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Growth, Yield and Nodulation Response of Green Beans (*phaseolus vulgaris* l.) to Different Indigenous Microorganism Fertilizer Concentrations in Bambili, Cameroon

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Abstract
Green bean (*Phaseolus vulgaris* L.) is a leguminous plant consumed worldwide with the capacity of fixing atmospheric nitrogen by forming a symbiotic relationship with nitrogen fixing bacteria (*Rhizobia*) in their root nodules. This study was carried out to determine the optimal concentration of indigenous microorganism (IMO) fertilizer for maximum productivity and its effect on formation of root nodules in Bambili village. An experimental farm was set up, laid out in a complete randomized design using four treatments (0g being the control, 5g, 10g and 15g of IMO) with four replications. Fertilizers were applied 5 days before planting and repeated every 2 weeks up to 8 weeks after planting (WAP). Growth parameters were measured every 2 weeks. The data were analyzed using Analysis of Variance (ANOVA). Duncan's multiple range test was used to determine specific differences between group pairs.
Plants grown with IMO did better in terms of growth and yield as compared to the control. Plants with 15 g of IMO produced the best results which were significantly different at $P \leq 0.05$ for number of leaves (47.31 ± 4.347), number of branches (14.25 ± 2.145), plant height (90.719 ± 0148.154), number of pods ($29{,}81 \pm 12.608$) weight of pods (0.903 ± 0.303) and number of root nodules (79.18 ± 15.633). These results suggest that 15 g of IMO per plant is suitable for green bean cultivation. Farmers should prioritize the use of IMO to grow crops as it can substitute for chemical fertilizers which are not environmentally friendly. More research should be carried out using higher doses of IMO to know the optimal concentration of IMO that can be used for the cultivation of green beans and other crops.
Keywords: Green beans, IMO, fertilizer, Yield, Nodulation

1. Introduction

Green beans (*Phaseolus vulgaris* L.) are grown for its unripe freshly eaten fibreless succulent pods. It is cultivated globally as an annual herbaceous plant for both its green, immature pods (green beans) and its dry, mature seeds (Kostrakiewicz-Gierałt, 2023). It is a popular and nutritious vegetable consumed worldwide due to its contribution to soil fertility and having high market value (Demelash, 2018). Green beans have the capacity of fixing atmospheric nitrogen by forming a symbiotic relationship with *Rhizobia* in their root which can then converts atmospheric nitrogen into a usable form (Shamseldin and Velázquez, 2020). The mineral nitrogen in the soil is mainly NO^{3-} and to a lesser extent ammonium NH^{4+}. Nitrogen (N) plays an important role in the growth and development of plants and is also responsible for many physiological and biochemical functions in plants (Nand, 2014). Nevertheless, compared to other legumes, green beans are weak fixers of atmospheric

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nitrogen. and it often requires supplemental nitrogen fertilization to achieve optimal yields, in contrast to heavy-feeding legumes like soybeans or cowpeas, which can meet most of their own nitrogen needs through robust symbiotic relationships with *Rhizobia* bacteria (Farid and Navabi 2015). However, the use of chemical fertilizers and pesticides in crop cultivation has raised concerns about environmental pollution and food safety (Damián *et al.,* 2013). Fossil fuel-derived inputs (fertilizers, pesticides, herbicides) boost yields but harm soil health (reducing microbes, organic matter, pH balance) and the environment. This growing concern pushes for sustainable alternatives, like using beneficial microbes (biofertilizers) and organic practices, to balance productivity with ecological preservation. The high cost of these products, the difficulties of meeting demand for them, and their harmful environmental legacy have encouraged scientists to develop alternative strategies to raise productivity, with microbes playing a central role in these efforts (Vaxevanidou *et al*., 2015). According to Richardson *et al* (2017), the residual effects of synthetic chemicals on green beans cultivation and yield is alarming. Agriculture products with pesticide residues can harm the consumer's health (Damalas and Eleftherohorinos, 2011) not only human beings but also the useful microorganisms and other beneficial insects will be impaired too (Altieri, 2018). Chemical compounds from pesticides survive in the environment for a more extended period, the continuous use of pesticides also aggravates global warming and climate change (Richardson, *et al* 2017). The demand for green beans in Cameroon has increased over time due population increase and export to neighboring countries (Siri *et al.*, 2020). Yet, green bean crops are negatively affected by biotic and abiotic factors, including pests, nutritional imbalance, inadequate development of root nodules, scarcity of water, and elevated temperature (Melkamu *et al*., 2023). There is a need to increase crop production to meet the demand for the local market (Refaay *et al*., 2021). Indigenous microorganisms (IMOs) fertilizer has been proposed as a potential and effective means to address these issues since they can decompose organic matter thereby releasing nutrients and organic compounds into forms that are accessible to plants hence improving soil fertility and promoting healthy plant growth (Ngueleu *et al*., 2023). Microbial stimulants have been proven to improve crop yield, but more experiments are required to evaluate the optimum quantities needed to get the maximum yields (Tahiri *et al*., 2024). This research seeks to determine the range or optimal concentration of IMO that can be used in green beans cultivation and its effect on nodulation.

2. Materials and Methods

Study area

This study was carried out in Bambili, a village located in the Tubah Sub-Division of the Northwest region of Cameroon. The topography consists mainly of hilly terrains. The village is 1100 m above sea level. The climate is typically cool and temperate due to its high elevation with average temperatures ranging from 15 ^{0}C to 25 ^{0}C. Average rainfall is about 2400 mm. There are two main seasons; the rainy season which starts from April and ends in October, and dry season from November to February. Bambili has a vegetation made up of montane forests and grasslands, with a variety of plant species adapted to the highland environment. Common tree species found in Bambili include bamboo, and *Eucalyptus*. Activities carried out in Bambili include agriculture with crops like maize, beans, potatoes and vegetables being cultivated by local farmers. Natives of Bambili village also rear livestock particularly poultry (Weather and Climate, 2023).

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Experimental design
The study was conducted in a complete randomized design consisting of 4 treatments. These treatments were replicated 4 times. making a total of 16 ridges. The ridges were 4m long, having 0.5 m spacing between them. The treatments were as follows;
Treatment 1 (T_1): 0 g (control),
Treatment 2 (T_2): 5g of indigenous microorganism fertilizer (IMO) per plant,
Treatment 3 (T_3): 10g of indigenous microorganism fertilizer (IMO) per plant,
Treatment 4 (T_4): 15g of indigenous microorganism fertilizer (IMO) per plant.
Plant material
The "DOLLY" green beans were used for this study. It is a high yielding French bean variety (*Phaseolus vulgaris*). It is a dwarf self-supporting plant and matures early. The pods are highly tender giving them an excellent crisp hence the name snap beans. Each pod contains multiple soft seeds that remain small and immature during the ideal harvest window. The seeds were bought from a famer's shop at Mile 4 Nkwen.
Preparation of IMO fertilizer
The IMO fertilizer (solid form) was prepared using the Korean natural farming method as proposed by Taffouo *et al*., (2018). White rice (10kg) was partly boiled for about 15 minutes and then placed in trays for 1 hour. After cooling, the rice was moved into wooden boxes and covered with clean sheets of paper. The top part of the boxes was tied with rubber band and buried 5 cm deep in the soil and covered with dry thick leaves for 5 days. After 5 days, whitish moldy filaments grew on the rice surface indicating that fermentation of IMO1 was complete. IMO1 (10 kg) was mixed with 10 kg of brown sugar in a plastic bowl. The mixture was transferred to a clay pot and firmly covered with a clean piece of soft white paper and kept in a dark cupboard for seven days. This is IMO2. It was transferred to a 30 L drum to which 10 L of chlorine free water was added and stirred thoroughly, then wheat brand was added and mixed further to ensure proper distribution of the liquid The mixture was then covered in the bucket tightly and allowed to undergo anaerobic fermentation for 7 days. After 7 days, white mold filaments were visible on the mixture, indicating that the fermentation process was complete.
Land preparation and application of biofertilizers
A land surface of 10 m × 8.5 m was cleared with a cutlass and racked. A hoe was used to till the soil, and 16 ridges were formed in two rows. Each ridge was 4 m by 1 m. On each ridge, 14 holes of 5 cm depth were made in 2 lines with each line having 7 plants giving a total of 14 plants per ridge. The different concentrations of IMO were measured and applied in the holes and allowed for 5 days before planting. This was repeated for 2 weeks, 4 weeks, 6 weeks and 8 weeks after planting (WAP).
Planting and weed control
The green bean seeds were selected to allow healthy ones, and 2 bean seeds were planted per hole about 2.5 cm deep and 5cm distance between each crop for all 16 ridges. The farm was watered using a watering can and after 2 weeks, weeding and mulching was done to retain soil moisture and keep the roots cool. The IMO fertilizer was applied around the surface of each plant without touching the roots. This process was repeated every two weeks till the 8th WAP.
Data collection

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Data was collected from 16 selected plants from each treatment labeled plant one (1) to sixteen (16). These plants were tagged using black thread. Data was collected every 2 weeks from 2 WAP to 12 WAP.

Measurement of growth parameters

The growth parameters measured were number of leaves, number of branches and plant height. The number of leaves and number of branches were counted. The plant's height was measured from the bottom to the top using a measuring tape.

Harvesting green bean pods

Green bean pods were harvested every 2 weeks, from the 8^{th} WAP and ended at 12 WAP. The number of pods were counted and weighed using a Gram Core S3 Digital Check weigher after each harvest.

Nodulation

By the end of the harvest, the selected 16 plants per treatment were carefully uprooted with their root nodules still attached, roots were washed gently and the root nodules counted.

Data analysis

Data was recorded in Microsoft Excel 2017. Data analysis involved a one-way ANOVA (SPSS 2006 version). To compare means across groups, Duncan's multiple range test (DMRT) was used to determine specific differences between group pairs, with significance set at $P \le 0.05$

3. Results

Number of leaves per plant

There was a significant increase in the number of leaves from 2 WAP to 8 WAP for all the treatments. The mean number of leaves at 8 WAP, for T_4 (47.31 ± 4.3) was significantly different at $P \le 0.05$ to the control (28.88 ± 9.337) (Table 1).

Number of branches of bean plants for different treatments

The number of branches increased significantly across the different concentrations. Plants of T_4 (14.25 ± 2.145) and T_3 (14.19 ± 1.759) produced branches which were not significantly different at $P \le 0.05$ but were both significantly different from T_1 (13.00 ± 1.633) and T_2 (11.75 ± 1.915) (Table 2).

Effects of IMO fertilizer on plant height

The mean plant height increased for all treatments from 2 WAP to 4 WAP. At 6 WAP, T_4 (48.131 ± 5.883 cm) was significantly different at $P \le 0.05$ from T_1 (42.481 ± 5.101 cm) but was not significantly different from T_2 (45.219 ± 5.101 cm) and T_3 (44.375 ± 5.830 cm). There was no significant difference in mean plant height at $P \le 0.05$ for all the treatments at 8 WAP. (Table 3).

Number of pods per plant for various treatments

The first harvest was done at 8 WAP, and the mean number of pods for all treatments were significantly different from each other at $P \le 0.05$, with T_4 plants recording the highest mean number of pods (15.38 ± 5.943) which were significantly different at $P \le 0.05$ from all the other treatments. The control plants had no mature pods. T_4 plants had the highest mean number of pods (33.50 ± 6.346) at 10 WAP which was significantly different from T_3 (31.94 ± 8.250) but were both significantly different at $P \le 0.05$ from T_2 (24.88 ± 3.810) and T_1 (18.38 ± 5.09). (Table 4).

Effects of IMO on the fresh weight of pods

There was a general increase in the mean fresh weight of pods for all the different treatments from the first harvest at 8 WAP to the second at 10 WAP. T_4 plants (1.176 ± 0.247 kg) were not significantly different at $P \leq 0.05$ from T_3 (1.072 ± 0.186 kg) at 10 WAP but were both significantly different from T_2 (0.756 ± 0.186 kg) and T_1 (0.675 ± 0.204 kg) (Table 5).

Number of root nodules per treatment

There was no significant difference at $P \leq 0.05$ in the mean number of nodules between T_1 (29.94 ± 14.503) and T_2 plants (32.19 ± 17.174), but there was a significant difference at $P \leq 0.05$ between T_2, T_3 and T_4. Treatment 4 plants had a significant mean number of root nodules (79.18 ± 15.633) over the control (29.94 ± 14.503) (Table 6).

Discussion

The number of leaves and branches increased significantly with increase in IMO concentration, the plant height also increased significantly. Plants grown with 15 g of IMO (T_4) performed best in terms of number of leaves, number of branches and plant height. These findings are similar to those of Maryam *et al* (2018) who worked on growth and quality of green beans under application of organic chelate fertilizers and reported that organic fertilizers have a positive effect on the various growth traits of green beans. Yaser and Yusop (2019) worked on Response of IMO on *Brassica chinensis* seedlings and reported that greater number of leaves were recorded in the higher IMO treatment (3.46) with 600 g of IMO. These results may be due to the increase in number of indigenous microorganisms found in the 15 g of fertilizer which are able to rapidly decompose organic matter and provide the required nutrients for growth of green bean plant.

The highest number and weight of green bean pods was obtained by T_4 plants. These results are comparable with the work of Jeffrey *et al*., 2018 who had an increase in the growth and yield of sweet corn and peanut grown with IMO 7. This could be attributed to the availability of bio-nutrients and minerals in the treated soil, and improved soil structure.

At the final harvest (12 WAP), the number of root nodules of the plants from the different treatments were significantly different at $P \leq 0.05$ with plants treated with 15 g IMO having more root nodules than plants under the other treatments. The results are similar to that of Oldryod and Dixon (2014) who worked on the biotechnological solutions to the nitrogen problem and Melkamu *et al*, 2023 who worked on Bio-fertilizer and NPSB fertilizer application effects on nodulation and productivity of common bean (*Phaseolus vulgaris* L.) at Sodo Zuria, Southern Ethiopia. They noticed that organic fertilizers have a positive influence on legume root nodules by improving soil conditions and nutrient availability thereby increasing productivity. When compared to HB-A15, Rhizobia strain type and control treatment, inoculation with ***Rhizobium etli*** resulted in a higher number of nodules per plant (Melkamu *et al*., 2023).

This increase in nodulation in the higher treatments could suggest increased microbial activity, nitrogen fixation and nutrient availability for bean plants which was caused by the IMOs.

4. Conclusion

The aim of this research was to determine the ideal concentration of IMO for green bean cultivation. The results from this research demonstrated that plants grown with IMO produced better than the control. Amongst the treatments with IMO, 15 g produced the best results in terms of growth and yield parameters. The mean number of root nodules was also

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more in for plants with 15 g of IMO. This shows that green bean productivity increases with an increase in IMO concentration.

Perspectives

Further research should be carried out using higher doses of IMO to know the optimal concentration of IMO that can be used for the cultivation of green beans and other crops. Also, studies should be carried out on the identification of different microorganisms present in the different treatments.

Competing Interests

The authors declare that they have no competing interest.

Authors' Contributions

MRF: Conceptualization, investigation, original write up and editing. ANT: Review and data collection NGP: Data analysis, methodology and editing. CBC: Establishment of the field and collection of data. F: Investigation and editing. TVD: Supervision and review.

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Table 1: Number of leaves of bean plants under different treatments

Treatment /g	2 WAP	4 WAP	6 WAP	8 WAP
T_1 (0g)	5.13±1.455^a	10.44±0.727^{ab}	23.81±10.061^a	28.88±9.337^a
T_2 (5g)	5.63±2.156^a	10.81±2.287^{ab}	19.94 ± 5.603^a	26.31±5.986^a
T_3 (10g)	5.38±1.893^a	9.81 ± 1.167^{ab}	32.81 ±7.545^b	41.25±7.076^b
T_4 (15g)	6.00±2.449^a	11.38 ±1.258^b	36.06±11.216^b	47.31±4.347^c

Means followed by the same letter within each week are not significantly different at P ≤ 0.05 (DMRT)

Table 2: Number of branches of bean plants under different treatments

Treatment/g	2 WAP	4 WAP	6 WAP	8 WAP
T_1 (0g)	2.19 ± 0.544^a	4.38 ± 0.500^{ab}	6.75 ± 1.291^a	13.00 ± 1.633^{ab}
T_2 (5g)	2.44 ± 0.727^a	4.31 ± 0.602^a	6.88 ± 1.455^a	11.75 ± 1.915^a
T_3 (10g)	2.56 ± 0.964^a	4.88 ± 0.500^c	9.75 ± 3.256^b	14.19 ± 1.759^b
T_4 (15g)	2.44 ± 0.829^a	4.81 ± 0.911b^c	8.50 ± 1.317^b	14.25 ± 2.145^b

Means followed by the same letter within each week are not significantly different at P ≤ 0.05 (DMRT)

Table 3: Plant height/cm of bean plants under different treatments

Treatment/ g	2 WAP	4 WAP	6 WAP	8 WAP
T_1 (0g)	3.706 ±0.254^a	16.5 5± 2.901^b	42.481 ± 5.101^a	73.031 ± 110.788^a
T_2 (5g)	4.931 ±1.311^a	13.450 ± 2.954^a	45.219 ± 5.101^{ab}	48.6 ± 4.924^a
T_3 (10g)	3.931 ± 0.480^a	17.444 ± 4.423^b	44.375 ± 5.830^{ab}	90.719 ± 148.154^a
T_4 (15g)	3.969 ± 0.435^a	16.963 ± 5.500^b	48.131 ± 5.883^b	90.719 ± 148.154^a

Means followed by the same letter within each week are not significantly different at P ≤ 0.05 (DMRT)

Table 4: Number of pods of bean plant under different treatments

Treatment/g	8 WAP	10 WAP	12 WAP
T1 (0g)	0	18.38±5.097^a	18.50±5.073^a
T_2 (5g)	2.63±3.384^a	24.88±3.810^b	17.88±7.173^a
T_3 (10g)	8.25±4.01^b	31.94±8.250^c	25.63±7.588^b
T_4 (15g)	15.38±5.943^c	33.50±6.346^c	29.81±12.608^b

Means followed by the same letter within each week are not significantly different at P ≤ 0.05 (DMRT)

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Table 5: Weight of green bean pods under different treatments

Treatment/g	8 WAP	10 WAP	12 WAP
T_1 (0g)	0	0.675 ± 0.204^a	0.604 ± 0.149^a
T_2(5g)	00.027 ± 0.460^a	0.756 ± 0.186^a	0.647 ± 0.167^a
T_3(10g)	0.286 ± 0.141^b	1.072 ± 0.186^b	0.916 ± 0.253^b
T_4(15g)	0.656 ± 0.196^c	1.176 ± 0.247^b	0.903 ± 0.303^b

Means followed by the same letter within each week are not significantly different at $P \leq 0.05$ (DMRT)

Table 6: Number of root nodules of bean plants under the different treatments

Treatments/g	12 WAP
T_1 (0g)	29.94 ± 14.503^a
T_2 (5g)	32.19 ± 17.174^a
T_3(10)g	65.50 ± 16.645^b
T_4 (15g)	79.18 ± 15.633^c

Means followed by the same letter are not significantly different at $P \leq 0.05$ (DMRT).