup>c</sup>-<sup>d</sup> |
| CP-37      | 0.59<sup>d</sup>-<sup>h</sup> | 1.09<sup>a</sup>-<sup>c</sup> | 0.95<sup>a</sup>-<sup>e</sup> | 0.82<sup>c</sup>-<sup>f</sup> | 0.82<sup>c</sup>-<sup>f</sup> |
| GN-102     | 0.37<sup>c</sup>-<sup>i</sup> | 0.80<sup>c</sup>-<sup>i</sup> | 0.56<sup>e</sup>-<sup>i</sup> | 0.79<sup>c</sup>-<sup>f</sup> | 1.41<sup>ab</sup> |
| GN-33      | 0.55<sup>e</sup>-<sup>c</sup> | 0.80<sup>c</sup>-<sup>i</sup> | 0.46<sup>i</sup> | 0.53<sup>e</sup>-<sup>h</sup> | 0.29<sup>f</sup>-<sup>g</sup> |
| +ve control | 0.00<sup>i</sup> | 0.00<sup>j</sup> | 0.00<sup>j</sup> | 0.00<sup>j</sup> | 0.00<sup>j</sup> |
| -ve control | 0.00<sup>i</sup> | 0.00<sup>j</sup> | 0.00<sup>j</sup> | 0.00<sup>j</sup> | 0.00<sup>j</sup> |

Note: +ve control- given with solution 0.5 g KNO<sub>3</sub> per 1 liter of sterilized distilled water, -ve control- given only N-free nutrient solution. Means followed by dissimilar letters in the columns and rows are significantly different at p = 0.05.

Table 5. Mean nodule dry weight of five cowpea varieties inoculated with *Bradyrhizobium* strains during 2018

| Treatment | Black eye bean | White wonderer trailing | TVU | Keti (IT99K-1122) | Bole |
|-----------|----------------|-------------------------|-----|------------------|------|
| CP-24     | 0.16<sup>a</sup>-<sup>d</sup> | 0.12<sup>a</sup> | 1.21<sup>a</sup> | 0.17<sup>c</sup>-<sup>d</sup> | 0.57<sup>b</sup> |
| CP-10     | 0.15<sup>c</sup> | 0.13<sup>c</sup> | 0.09<sup>d</sup> | 0.19<sup>c</sup> | 0.17<sup>c</sup> |
| CP-37     | 0.13<sup>c</sup> | 0.13<sup>c</sup> | 0.05<sup>d</sup> | 0.21<sup>c</sup> | 0.16<sup>c</sup> |
| GN-102    | 0.08<sup>c</sup>-<sup>d</sup> | 0.10<sup>c</sup> | 0.11<sup>c</sup> | 0.07<sup>c</sup>-<sup>d</sup> | 0.15<sup>c</sup> |
| GN-33     | 0.11<sup>c</sup> | 0.10<sup>c</sup> | 0.09<sup>d</sup> | 0.16<sup>c</sup>-<sup>d</sup> | 0.10<sup>c</sup> |
| +ve control | 0.00<sup>e</sup> | 0.00<sup>e</sup> | 0.00<sup>e</sup> | 0.00<sup>e</sup> | 0.00<sup>e</sup> |
| -ve control | 0.00<sup>e</sup> | 0.00<sup>e</sup> | 0.00<sup>e</sup> | 0.00<sup>e</sup> | 0.00<sup>e</sup> |

Note: +ve control- given with solution 0.5 g KNO<sub>3</sub> per 1 liter of sterilized distilled water, -ve control- given only N-free nutrient solution. Means followed by dissimilar letters in the columns and rows are significantly different at p = 0.05.

Shutsrirung et al. (2002) also reported an improved nodulation performance of soybean with the presence of rhizobia. Improved nodulation performance of cowpea plants when inoculated with *Bradyrhizobium* strains were also reported by Ndungu (2017) although the performance varies across seasons. Kyei-Boahen et al. (2017) confirmed the effect of *Bradyrhizobium* on dry weight of nodule in their study aimed to evaluate the growth and yield responses of cowpea to inoculation and phosphorus fertilization in different environments.

A higher nodule dry weight of 1.2 (g plant<sup>-1</sup>) was recorded from CP-10 strain inoculated White wonderer trailing variety. Moreover, all the *Bradyrhizobium* isolates induced nodule fresh and dry weight formation, where significantly higher nodule number in all the investigated cowpea genotypes with none on the control and N<sup>+</sup> treatment without inoculation was recorded. Weber et al. (1971) reported improved nodule dry weight due to inoculation with *Bradyrhizobium* isolates. Similarly, Argaw (2014) revealed a significant improvement on the nodule dry weight formation due to *Bradyrhizobium* inoculation. Ndungu (2017) reported a significant difference in nodule dry weight of cowpea plant amongst inoculated and uninoculated treatments, where, inoculation found to result with increased nodule dry weight. Similarly, an experiment conducted in Nigeria reported a maximum nodule dry weight of 1.177 (g plant<sup>-1</sup>) due to inoculation (Okerere & Unaegbu, 1992), which is comparable with the highest nodule dry weight of (1.21 g plant<sup>-1</sup>), recorded from the current experiment. Stamford et al. (2013) proved the increment in nodule dry biomass of cowpea in the experiment conducted to assess the nodulation, yield and nutrient uptake response of cowpea to *Bradyrhizobium* inoculation in a sodic soil.
3.5. Effects of inoculation on cowpea shoot fresh and dry weight g plant$^{-1}$

The five-cowpea varieties investigated showed significant variation in shoot fresh and dry weight formation among inoculation treatments (Tables 6 and 7). The highest fresh weight observed when Keti (IT99K-1122) inoculated with CP-24 (11.08), CP-10 (9.19), CP-37 (9.21) and GN-33 (8.81 g plant$^{-1}$), White wonderer trailing inoculated with CP-24 (9.94 g plant$^{-1}$), Black eye bean inoculated with CP-24 (9.30), CP-10 (7.54) and CP-37 (7.77 g plant$^{-1}$), and TVU inoculated with CP-24 (8.66 g plant$^{-1}$) compared with the remaining treatment combinations and the control treatments (Table 6).

Inoculating cowpea varieties with the *Bradyrhizobium* isolates improved shoot dry weight (Table 7). Relatively higher, shoot dry weight recorded from the treatment combinations of White wonderer trailing with CP-24, Black eye bean with CP-24, and Keti (IT99K-1122) with CP-24 and the least recorded from the control treatments. Significantly, lower shoot dry weight of 0.12 recorded from the Keti (IT99K-1122) given only N-free nutrient solution; where the higher shoot dry weight of 1.59 g plant$^{-1}$ obtained from the same variety when inoculated with CP-24 *Bradyrhizobium* strain. The finding is in agreement with the previous reports by Stamford et al. (2013), who revealed an increased shoot dry weight when cowpea inoculated with different *Bradyrhizobium* isolates. Kyei-Boahen et al. (2017) also reported a significant increase in shoot dry biomass of cowpea due to inoculation.

Argaw (2014) reported a significant effect of *Bradyrhizobium* inoculation on shoot dry weight accumulation. Higher shoot dry weigh of cowpea plant revealed by Ndungu (2017) due to *Bradyrhizobium*

### Table 6. Mean shoot fresh weight of five cowpea varieties inoculated with Bradyrhizobium strains during 2018

| Treatment  | Black eye bean | White wonderer trailing | TVU | Keti (IT99K-1122) | Bole |
|------------|----------------|-------------------------|-----|------------------|------|
| CP-24      | 9.30$^{ab}$    | 9.84$^{bc}$             | 8.66$^{a-f}$ | 11.08$^{a}$     | 6.39$^{a-j}$ |
| CP-10      | 7.54$^{ab}$    | 6.03$^{c}$              | 4.68$^{a-j}$ | 9.19$^{a-d}$    | 6.57$^{h-j}$ |
| CP-37      | 7.7$^{a-b}$    | 7.78$^{a-b}$            | 4.82$^{a-j}$ | 9.21$^{a-d}$    | 8.94$^{a-e}$ |
| GN-102     | 4.28$^{g-m}$   | 4.73$^{a-f}$            | 7.49$^{a-i}$ | 5.05$^{i}$      | 8.97$^{a-e}$ |
| GN-33      | 5.49$^{k}$     | 4.73$^{a-f}$            | 5.6$^{i}$   | 8.8$^{a-e}$     | 5.73$^{g-f}$ |
| +ve control | 3.54$^{n}$    | 2.00$^{k-n}$            | 2.16$^{n}$  | 6.18$^{i}$      | 4.18$^{a-m}$ |
| -ve control | 4.09$^{m-n}$  | 1.67$^{n}$              | 0.00$^{n}$  | 0.73$^{m}$      | 2.34$^{n}$  |

**Note:** +ve control given with solution 0.5 g KNO$_3$ per 1 liter of sterilized distilled water, -ve control given only N-free nutrient solution. Means followed by dissimilar letters in the columns and rows are significantly different at $p = 0.05$.

### Table 7. Mean shoot dry weight of five cowpea varieties inoculated with Bradyrhizobium strains during 2018

| Treatments  | Black eye bean | White wonderer trailing | TVU | Keti (IT99K-1122) | Bole |
|-------------|----------------|-------------------------|-----|------------------|------|
| CP-24       | 1.68$^{a}$    | 1.74$^{a}$              | 1.31$^{a-g}$ | 1.59$^{a-b}$     | 1.22$^{h-n}$ |
| CP-10       | 1.41$^{c}$    | 0.95$^{d}$              | 0.87$^{d}$  | 1.45$^{a-e}$     | 1.26$^{g}$  |
| CP-37       | 1.56$^{c-e}$  | 1.32$^{a-g}$            | 0.77$^{f}$  | 1.42$^{a-d}$     | 1.22$^{h-n}$ |
| GN-102      | 0.71$^{g-i}$  | 0.70$^{g-i}$            | 1.29$^{g-i}$ | 0.8$^{i}$      | 1.55$^{c}$  |
| GN-33       | 1.08$^{g-i}$  | 0.70$^{g-i}$            | 0.94$^{k}$  | 1.31$^{a-g}$     | 1.09$^{g-f}$ |
| +ve control | 0.51$^{m}$    | 0.29$^{m}$              | 0.41$^{m}$  | 1.18$^{i}$      | 0.75$^{f}$  |
| -ve control | 0.50$^{m-n}$  | 0.15$^{m-n}$            | 0.00$^{n}$  | 0.12$^{m}$      | 0.55$^{m}$  |

**Note:** +ve control given with solution 0.5 g KNO$_3$ per 1 liter of sterilized distilled water, -ve control given only N-free nutrient solution. Means followed by dissimilar letters in the columns and rows are significantly different at $p = 0.05$. 

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inoculation. Previous works of Shutsrirung et al. (2002), Gay et al. (1980), and Smith and Nelson (1986) supported the results observed in this study. There is a variation in shoot dry weight also among the genotypes, where, relatively higher dry weight obtained from White wonderer trailing (1.74 g plant⁻¹) and Black eye bean (1.68 g plant⁻¹) both inoculated with CP-24. A variation on shoot dry weight among varieties receiving the same inoculation treatment reported by Argaw (2014). The result is also in agreement with the findings of Papakosta and Veresoglou (1989), who reported a significant variation in shoot dry weight of legumes.

Variable shoot dry weight also recorded from the same varieties when inoculated with different strains. For instance, the highest shoot dry weight of 1.74 g plant⁻¹ from White wonderer trailing obtained when inoculated with CP-24, whereas, the least shoot dry weight of 0.70 g plant⁻¹ obtained from the same genotype when inoculated with GN-33 and GN-102. This finding supported by Degifu et al. (2018), who reported a significant variation on the shoot dry weight of the same genotype when inoculated with different *Bradyrhizobium* strains.

4. Conclusions

The experiment was conducted to evaluate the symbiotic effectiveness of inoculation with *Bradyrhizobium* strains on cowpea varieties. For this purpose, a treatment consisting of five cowpea varieties, five *Bradyrhizobium* isolates and two controls experimented on sand filled pot. The findings of the experiment clearly indicated the significant effects of *Bradyrhizobium* inoculation on the growth, biomass accumulation and nodulation performance of the tested cowpea varieties. Basing on the results of the current experiment, it can be suggested that, the studied *Bradyrhizobium* isolates can be used for improved cowpea production.

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