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EFFECT OF INOCULATING *Bradyrhizobium liaoningense* ON GROWTH, CHLOROPHYLL CONCENTRATION, NODULATION AND YIELD OF SOYA BEANS

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ABSTRACT

Soya bean is important staple food crops in Western Kenya. Legumes play a significant role in agriculture by fixing nitrogen. Currently Kenya is experiencing low yield of soya beans. Approximately 0.8 t/ha is under production and this is due to low soil fertility. This has posed a threat to agricultural productivity. Symbiotic rhizobia inoculation increases crop yields through biological nitrogen fixation. However, the potential of *Bradyrhizobium liaoningense* in improving yield and productivity of soya beans has not been documented. Rhizobia bacteria are known to improve growth and yield of several other crops but the effect of inoculating soya beans with *B. liaoningense* on growth, chlorophyll concentration, nodulation and yields has not been determined. This study was conducted at Maseno University under greenhouse conditions. Previously purified local isolates of *B. liaoningense* (S_3) from wild soya beans plants were used to inoculate the soya bean seeds. 4.5 litre plastic pots were filled with 7kg of top soil. Ten seeds of Soya beans coated with *Bradyrhizobium liaoningense* inoculants were sown in each pot except the control. The seeds were treated with the isolates inoculants as: un-inoculated (control), $1.07x10^7$ cfu/ml, $1.19x10^7$ cfu/ml, $1.31x10^7$ cfu/ml and $2.67x10^7$ cfu/ml inoculation of *B. liaoningense*. The experiment was laid out as Completely Randomised Design (CRD). The treatments were replicated three times. After two weeks of germination, the seedlings were thinned to three plants per pot. Watering was done daily with 200 ml of water per pot up to the end of the experiment. Data on plant height, number of leaves, leaf area, shoot and root fresh weight and dry weight, chlorophyll concentration, number of nodules and number of pods were determined. Data was subjected to Analysis of Variance (ANOVA). Treatments means were separated using Fisher's Least Significant Difference at (P = 0.05). Soya bean growth, chlorophyll concentration, number of nodules and number of pods after inoculation treatments were significantly different among the treatments ($P \leq 0.05$). Plant height, number of leaves and leaf area were highest in 2.67 x 10^7 cfu/ml of *B. liaoningense* inoculation followed by 1.31x 10^7 cfu/ml inoculation, $1.19x10^7$ cfu/ml, $1.07x10^7$ cfu/ml and lowest in un-inoculated. Inoculation at 2.67 x 10^7 cfu/ml significantly increased soya bean root and shoot fresh weight and dry weights. Chlorophyll concentration was found to be highest in 2.67 x 10^7 cfu/ml treatment and lowest in un-inoculated control treatment. Inoculation at 2.67 x 10^7 cfu/ml increased the number of nodules of soya beans. Number of pod per plant were highest at 2.67 x 10^7 cfu/ml treatment and lowest in un-inoculated (control treatment). These findings showed that *B. liaoningense* inoculation was effective in enhancing growth, chlorophyll synthesis, formation of root nodules and pods in soya beans. From the study it can be recommended that *B. liaoningense* from wild soya bean may be used as a biofertiliser to improve productivity of soya beans by smallholder farmers in Kenya.

Keywords: *Bradyrhizobium liaoningense, Glycine soja,* Chlorophyll content, Innoculation, Soya bean

INTRODUCTION

Soya bean (*Glycine max*) is one of the world's most important legume in terms of production and trade and a dominant oil seed since 1960s (**Getnet, 2019**). The data on Kenya's soya bean production has remained poor and scanty (**Nyaguthii, 2017**). The soya bean production area and yield have remained almost stagnant, with little annual change (**Nyaguthii, 2017**). Currently Kenya is experiencing low yield of soya beans, approximately 0.8 t/ha, due to low soil fertility and high cost of inorganic fertilizers and it is a serious threat to agricultural productivity (Chianu *et al.*, 2008). The production of soya bean is low compared to food crops like corn (3t/ha) and wheat (2.5t/ha) (**Nyaguthii, 2017**). For better plant growth and yield of plants such as soya beans, physiological processes such as chlorophyll synthesis is key determining photosynthetic capacity and hence plant growth. The synthesis of chlorophyll molecules is pegged on essential nutrients such as nitrogen and magnesium, a deficit in the soils which leads to deficiencies in soya bean chlorophyll and overall plant growth (**Gwenzi *et al.*, 2015**). Previous studies by **Ntambo *et al*. (2017)** indicated that inoculation of soya bean with *Bradyrhizobium japonicum* increased growth rate of soya beans significantly by introducing more numbers of viable and efficient rhizobium bacteria in the soil rhizosphere; which promoted nitrogen fixation, and uptake of minerals.

Low crop productivity is a general problem facing most farming systems in sub-Saharan Africa. Unless the soil fertility is restored, farmers will gain little from the use of improved varieties and even more productive agricultural technologies (**Vanlauwe *et al*., 2015**). Application of inorganic fertilizers may be a remedy to soil infertility, however, long term use of inorganic fertilizers causes decline in soya bean productivity due to their negative environmental effects (**Gwenzi *et al.*, 2015**). Legumes inoculated with rhizobium species and supplemented with P and K, respond differently in the growth, yield and nitrogen fixation (**Yanni *et al*., 2001**). Biofertilizer have recently emerged as a promising component of integrating nutrient supply system in agriculture (**Itelima *et al*., 2018**). Biofertilzers offer environmentally friendly and sustainable agricultural practices (**Itelima *et al*., 2018**). Sustainable agriculture based on the use of microbial products is an effective option for overcoming problems of soil fertility (**Hassen *et al*., 2014; Masso *et al*., 2015**). A great advantage of rhizobial inoculation is that it is much cheaper than mineral nitrogen fertilizer (**Chianu *et al*., 2008; Chianu *et al*., 2011**). Previous findings by **Zimmer *et al*. (2016) and Htwe *et al*. (2015)** indicated that inoculation of soya beans with rhizobia species such as *Bradyrhizobium japonicum* improved soya bean yield. Optimal soils conditions especially soil nitrogen levels are however important for the success of any inoculant application to boost soya bean yield. Inoculation of legume seeds with appropriate *Rhizobium* strains for enhanced nitrogen fixation provides an alternative to the application of nitrogenous fertilizers. However, legumes express host specificity, meaning that only certain species or subspecies of rhizobia will infect certain species of legume. However, there is limited research on the effects of inoculation of soya beans with *Bradyrhizobium li*aoningense on growth, chlorophyll content and yield of soya beans.

MATERIALS AND METHODS

Experimental treatments and design

Growth tests were carried out in the greenhouse whose daily mean temperature was maintained at 29 ^{0}C with diurnal amplitude of ± 5 ^{0}C at Maseno University, Kenya. Previously purified local isolates of *Bradyrhizobium liaoningense* (S_3) from wild *G. soja* plants were used for the inoculation treatments. Ten sterilized soya bean seeds were coated with *Bradyrhizobium liaoningense* isolate inoculants before sowing as follows, 2.67 $x10^7$ cfu/ml, 1.31 $x10^7$ cfu/ml, 1.19 $x10^7$ cfu/ml, 1.07 $x10^7$ cfu/ml and (un- inoculated) control. Ten treated seeds of Soya beans were sown in 4.5 litre plastic pots with dimensions 21cm in height and 19cm in diameter were filled with top soil collected from Maseno University botanic garden for each treatment and replicated three times. The experiment was laid out as a completely randomized design. After two weeks of germination, the seedlings in each pot were thinned to three plants per pot. Watering was done every morning with 250ml tap water per pot up to the end of the experiment. The soils are classified as acrisol, deep reddish brown friable clay with pH ranging from 4.5 to 5.5, soil organic carbon and phosphorus contents are 1.8% and 4.5 mg kg-1, respectively (**Ambede *et al*., 2012**).

Growth, chlorophyll concentration and yields measurement

Plant height

Plant height was measured as described by **Khasabulli *et al*. (2017)**, from soil level to the upper point of the terminal bud of the seedling using a meter rule every fourteen days up to the end of the experiment. Two randomly selected plants in each pot were tagged and measured.

Number of leaves

Number of mature leaves of two randomly selected plants in each pot were counted and recorded after every fourteen days up to the end of experiment.

Leaf area

Leaf area of two randomly selected plants in each pot was determined after every fourteen days. Leaf area was calculated using the formula: LA=0.5(L X W) Where L=length of leaf W=maximum width, according to **Khasabulli *et al*. (2017).**

Root and shoot fresh weight

Ninety days after sowing, two randomly selected plants in each pot were carefully uprooted from the soil, cleared off debris, separated into shoot and root and weighed separately using electronic weighing balance.

Root and shoot dry weights

Ninety days after sowing, two randomly selected fresh plants in each pot were uprooted packed separately in envelopes and dried in an oven at constant temperature 60^0C for three days. The roots and shoot were allowed to cool in a dry environment then weighed on an electronic weighing balance and records were made.

Leaf chlorophyll concentration

Determination of chlorophyll followed the method of **Ali *et al.* (2012).** The third fully expanded leaf from shoot apex was collected from all the treatments after every 14 days. Three grams of leaves were grounded in 10 ml of 80% (V/V) acetone using mortar and pestle. They were left overnight for 24 hours to allow maximum extraction of chlorophyll. The resulting extracts were read at 645 nm and 664 nm using UV-visible spectrophotometer. Chlorophyll a, b and total concentration were calculated as follows:

Chlorophyll a =13.19 A664-2.57 A645 (mgg^{-1} fresh weight)
Chlorophyll b =22.1 A664-5.26 A664 (mgg^{-1} fresh weight)
Total Chlorophyll =7.93 A664+19.53 A645 (mgg^{-1} fresh weight)
Where A664 is the absorbance at 664nm and A645 is the absorbance at 645 nm.

Number of nodules per plant

Mature nodules from two randomly selected plants in each pot were counted and recorded 90 days after sowing. This was achieved by uprooting the plants and removing all the soil from the roots of selected plants by washing before counting (**Hao *et al*. 2014)**.

Number of pods per plant

At the end of the experiment which was 90 days after planting, mature pods of soya bean plant of two randomly selected plants in each pot were counted for each plant and recorded according to **Yang *et al*. (2018)**.

Data Analysis

Data was subjected to Analysis of Variance (ANOVA). When significant differences were detected, the means of the treatments were compared using Fisher's Least significant difference (F-LSD) at 0.05 probability level.

RESULTS

Effect of inoculating Soya beans with *Bradyrhizobium liaoningense* on growth parameters

Plant height

Soya bean plant height showed significant differences (P≤0.05) among treatments of rhizobia inoculation (Table 1). At 14 days after emergence the soya bean plant height recorded significantly taller plants at $2.67x10^7$ cfu/ml rhizobia inoculation and was not significantly different from $1.31x10^7$ cfu/ml, $1.19x10^7$ cfu/ml $1.07x10^7$ cfu/ml, and un-inoculated (control). At $1.31x10^7$ cfu/ml rhizobia concentration soya bean plant height was higher than $1.19x10^7$ cfu/ml rhizobia inoculation `1which was also higher than the $1.07x10^7$ cfu/ml rhizobia inoculation. Un-inoculated (control) recorded the lowest soya bean plant height which was not significantly different with $1.07x10^7$ cfu/ml rhizobia inoculation but significantly different with $2.67x10^7$ cfu/ml, $1.31x10^7$ cfu/ml and $1.19x10^7$ cfu/ml

Table 1 Effect of inoculating soya beans with Bradyrhizobium liaoningense on plant height (cm) of soya beans

	Days after Treatment (DAT)					
Inoculation treatments	14DAT	28 DAT	42 DAT	56 DAT	70 DAT	Overall mean
Un-inoculated (control)	11.87d	21.13e	28.37e	36.40e	45.20e	28.59e
$1.07x10^7$ cfu/ml	14.80d	23.70d	31.67d	41.17d	52.10d	32.69d
$1.19x10^7$ cfu/ml	16.80c	25.60c	34.90c	45.97c	58.47c	36.35c
$1.31x10^7$ cfu/ml	20.03b	29.73b	39.73b	50.23b	62.83b	40.51b
$2.67x10^7$ cfu/ml	23.00a	34.60a	44.10a	56.10a	67.70a	45.10a
LSD	**0.8937**	**0.6285**	**0.6758**	**0.9133**	**0.9987**	**1.8193**
P.value	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0001**

Means with the same letter down the column are not significantly different at P≤0.05.

Effect of inoculating Soya beans with *Bradyrhizobium liaoningense* on Leaf number of soya beans

There were significant differences (P≤0.05) on the number of leaves among the treatments (Table 2). The number of leaves of soya beans increased steadily from 14 DAP to 70 DAP with increase in concentration of rhizobia inoculant. $2.67x10^7$ cfu/ml rhizobia inoculation had highest and significantly different soya bean plant leaf number across treatments while $1.31x10^7$ cfu/ml, $1.19x10^7$ cfu/ml, $1.07x10^7$ cfu/ml and un-inoculated (control) had no significant differences in leaf number at day 14. At day 28, $2.67x10^7$ cfu/ml rhizobial concentrations showed the highest and significantly different (P≤0.05) soya bean plant leaf number while $1.31x10^7$ cfu/ml, $1.19x10^7$ cfu/ml and $1.09x10^7$ cfu/ml rhizobial inoculation registered no significant differences (P≥0.05). At day 42, $2.67x10^7$ cfu/ml and $1.31x10^7$ cfu/ml inoculation was not significantly different (P≥0.05) but significantly different from other treatments, $1.31x10^7$ cfu/ml and $1.19x10^7$ cfu/ml were not significant (P≥0.05) while un-inoculated (control) had the lowest soya bean leaf number means, which was significantly different (P≤0.05) from other treatments. At day 56 and 70, the soya bean leaf number was highest at $2.67x10^7$ cfu/ml rhizobia inoculation which was significantly different (P≤0.05) across treatments and lowest in un-inoculated (control) except for day 56 where $1.07x10^7$ cfu/ml and un-inoculated (control) had no significant differences.

Table 2 Effect of inoculating soya beans with Bradyrhizobium liaoningense on leaf number of soya beans

	Days after treatment (DAT)					
Inoculation treatments	**14 DAT**	**28 DAT**	**42 DAT**	**56 DAT**	**70 DAT**	**Overall mean**
Un-inoculated (control)	3.00b	3.33c	5.00c	6.33d	8.00e	5.13c
$1.07x10^7$ cfu/ml	3.00b	4.33b	5.67bc	7.33cd	9.00d	5.87bc
$1.19x10^7$ cfu/ml	3.00b	4.33b	6.333b	7.667c	10.00c	6.27abc
$1.31x10^7$ cfu/ml	3.00b	5.00b	7.67a	8.67b	10.67b	7.00ab
$2.67x10^7$ cfu/ml	**4.00a**	**6.00a**	**8.00a**	**10.33a**	**11.66a**	**7.99a**
LSD	**0.9401**	**0.8136**	**1.1506**	**1.0504**	**0.6643**	**1.8193**

Means with the same letter down the column are not significantly different at P≤0.05.

Effect of inoculating soya beans with *Bradyrhizobium liaoningense* on Leaf area of soya beans

Significant differences were observed in the leaf area among treatments (Table 3). The $2.67x10^7$ cfu/ml rhizobia concentration treatment recorded significantly higher (P≤0.05) leaf area compared to the rest of the treatments ($1.31x10^7$ cfu/ml, $1.19x10^7$ cfu/ml, $1.07x10^7$ cfu/ml and control) of rhizobia inoculation as the days increased. Un-inoculated (control) treatment showed the lowest leaf area in all the days of data collection.

Table 3 Effect of inoculating soya beans with Bradyrhizobium liaoningense on leaf area of soya beans

	Days after treatment (DAT)					
Inoculation treatments	**14 DAT**	**28 DAT**	**42 DAT**	**56 DAT**	**70 DAT**	**Overall mean**
Un-inoculated (control)	26.27e	34.27d	35.23e	39.17e	43.27e	35.642d
$1.07x10^7$ cfu/ml	33.40d	37.50c	40.67d	45.43d	48.83d	43.108c
$1.19x10^7$ cfu/ml	35.53c	40.00c	43.87c	48.50c	53.13c	44.210c
$1.31x10^7$ cfu/ml	38.53b	43.20b	48.27b	52.93b	57.53b	48.090b
$2.67x10^7$ cfu/ml	41.97a	46.00a	50.40a	56.17a	62.10a	51.330a
LSD	**0.7589**	**2.7086**	**0.5167**	**0.6372**	**0.6267**	**2.3012**
P.value	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**

Means with the same letter down the column are not significantly different at P≤0.05.

Effect of inoculating Soya beans with *Bradyrhizobium liaoningense* on Shoot fresh and dry weight

The shoot fresh weight increased steadily with increase in the inoculation treatment. There was significant difference (P≤0.05) of root fresh weight at different treatment of rhizobia inoculant. $2.67x10^7$ cfu/ml inoculation recorded the highest shoot fresh weight and was significantly different from the rest of the treatments. $1.09x10^7$ cfu/ml inoculation rhizobia had the lowest shoot fresh weight which was significantly different with the other treatments. The shoot dry weight of soya bean increased with increase in the concentration of rhizobial inoculant. There were significant differences (P≤0.05) among different treatments of rhizobia inoculant The $2.67x10^7$ cfu/ml rhizobia concentration had significantly higher shoot dry weight compared to other treatments. The un-inoculated treatment had the lowest shoot dry weight which was significantly different from other treatments (Table 4).

Table 4 Effect of inoculating Soya beans with Bradyrhizobium liaoningense on shoot fresh and dry weight

Inoculation treatments	**Shoot fresh weight (g)**	**Shoot dry weight (g)**
Un-inoculated (control)	41.33e	18.00e
$1.07x10^7$ cfu/ml	55.73d	24.80d
$1.19x10^7$ cfu/ml	58.10c	26.53c
1.31x107 cfu/ml	61.43b	28.03b
$2.67x10^7$ cfu/ml	64.43a	29.46a
LSD	**1.3127**	**0.7675**
P.Value	**<.0001**	**<.0.0001**

Means with the same letter down the column are not significantly different at P≤0.05

Effect of inoculating Soya beans with *Bradyrhizobium liaoningense* **on Root fresh and dry weight**

The root fresh weight of soya bean increased with increase in the rhizobium inoculant. There was significant differences (P≤0.05) among different treatment of rhizobia inoculant with $2.67x10^7$ cfu/ml rhizobia inoculant concentration registering the highest root fresh weight which was significantly different from the rest of the treatments. Un-inoculated (control) treatment had the lowest root fresh weight which was significantly lower than compared to other treatments. Root dry weight of the soya beans increased with the increase in the rhizobium inoculant concentration. There was significant difference (P≤0.05) of root dry weight in different treatment of rhizobia inoculant. The $2.67x10^7$ cfu/ml rhizobia concentration had significantly highest root dry weight than other treatments. Un-inoculated (control) treatment recorded the lowest root dry weight which was also significantly lower than the rest of the treatments (Table 5).

Table 5 Effect of inoculating Soya beans with Bradyrhizobium liaoningense on root fresh and dry weight

Inoculation treatments	**Shoot fresh weight (g)**	**Shoot dry weight (g)**
Un-inoculated (control)	12.80e	3.86e
$1.07x10^7$ cfu/ml	15.56d	5.40d
$1.19x10^7$ cfu/ml	16.73c	5.90c
1.31x107 cfu/ml	18.96b	6.50b
$2.67x10^7$ cfu/ml	20.33a	7.86a
LSD	**0.5997**	**0.3787**
P.Value	**<.0.0001**	**<.0.0001**

Means with the same letter down the column are not significantly different at P≤0.05.

Effect of inoculating Soya beans with *Bradyrhizobium liaoningense* **on Chlorophyll concentration**

There was increase in the chlorophyll concentration with increase in the inoculant concentration (Table 6). Inoculation of soya beans at $2.67x10^7$ cfu/ml had the highest and significantly different (P≤0.05) chlorophyll concentration than the rest of the treatments during the study period. The un-inoculated had the lowest chlorophyll concentration in all the days of treatments. The highest chlorophyll concentration was higher at day 56 in all the treatments.

Table 6 Effect of inoculating soya beans with Bradyrhizobium liaoningense on chlorophyll concentration of soya bean plants

Inoculation treatments	**Days after treatment (DAT)**					
	14 DAT	**28 DAT**	**42 DAT**	**56 DAT**	**70 DAT**	**Overall mean**
Un-inoculated (control)	35.07e	36.00e	37.47e	39.27e	37.53e	37.068c
$1.07x10^7$ cfu/ml	36.70d	37.30d	38.33d	39.53d	38.57d	38.086bc
$1.19x10^7$ cfu/ml	38.40c	39.50c	40.30c	41.27c	40.57c	40.008abc
$1.31x10^7$ cfu/ml	39.57b	40.70b	41.47b	42.30b	41.60b	41.128ab
$2.67x10^7$ cfu/ml	40.90a	42.07a	42.63a	43.33a	42.00a	42.186a
LSD	**0.1558**	**0.1694**	**0.1243**	**0.1243**	**0.1819**	**3.0442**
P. Value	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**	**<.0.0001**

Means with the same letter down the column are not significantly different at P≤0.05.

Effect of inoculating Soya beans with *Bradyrhizobium liaoningense* on Number of nodules

The number of nodules increased with the increase in rhizobia inoculant concentration (Table 7). The $2.67x10^7$ cfu/ml treatment recorded significantly higher (P≤0.05) chlorophyll content than the rest of the treatments. Un-inoculated showed the lowest number of nodules among all the treatments.

Table 7 Effect of inoculating Soya beans with Bradyrhizobium liaoningense on number of nodules of soya bean plant

Inoculation	**Number of nodules**
Un-inoculated (control)	10.33e
$1.07x10^7$ cfu/ml	12.67d
$1.19x10^7$ cfu/ml	15.67c
$1.31x10^7$ cfu/ml	18.33b
$2.67x10^7$ cfu/ml	21.00a
LSD	**1.2428**
P.Value	**<.0.0001**

Means with the same letter down the column are not significantly different at P≤0.05

Effect of inoculating Soya beans with *Bradyrhizobium liaoningense* on Number of pods

The number of pods increased linearly with the increase in the concentration of the inoculant (Table 8). The number of pods of soya bean plants recorded a significantly (P≤0.05) higher number pods under $2.67x10^7$ cfu/ml inoculant concentration compared to the rest of the treatments. The un- inoculated treatment showed the lowest soya bean plant pods number.

Table 8 Effect of inoculating Soya beans with Bradyrhizobium liaoningense on number of pods in soya beans

Inoculation treatments	**Number of pods**
Un-inoculated (control)	14.33e
$1.07x10^7$ cfu/ml	18.33d
$1.19x10^7$ cfu/ml	21.67c
$1.31x10^7$ cfu/ml	23.33b
$2.67x10^7$ cfu/ml	26.00a
LSD	**1.2428**
P.Value	**<.0.0001**

Means with the same letter down the column are not significantly different at P≤0.05.

DISCUSSION

Soya bean plant height significantly increased under *Bradyrhizobium liaoningense* inoculation. The soya bean plant height increased with the increase in the inoculant concentration where $2.67x10^7$ cfu/ml inoculum resulted in highest height and lowest in un-inoculated treatment. The findings are in agreement with **Mondal and Bose (2019)** who reported that plant height is an indicator of vegetative growth and that reduced plant height might be due to the result of unavailability of nitrogen and other nutrients required by the plants for their normal growth and development. The significant increase in plant height because of inoculation was probably due to nitrogen fixation induced by inoculated isolates of *Bradyrhizobia liaoningense*, which increased nitrate supply in the soils which played a vital role in the vegetative growth of soybean.

Soya bean inoculation had a significant increase in number of leaves with $2.67x10^7$ cfu/ml inoculum giving the highest number of leaves and lowest in un-inoculated treatment. These results are in agreement with earlier findings of **Kumawat *et al*. (2017)** who indicated that application of *Bradyrhizobium liaoningense* inoculants had optimum growth of soya bean leaves. Several researchers have also reported on the importance of inoculation on number of leaves and branches. This is supported by the findings of **Safir *et al.* (2003)** who obtained higher growth and biomass yield of soybeans plants inoculated with *Rhizobium* than the non-inoculated ones. This may be explained by the ability of the inoculated plants to fix more nitrogen as a result of improved soil nutrient status as compared to the non inoculated ones. **Turuko and Mohammed (2014)** reported that inoculation of seeds before planting increased the soya beans number of leaves, leaf area and branches. The increase in leaf number could be attributed to the availability of nitrogen source resulting from *Bradyrhizobium liaoningense* inoculums which enhanced the formation of nodules enhancing nitrogen fixation while un-inoculated soya beans lacked adequate supply of nitrogen required for multiplication of leaves number. Inoculation with *Rhizobium liaoningense* enhanced the growth of soya bean plants as a result of their ability to fix atmospheric nitrogen.

Inoculated soya bean plants recorded larger soya bean leaf area with $2.67x10^7$ cfu/ml inoculum giving the highest leaf area and lowest in un-inoculated treatment. The findings are in-line with those of **Jaga and Sharma (2015)** who reported that increased nitrogen uptake by soya bean plants resulted in higher leaf area per plant. *Bradyrhizobium liaoningense* inoculation may have enhanced nitrogen fixation rates and thus led to development of leaves. Leaf area is directly related to shoot growth and the two are directly influenced by root growth. Large root system and continued production of root hairs in plants are required for maximum response to nutrient supply that positively correlates with improved shoot growth and consequently increase in leaf area. Such results were observed in inoculated soya bean plants that produced larger leaves than the control experiment.

Inoculated soya beans recorded a higher root and shoot fresh weight with $2.67x10^7$ cfu/ml inoculum giving the highest root and shoot fresh weight and lowest in un-inoculated treatment. The findings are in agreement with **Yang *et al*. (2018)** who showed that effective rhizobia bacteria isolates were able to fix nitrogen, increase soya bean root and shoot fresh weight and nitrogen content of the roots and shoots. Active shoots ensure a sufficient supply of carbohydrate to roots and maintain root function which can in turn, improve shoot characteristics by supplying sufficient amount of nutrients, water, and phytohormones to shoots which improved shoot and root fresh weight of inoculated soya beans in comparison to un-inoculated soya bean plants.

Inoculated soya beans recorded a higher root and shoot dry weight with $2.67x10^7$ cfu/ml inoculum giving the highest root and shoot dry weight and lowest in un-inoculated treatment. The results are in agreement with the study conducted by **Samago *et al*. (2018)** who revealed that root and shoot dry matter of the inoculated treatments was significantly greater than that of the control because of an increase in nodulation. Based on the levels of nitrogen in plant shoots, it was evident that the symbiotic effectiveness was high when high concentration of rhizobial inoculant was applied on soya bean. Bring out how different treatments performed. The root and shoot dry weight obtained from soya beans confirmed an increase in biomass through inoculation. The results are in agreement with those of **Koskey *et al*. (2017)** who indicated that nitrogen accumulation in legumes affect positively the root and shoot dry weight of soya beans plants. The significant improvement in growth parameters may have been as a result of more readily available essential elements in the soil. Because the results of this study were only obtained from pot experiments under greenhouse conditions, the possibility of more favourable responses to *Bradyrhizobium liaoningense* inoculation under field conditions should not be discounted.

Inoculating soya beans with *Bradyrhizobium liaoningense* increased chlorophyll concentration in the soya bean leaves with $2.67x10^7$ cfu/ml inoculum giving the highest chlorophyll concentration and the lowest in un-inoculated treatment. **Bejandi et al. (2012)** while studying on the effects of seed inoculation and microelement supplementation in Chickpea (*Cicer arietinum* L.) reported that plants inoculated with *Rhizobium cicerea* were characterized by a higher chlorophyll concentration. **Kalaji *et al*. (2018)** indicated that optimum availability of nutrients such as nitrogen play a vital role in the formation of photosynthetic pigments such as chlorophyll. The higher chlorophyll content in the inoculated soya beans can be attributed to applied inoculation which readily supplied important nutrients such as nitrogen, which is important in the synthesis of chlorophyll in the chloroplast. Nitrogen is a component of the enzymes associated with chlorophyll synthesis and chlorophyll concentrations reflects relative N status in the soil that was greatly enhanced through soya bean inoculation. Nitrogen is a building block of proteins which is highly required for all enzymatic reactions in a plant (**Ayoola, 2010**). It is a major part of the chlorophyll molecules and plays a necessary role in photosynthesis and also is a major component of several vitamins.

Inoculating soya bean with *Bradyrhizobium liaoningense* increased nodulation significantly with $2.67x10^7$ cfu/ml inoculum giving the highest number of nodules and lowes tin un-inoculated treatment. This is in agreement with previous findings by **Yang *et al*. (2018)** who indicated that improvement in nodulation resulted from inoculation of the soya bean. Yadav (1996) demonstrated a significant increase in the number and size of nodules after seed inoculation. Similar results have been reported by **Ahmad *et al*. (2001)**.

The numbers of pods per plant were more in the inoculated soya bean plants with $2.67x10^7$ cfu/ml inoculum giving the highest pod number and lowest in un-inoculated treatment. The increase in pod number in this study could be attributed to the complementary effects elucidated by the rhizobium to enhance the nitrogen fixation performance. The increase in pod number in inoculated soya bean plants over un-inoculated may also be attributed to rhizobium inoculant, which increases the level of nitrogen absorption by the soya bean plant, that has a direct effect in the biomass accumulation in the plant parts including the pods. This shows that rhizobium bacteria within the root nodules affect plants in forming pods. Similar studies by **Bhuiyan *et al.* (1998), Khanam *et al.* (1999) and Khanam *et al.* (1994)** found that *Rhizobium* inoculation increased nodule number, nodule dry weight, stover yield and seed yields. Seed development is more influenced by N supply during seed formation. Increase in the number of pods and number of nodules may be attributed the increasing number of leaves, leaf area and plant height, as explained by the source sink relationship. More carbohydrates were produced due to more number of leaves and increased leaf area, sank into the root zone hence increasing the number of nodules.

CONCLUSION

Inoculation of soya beans with *Bradyrhizobium liaoningense* recorded increased plant growth, chlorophyll content, nodulation and yield of soya beans. Inoculation of soya bean with *Bradyrhizobium japonicum* introduced more viable and efficient rhizobium bacteria in the soil rhizosphere which promoted nitrogen fixation and mineral uptake. This promoted timely supply of the primary nutrients required for plant growth and development.

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