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RESEARCH ARTICLE

Evaluation of Rhizobial Inoculation in Comparison to Urea Fertilizer Application of Vegetable Bean (*Phaseolus vulgaris* L)

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Abstract: A field trial was conducted to evaluate the effects of Rhizobium inoculation in comparison to nitrogen fertilizer (NF) application on vegetable bean in the Ankumbura area, Kandy district during the Maha 2018/19 season. Six treatments were employed comprising four NF levels (55, 110, 165, and 220 kg urea/ha), rhizobium inoculation without NF, and control with neither NF nor inoculant. The treatments were set out in a randomized complete block design with three replicates. Four NF levels were 25%, 50%, 75%, and 100% of the recommended level for vegetable bean by the Department of Agriculture (DOA). All the treatments received potassium and phosphorous according to DOA recommendations. Pod yield/m^2, growth parameters, several nodule parameters, and weed biomasses were recorded. Total yield (t/ha) was calculated based on the pod harvest. A yield versus fertilizer response curve was developed using calculated yield data. The highest total yield (3.03t/ha) with the application of 220kg/ha of urea was not significantly different from the yield produced under inoculation without NF (2.93 t/ha). This implies that inoculation facilitates a possible replacement of NF. Number of root nodules (30) and number of leaves (36) were significantly higher with inoculation. Higher levels; of 75% and 100% of recommended NF reduced nodule dry weight. Pod length, diameter, and dry weight with 100% and 75% NF levels were not significantly different ($p<0.05$) from those with inoculation. The highest pod N-yield (0.0586 g) and shoot N-yield (0.253 g) were recorded with inoculation and 220kg/ha urea application respectively. Weed biomass was significantly reduced (70%) with inoculation compared to the highest NF treatment. The results indicate a potential avenue to decrease the need for chemical nitrogen fertilizer and use inoculation to manage weed growth in vegetable beans in the Ankumbura area.

Keywords: *N-Fertilizer, Rhizobium, Seed inoculation, Vegetable bean, Yield improvement*

Introduction

Crops have specific mineral requirements for optimal growth and development. Modern agricultural practices use lots of synthetic fertilizers with essential nutrients to maximize production per unit area. However, not more than 30% of synthetic fertilizers are utilized by plants, and the bulk is released into the environment (Cheng 2008). Leaching of chemical fertilizers (CF) into drinking water supplies poses immediate risks to human health. Misuse of synthetic fertilizers and other agrochemicals is responsible for environmental issues such as global warming, eutrophication, and depletion of the ozone layer (Kulasooriya 2008). Plant growth promoting rhizobacteria plays a significant role in eco-friendly agricultural production since they aid in the movement and availability of minerals essential for plant growth, reduce disease incidences, improve crop yields on a long-term basis, and minimize the need for CF application. Rhizobia, a type of diastrophic bacteria, is involved in the most efficient biological nitrogen fixation (BNF) mechanism in legume root nodules (Santi et al. 2013). *Phaseolus vulgaris* is a legume crop that fixes nitrogen in symbiosis with rhizobia (Zahran 1999). Application of higher doses of NF adversely affects nodulation and nitrogen fixation of legumes including vegetable beans. Increased rates of inorganic nitrogen have decreased the nodule number and nodule dry weight of common beans in soils with varied rhizobia populations (Argaw and Muleta 2017).

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This is because synthetic nitrates reduce root hair formation, limit attachment of rhizobia onto the root hair, and infection thread formation is discouraged. The application of rhizobium strains as inoculants has reduced chemical nitrogen fertilizer usage with bean production (Morad et al. 2013). Rhizobium strains are cultured and developed as bioformulations called rhizobium inoculants. Inoculants are incorporated into suitable carrier materials and used as biofertilizers. The application of inoculants for legumes ensures a large population of rhizobia in the targeted host rhizosphere for better nodulation (Kulasooriya, 2008). Locally produced rhizobial bio-fertilizers have the potential to replace N- N-fertilizer applications to *Trifolium repens* L. in the Sri Lankan highland farming systems (Kulasooriya et al. 2017). It is essential to try this approach for vegetables as well. Therefore, the objective of this study was to compare the application of inoculants and nitrogen fertilizers to vegetable beans. This will provide an idea about the extent to which CF can be replaced with inoculation of vegetable beans in the study area.

Materials and Methods

A field experiment was conducted during the *Maha* season 2018/19 from September to December in the Ankumbura area in Kandy District in Sri Lanka. Six treatments comprising four urea levels; 55, 110,165 and 220 kg/ha, a rhizobium inoculation treatment, and a control (without NF and inoculation) were set out in a randomized complete block design with three replications per treatment. The four NF levels were 25%, 50%, 75%, and 100% of the recommended urea fertilizer for vegetable beans by DOA. The rhizobial inoculant in a coir dust-based carrier was produced by the National Institute of Fundamental Studies (NIFS), Kandy. These Inoculants were prepared by screening several rhizobial isolates obtained from different locations and selecting the most efficient strain. Three seeds of the bean variety '*Cora black*' (an imported bean variety recommended by DOA) were planted manually per hole in 2 m x 1 m size plots maintaining a spacing of 40 cm x 50 cm between holes. In the inoculation treatment, moistened seeds were mixed with the coir dust-based inoculant before planting. One plant was thinned out three weeks after seeding, maintaining two plants per hole. Recommended full doses of potassium and phosphorous were applied two days before sowing in the form of Muriate of Potash (MOP: 75 kg/ha) and Triple Super Phosphate (TSP: 280 kg/ha) respectively for all the treatments. Another application of MOP (75 kg/ha) was done four weeks after planting as a top dressing. Carbosulfan 25% (384-480 ml ha^{-1}) was sprayed across the plots to control bean flies. Manual weeding was done after the fifth week of planting and weeds in each plot were collected separately and oven dried at 65°C for 72 hours for weed biomass. Initial soil analysis was done for soil pH, soil moisture, total nitrogen (Kjeldahl digestion method), soil available nitrogen (calorimetric method), total phosphorous (Ultraviolet Spectrophotometer method), soil exchangeable potassium (Atomic Absorption Spectrophotometer method) and organic carbon (colorimetric method). Leaf chlorophyll content was recorded using SPAD- 502 chlorophyll meter 40 days after planting at the full bloom stage. At maturity, 18 plants from each treatment were removed to determine growth and nodule parameters. Growth parameters such as the number of leaves, plant height (cm), shoot and root dry weight (g) (65°C for 72 hours) were recorded. Nodule parameters such as number of nodules and nodule dry weight (g) (65°C for 72 hours) were recorded. Yield and yield components were recorded 50 days after planting at the harvesting stage. Average pod yield per plant (g) and average number of pods per plant were recorded in harvested pods from the selected 18 plants in each treatment. Pod length (cm), pod diameter (mm), and pod dry weight (g) (65°C for 72 hours) were recorded from randomly selected 10 pods from each treatment. Plant total nitrogen (%) was separately determined for pods and above-ground plant parts using the Kjeldahl digestion method and the N- N-yield of the plant was calculated by the following equation.

$$N - Yield = \%\, Kjeldhal\ NItrogen\ x\ Dry\ weight$$

(Kulasooriya, 2008)

Pod protein yield was calculated by multiplying the % Kjeldhal nitrogen by 6.25 factor.

Data was subjected to Analysis of Variance (ANOVA) using the SAS Package. The Duncan Multiple Range Test (DMRT) at a 5 % significant level was used to compare treatment means. Since all treatments received potassium and phosphorous at the same rate, discussion was limited to the urea-N application and inoculation.

Results and Discussion

Initial soil characteristics

The initial soil characteristics of the field are given in Table 01. The soil type of this area is reddish brown latosolic soil. According to these results, the soil was acidic during the experimental period.

Table 1: Soil chemical properties (30 cm depth: n = 06)

Chemical property	Value
pH	4.430
Total nitrogen %	0.118
Available nitrogen %	0.038
Total phosphorus %	0.014
Potassium (mmol/kg)	0.046
Organic carbon %	0.334

Growth and nodule parameters of vegetable bean

Growth and nodule parameters of vegetable beans at 40 DAP as affected by different treatments are presented in Table 02. Nodule number and dry weight are the most important nodule parameters to determine the effectiveness of BNF of legumes. Inoculated vegetable bean plants produced a significantly higher number of nodules compared to the other treatments.

Table 2: Growth parameters and nodule parameters of vegetable beans as affected by different treatments.

Treatment	Number of leaves/plant	Chlorophyll content (μg/cm^2)	Plant height (cm)	Shoot dry weight/ plant (g)	Root dry weight/ plant (g)	Total dry weight/ Plant (g)	Nodule number/ plant	Nodule Dry weight/ plant (g)
Control	27^c ±1.52	34.26^b ±1.22	190.83^a ±16.26	5.17^c ±0.300	0.86^b ±0.0200	6.03^{bc} ±0.291	16^{bc} ±1.50	0.027^b ±0.0047
25% NF (55 kg/ha)	31^b ±1.52	43.66^a ±2.47	239.50^a ±23.3	3.93^c ±0.875	1.20^a ±0.0306	5.59^c ± 0.905	12^c ±1.50	0.056^a ±0.0142
50% NF (110 kg/ha)	27^c ±1.52	39.16^b ±6.05	229.33^a±5 .92	4.63^c ±0.420	0.70^c ±0.0550	5.54^c ±0.395	17^b ±3.46	0.015^d ±0.0050
75% NF (165 kg/ha)	30^b ±1.52	34.03^b ±2.95	219.38^a ±31.2	6.46^b ±1.060	0.58^d ±0.0115	6.54^b ± 1.059	21^b ±20	0.012^c ±0.0013
100% NF (220 kg/ha)	32^b ±4.0	35.40^b ±4.41	178.17^a ±5.77	7.81^a ±0.550	0.70^c ±0.0650	8.24^a ±0.585	20^b ±5.51	0.013^c ±0.0014
Inoculant	36^a ±0.57	38.63^{ab} ±1.46	218.33^a ±5.5	4.76^c ±0.450	0.65^c ±0.030	5.62^c ±0.420	30^a ±3.50	0.049^a ±0.0097
P value	0.0025	0.0481	0.7531	0.0006	<0.0001	<0.0001	0.0020	<0.0001
CV% (0.05)	*6.12*	*9.33*	*21.70*	*11.91*	*5.58*	*4.78*	*16.49*	*18.89*

Means followed by the same superscripts are not significantly different at p>0.05.

Dogra and Dudeja (1993) report that nodule dry weight is significantly suppressed by higher doses of CF while inoculation of Rhizobium bacteria increased the number of nodules per plant in bean. The same finding has been reported by Habete and Buraka (2016), that the use of CF notably reduced the nodule count, whereas the introduction of rhizobium bacteria significantly elevated the number of nodules per plant for both varieties utilized in an experiment of P. vulgaris in Ethiopia. Vegetable bean in the control treatment produced nodules with significantly higher dry weight than the 50%, 75%, and 100% NF treatments and lower than the 25% NF and the inoculant treatment. According to Tahir et al. (2009) application of Rhizobium inoculation alone or in combination with P and N significantly increased nodulation of soybean. Since the 25% NF treatment produces the highest nodule biomass it is assumed that this small dose of NF was used by the plants as a starter dose. Dogra and Dudeja (1993) also state that the initial nodulation process and the onset of fixation are enhanced with a small amount of nitrogen in the vegetable bean.

Significantly highest ($p<0.005$) number of leaves per plant in *P.vulgaris* was observed with the inoculated treatment. The highest chlorophyll content was observed in 25% NF treatment, and it was not significantly different from inoculated vegetable bean. Plant height was significantly similar among

all the treatments. There is a positive response of increasing NF rates to the shoot dry weight of vegetable bean. The highest shoot dry weight was observed in 100% NF treatment which was significantly higher than the inoculated vegetable bean. The same result was explained by Müller et al. (1993) that the shoot dry weight of common bean is enhanced with an increasing rate of NF application. Also, the lowest shoot dry weight and highest root dry weight were observed in the 25% NF treatment. Though 25% NF treatment produced a significantly higher nodule biomass, shoot dry weight was not increased proportionately. Therefore, it is presumed that native rhizobia is highly infective but less effective. According to (Kellman 2008) plants with functional nodules on the roots should direct adequate N supply predominately to the shoots. Since the shoot biomass of 25% treatment is low, biomass direction from roots to the shoots may have not taken place properly. The control treatment also recorded a higher root and shoot biomass than under inoculation indicating the presence of an effective rhizobial population in the field. The competitive effect between the native rhizobia and the strain in the inoculum may be affected by inoculated treatment to reduce the result. Also, Gicharu et al. (2013) report that the bean varieties that were treated with a combination of the three rhizobia strains in their experiment exhibited the greatest overall dry weight among the plants.

Total yield and yield parameters of vegetable bean

Total pod yield at the first harvest

The total yield and other yield parameters of the vegetable bean as affected by different treatments are represented in Table 03. Inoculation and NF had different effects on the formation of yield parameters. At the first harvest, the 100% NF treatment produced the highest total yield (598.6 g/m^2), but this was not significantly different from the yield in the inoculated treatment (571.6 g/m^2). Similar to this finding (Morad et al. 2013) reported that rhizobium strains can produce pods similar to NF application. The control treatment, which was not significantly different from the 25% NF treatment, provided the lowest yield/m^2. Despite the fact that the 25% NF treatment produced significantly more nodule biomass, the yield was lower than the inoculated treatment.

Number of pods per plant at the first harvest

A significantly higher number of bean pods at the first harvest was produced under the inoculant treatment (12.66), which is not significantly different from the 50% NF application treatment (10.33). The control treatment produced the lowest number of pods/ plant (4.33). In contrast to this finding, (Jarecki et al. 2016), reports that seed inoculation alone has a minimal impact on the pod count per plant of soybean, but when combined with foliar application of micronutrients, it can enhance this particular parameter. In line with our results, Tahir et al. (2009) report that the sole application of Rhizobium inoculation resulted in a significant increase in the number of pods per soybean plant compared to the control treatments. Also, Morad et al. (2013) report that seed inoculation performed over control and NF treatment in relation to seed yield, pod number per plant in bean

Table 3: Total yield and other yield parameters of vegetable bean as affected by different treatments.

Treatment	Total pod yield at first harvest (g/m^2)	Number of Pods/plants at first harvest	Pod Yield /plant at first harvest (g)	Average Pod dry weight (g)	Pod length (cm)	Pod diameter (mm)
Control	379.33^{d} ±25.3	4.33^{c} ± 0.57	23.44^{c} ± 1.01	0.52^{d} ± 0.03	14.66^{b} ±0.15	6.32^{b} ± 0.08
25% NF (55kg/ha)	414.00^{cd} ± 25.5	7.33^{b} ± 0.55	30.88^{b} ±7.04	0.71^{cd} ± 0.03	13.46^{c} ±0.70	7.47^{a} ±0.24
50% NF (110kg/ha)	441.33^{c} ±27.7	10.33^{a} ± 2.52	33.42^{b} ±3.25	0.92^{bc} ± 0.01	15.10^{b} ±0.36	6.59^{b} ± 0.21
75% NF (165/ha)	508.00^{b} ±59.1	6.00^{c} ±1.00b	43.19^{a} ±2.71	1.13^{ab} ± 0.07	16.20^{a} ± 0.30	7.30^{a} ±0.34
100% NF (220kg/ha)	598.67^{a} ±24.1	5.33b^{c} ±0.57	43.53^{a} ± 0.50	1.30^{a} ± 0.05	16.03^{a} ±0.25	6.57^{b} ±0.18
Inoculant	571.67^{a} ±25	12.66^{a} ±2.31	47.86^{a} ±4.5	1.38^{a} ± 0.33	15.96^{a} ± 0.20	7.62^{a} ±0.09
P value	< 0.0001	0.0009	< 0.0001	0.0005	0.0002	< 0.0001
CV%	*4.9*	*19.78*	*9.1341*	*14.95*	*2.65*	*2.72*

Means followed by the same superscripts are not significantly different at p>0.05

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Pod length and pod diameter at the first harvest

The 75% urea treatment produced the longest bean pods which was not significantly different from the inoculation and 100% urea application treatments. The highest pod diameter was observed in the inoculated vegetable bean. It was not significantly different from 25% and 75% urea treatments. The 25% treatment functions as a starter-N treatment, and in accordance with Van Kessel and Hartley (2000), a starter nitrogen effect will manifest when the host plant exhibits inadequate nodulation or when ineffective rhizobia is present in the soil.

Average pod dry weight at the first harvest

The inoculant treatment produced the longest pods with maximum weight which was not significantly different from 100% and 75% urea application. The control treatment produced the pods with lowest dry weight. Mrkovački et al. (2008) report that the best results in terms of seed and dry matter yield were achieved when 30 kg/ha of nitrogen was applied to inoculated soybeans, as opposed to using higher nitrogen rates. As reported by Kovács et al. (2008), the most substantial doses of N caused a delay in the ripening of the beans

The inoculant treatment produced the longest pods with maximum weight which was not significantly different from 100% and 75% urea application. The control treatment produced the pods with the lowest dry weight. Mrkovacki et al, (2008) report that the best results in terms of seed and dry matter yield were achieved when 30 kg/ha of nitrogen was applied to inoculated soybeans, as opposed to using higher nitrogen rates.

There is a positive response in yield with the advancement of the urea fertilizer rates (Figure 1). According to the curve, the bean pod yield produced by the inoculated treatment is not significantly different from the yield obtained with the highest level of recommended urea fertilizer application (220 kg/ha).

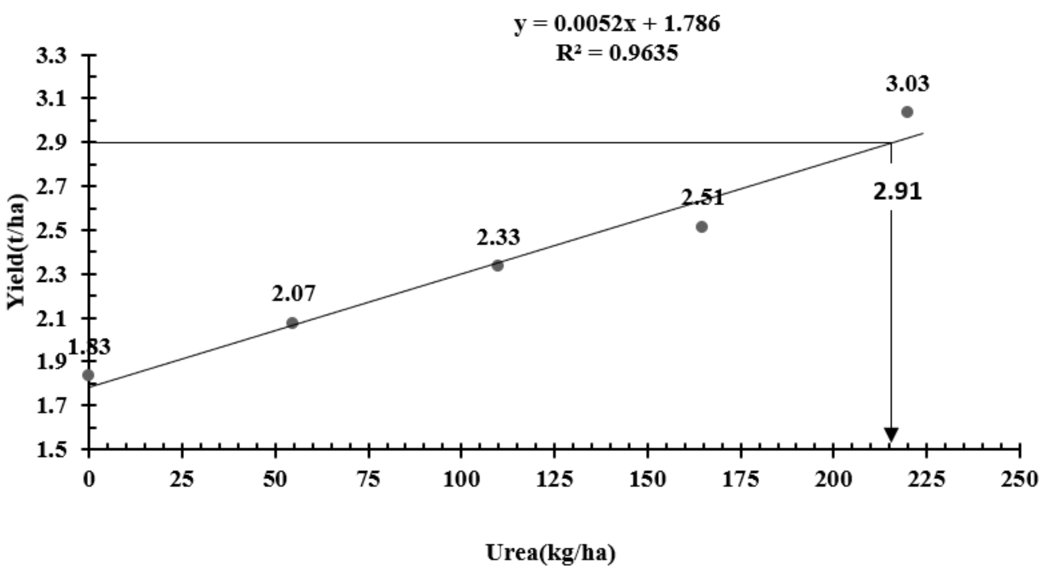

Figure 1: Vegetable bean yield response curve to urea application (arrow depicts the yield with inoculant)

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Weed biomass under different treatments.

Weed biomass in different vegetable bean plots as affected by different treatments is given in Table 04. Weed biomass responds positively to an increase in the NF rate. Significantly highest weed biomass is observed in 100% and 75% NF treatments. Inoculation has resulted in the significantly lowest weed growth and shows a 70% reduction in weed biomass from 100% NF. This result is understandable. Nitrogen fixed from the atmosphere by the rhizobia in the nodules is readily available to the host plant, but not to the other vegetation in the habitat. On the other hand, urea or any other chemical fertilizer added to the soil is freely available to all plants including weeds. Weeds being more aggressive, often outgrow the crop and could even smother it unless weed control measures are adopted. Under inoculation, this competition is minimized. This could be of advantage to the farmer because he could save money on weed control and minimizing applications of herbicides would also reduce environmental pollution.

Table 4: Weed biomasses under different treatments

Treatment	**Weed biomass (g)**
0% NF (Control)	$7.53^{c} \pm 0.03$
25% NF (55 kg/ha)	$12.72^{b} \pm 0.92$
50% NF (110 kg/ha)	$12.12^{b} \pm 3.48$
75% NF (165 kg/ha)	$16.81^{a} \pm 1.44$
100% NF (220kg/ha)	$18.60^{a} \pm 4.48$
Inoculant	$5.64^{c} \pm 1.13$
P value	0.0002
CV % (0.05)	*16.78*

Means followed by the same superscripts are not significantly different at p>0.05

Nitrogen (N) and protein yield of shoots and pods

Table 5: Nitrogen and protein yield of shoots and pods of vegetable bean

Treatment	Shoot N yield (g)	Pod N yield (g)	Pod protein yield (g)
Control	0.197	0.0219	0.1387
25% NF (55kg/ha)	0.123	0.0260	0.1637
50% NF (110kg/ha)	0.149	0.0346	0.2167
75% NF (165kg/ha)	0.206	0.0382	0.2391
100% NF (220kg/ha)	0.253	0.0402	0.2518
Inoculant	0.117	0.0586	0.3665

Rhizobial inoculation increased the protein content of bean pods over other treatments and control (Table 5). There was a positive response in shoot N and pod N content for the increased NF rates. Increased protein content under rhizobial inoculation might be due to rhizobium fixed atmospheric nitrogen and increased total nitrogen content in seeds. Inoculated plants have the highest N yield in the pods and the lowest N content in the shoots. This may be due to the nitrogen remobilization and compartmentalization in the different parts of the plant.

Conclusion

Inoculation of vegetable bean has the potential to produce a pod harvest equal to the full dose of recommended nitrogen fertilizer application. Inoculated vegetable bean increased nodulation and produced pods with high weight and protein yield. Weed growth was significantly reduced by rhizobial inoculation. Therefore, it is concluded that rhizobial inoculation is a low cost, eco-friendly technology that is possible to adopt for vegetable bean cultivation in the Ankumbura area in the Kandy district of Sri Lanka. In order to further establish these results, multiple repeated trials are essential to be conducted.

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