Pre-extension Demonstration and Evaluation of Biofertilizer (*Brhadirhizobium japonicum*) TAL-379 Strain on Soybean at Guto Gida and Wayu Tuka Districts, Oromia Region, Ethiopia

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Abstract: Declining of Soil fertility is the most known challenging factor of agriculture which limits crop production in Ethiopia. To increase agricultural productivity, farmers have to use fertilizers. There are many types of fertilizers in our world that enhances agricultural productivity even though many of them have drawback. The technology of using Integrated Plant Nutrient Management (IPNM) is the alternative method to increase sustainable productivity because of its zero negative impact on environment and soil. Among IPNM components, biological nitrogen fixation is the good method to fix nitrogen and improve soil fertility. This experiment was conducted in 2019 &2020 to evaluate the effectiveness of Birhadyrhzobium japonicum biofertilizer strain-379 and the result obtained from plant height, pod number/plant, branch number/plant, yields and yield components were shown significant difference ($P \leq 0.05$) with Birhadyrhzobium japonicum biofertilizer strain-379 comparing with the treatment with no any fertilizer and chemical N fertilizer at Wayu Tuka. This study present recommends using of rhizobium biofertilize TAL-379 strains in the production of soybean to increase grain yields and yield component at Wayu Tuka district, but in Guto Gida district, the experiment result shown chemical N fertilizer is significant difference at ($P \leq 0.05$) when comparing with the strain-379.

Keywords: Soil Fertility, Bio Fertilizer Strain-379, Nutrient Management, Inoculation

1. Introduction

Increasing Crop production is one is the most known challenging factor to achieving food security and poverty reduction in Ethiopia. Soil fertility is one of the main constraints to agricultural intensification in Ethiopia. Like in many East African countries, nutrient depletion rates are exacerbated in Ethiopia by high erosion rates, biomass and animal manure removal from farm plots and limited application of mineral and organic fertilizers [1]. Considering the fact that soil fertility is one of the biggest challenges, an obvious strategy is to increase fertilizer application and promote good agronomic practices to enhance productivity in Ethiopia particularly in the study area [2]. Many small scale and poor farmers could not use chemical fertilizer. Therefore, it is important to understand these constraints and develop low cost techniques that focus on development of appropriate Integrated Plant Nutrient Management (IPNM) for the Ethiopian farmers. One of the alternatives method to increase the biological inputs of nutrients is here that biological fixation of atmospheric N that contribute directly in increasing of soil fertility and sustainability of yields within the minimal external inputs [3]. The increasing need for environmentally friendly agricultural practice is driving the use offertilizers based on beneficial microorganisms [4] Bacteria that colonize plant roots and promote plant growth are referred to as plant growth promoting rhizobacteria (PGPR). They are a group of free living bacteria that colonize the rhizosphere and benefit the root growth. Rhizobacteria are belonging to the genera Pseudomonas and Bacillus. They are well known for their antagonistic effects and their ability to trigger Induced Systematic Resistance.

Resistance inducing and antagonistic rhizobacteria might be useful in formulating new inoculants with combinations of different mechanisms of action, leading to a more efficient use for bio control strategies to improve cropping systems [5]. Biological nitrogen fixation contributes directly and indirectly to the maintenance or enhancement of soil fertility and increasing productivity. Biological nitrogen fixation (BNF) by legumes is an indicator of their potential contribution to recycling nitrogen in cropping systems [6]. Bifertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or to soil. They accelerate certain microbial process in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. Very often microorganisms are not as efficient in natural surroundings as one would expect them to be and therefore artificially multiplied cultures of efficient selected microorganisms play a vital role in accelerating the microbial processes in soil. The uses of bio-fertilizer is one of the important component of integrated plant nutrient management, as they are cost effective and renewable source of plant nutrients supplement instead of the chemical fertilizers for sustainable agriculture [7]. Inoculation of biofertrilizer can be beneficial to the establishment of effective N-fixation on new seedlings of legume if done properly in areas where a legume of the cross inoculation group has not been grown previously or where N-fixing populations of soil bacteria have been severely reduced by adverse soil conditions such as drought or soil acidity. If a particular legume has not been grown in a field for several years, inoculation of seed is generally recommended as 'insurance' to ascertain maximum benefit from legume N-fixation [8]. Therefore by considering that there were no history with biofertilizer inoculation on soybean production at the study area. The study has been done by inoculating soybean seeds with biofertilizer TAL379 strain (*Birhadyrhzobium japonicum,*) during plantation to evaluate the effectiveness of the strain on the enhancement of soybean productivity being low cost and no negative impact on the farm and demonstrate the technology.

2. Materials and Methods

2.1. Description of the Study Area

2.1.1. Location

The study was conducted in 2019 and 2020 at Wayu Tuka and Guto Gida Districts. Both districts are located in East Wollega Zone of the Oromia Regional state, Ethiopia. Gute town, which is the capital town of Wayu Tuka district, is located at about 316 km distance from Finfine in western direction, while Nekemt is a capital for Guto Gida district. Geographically Wayu Tuka district is located between 36°40′0′′ and 36°50′0′′ E and 8°50′0′′ and 9°10′ 0′′ N while Guto Gida district is situated at an altitude of 1500–2170 m.a.s.l and lies between 8° 57′ 43.4′′ and 9° 30′ 58.5′′ N latitude and 36° 26′ 25.24′′ E and 36° 44′ 419.58′′ E longitude according to Garmin 60 GPS reading.
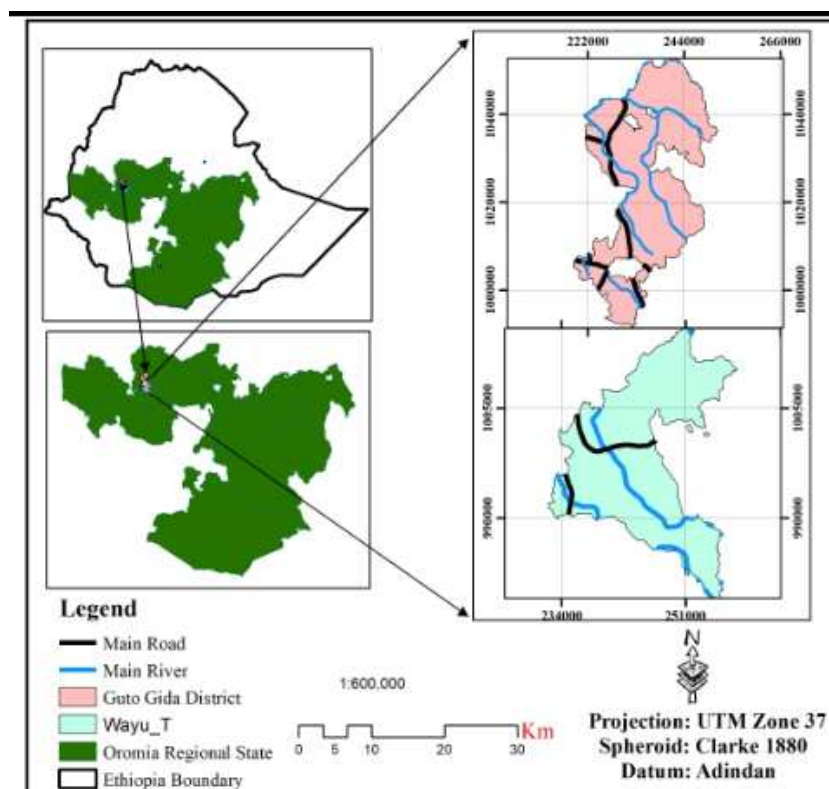

Figure 1. Location map of Guto Gida and Wayu Tuka Districts.

2.1.2. Agro Climate, Soil Type and Topography

The agro-climatic zone of the districts is falls in the highland (*badda*) and mid altitude (*Badda Daree*)) [9]. According to the ten years (2005-2014) weather data recorded at the Nekemte Meteorological Station, the average annual rainfall of the study areas is 2166.43mm and the monthly mean minimum and maximum temperatures ranges between 11.93 to 14.20 and 21.12°C to 28.210°C with unimodal rainfall pattern with monthly mean maximum rainfall (396.24mm) received in the month of june. While the average annual minimum and maximum temperatures of the districts are 13.05°C and 26.06°C, respectively. According to [10] classification, the dominant soil type in both districts is Nitisols. The district is topographically characterized by mountainous and gentle sloping landscapes.

Climatic data during the study period from experimental reas

Table 1. *Meanvalue of monthly rainfall, minimum and maximum temperature of the study area.*

Month	Rainfall (mm)	Minimum Temperature	Maximum Temperature
Jan	7.91	12.07	26.06
Feb	12.96	13.21	27.82
Mar	30.69	14.16	28.21
APR	93.52	14.20	27.38
May	320.40	13.68	24.53
Jun	396.24	13.01	22.69
Jul	374.65	12.93	21.14
Aug	387.73	13.00	21.12
+Sep	303.52	12.93	22.47
Oct	157.84	12.86	24.12
Nov	65.49	12.62	24.37
Dec	15.48	11.93	24.75

Total mean annual rainfall (mm) 2166.43. Source: Ethiopian National Meterology Agency, Nekemte Meterology station in 2006-2018.

2.2. Site Selection

Sites and farmers were selected in collaboration with key informant farmers, DAs, and the districts' agriculture and natural resource officers. The target sites were selected based on the high potential soybean production and access of road to the farm. The farmers were also selected based on their interest to accept the technology and willingness to provide their land for plantation. Total 20 farmers were selected from both districts, that means 10 farmers from each district.

2.3. Experimental Design

The lands have been prepared using hand hoe (three times ploughing) before planting. The treatments were three. They are chemical N fertilizer (NPS), TAL 379 strain of (*Bradyhizobium japonicum*) biofertilizer and without any fertilizer then replicated three times. The experimental plots were $36m^2$ (6m x 6m). Treatments and treatment combinations was laid out as randomized complete block design (RCBD) and assigned randomly to the experimental units (plots) within blocks. 1Kg/ha TAL 379 strain of (*Bradyhizobium japonicum*) biofertilizer has been applied and mixed with the seed under shadow to protect the rhizobium from direct sunlight. The plots with chemical N-fertilizer were applied after the seeds have been planted. The soybean variety used was *katta*.

2.4. Data Collection and Analysis Method

Qualitative data like farmers' perception towards the technology have been collected from farmers and DAs participated on field day by interviewing and discussing with them another day after they have visit the technology. While quantitative data like, Yield and yield related data such as, plant height, branch per plant, number of pods per plant, biomass and Grain yield have been collected manually. Five plants were sampled randomly at maturity time from each plot, plant height were measured in (cm) from each five plants and the average value was reported as plant height. The number of branch per plant were counted from each sampled plants and the average value was recorded and the pods were counted for all the five plants, and the average value was recorded as number of pods per plant. During the sampling, the plants were harvested by cutting with knife to the border of the ground to measure the above-ground dry biomass. The weight measurements of the five plants were done using an electronic digital balance and recorded as plant biomass. Finally the grain yields were weighted by threshing the seeds of five plants sampled from each plot and recorded as grain yield.

2.5. Statistical Analysis

The analysis of variance was carried out using the general linear model (GLM) procedure provided by SAS statistical software version 9.20 (SAS 2008). Means were separated using fisher's protected Least Significance Difference test (LSD) at 5% probability level.

3. Result and Discussion

3.1. Field Day

In this experiment the field day was held in W/Tuka district in 2019 on which large number of stake holders, FRG members farmers, their neighbor and experts who were working on the experiments were participated on the field day to learn and share their knowledge. During the field day twenty farmers were attended. Ten farmers are those the study has been conducted on their farm and the others ten were neighbors.

Table 2. *Field day participants by category.*

Participants	Female	Male	Total
Experts (DA, staffs, and other guests)	15	50	65
Farmers	5	15	20

3.2. Yield and Yield Related Parameters Performance

The results of combined analysis of variance revealed that

there are statistically significant difference between the treatments on the results of plant height, branches per plant, number of pod per plant, grain yield and biomass of soybean at Guto Gida and Wayu Tuka districts.

Table 3. Results of Combined ANOVA for plant height, branch per plant and pod per Plant of soybean at Guto Gida district In 2019/20 and 2020/21 cropping seasons.

Treatments	Parameters		
	Plantheight	Branchnumber/plant	Podnumber/plant
Cont	60.38[b]	6.55[b]	53.57[b]
Biofer	64.45[a]	6.92[ab]	69.09[a]
Chem. Fer	65.32	6.92[ab]	69.78[a]
Significance	**	NS	**
CV (%)	2.25	3.45	8.16
LSD	2.8546	0.47	10.46
Mean	63.39	6.85	64.14

Significant at P ≤ 0.05**; LSD: Least significant difference; CV: Coefficient of variation. NS, Non significant; che fer: Chemical fertilizer; Bio fer: Biofertilizer; Cont: control: (without any fertilizer).

3.3. Plant Height, Branch Number and Pod Number at Guto Gida

Chemical N fertilizer significantly (*P ≤ 0.05)* affect plant height. (65.32m) were recorded with the treatment of chemical N fertilizer. Branch per plant were not show significant (*P ≤ 0.05)* difference from each other even the treatment of *bradyrhizobium biofertilizer* (TAL379) and chemical N fertilizer show greater number (6.92) than the treatment with no fertilizer (6.55). The highest pod number/plant (69.78) were recorded at the treatment of chemical N fertilizer and significantly (*P ≤ 0.05)* different from the treatment with no fertilizer and numerically greater than treatment with (TAL379) strain of *bradyrhizobium biofertilizer* (69.09) at Guto Gida district (in table 3). This results is in agreement with [11]. Who reported similar findings on plant height at harvest, number of pods per plant, number of seeds per pod, that are show insignificance difference between inoculation of rhizobium biofertilizers.

Table 4. Results of Combined ANOVA for Effect of rhizobial inoculation and chemical fertilizer on Biomass and yield of soybean at Guto Gida district in 2019/20 and 2020/21 cropping seasons.

Treatments	Parameters	
	Biomass (Kg/ha)	Grainyield (Kg/ha)
Cont	7936.00	4078.90[b]
Biofer	7977.40	4865.60[a]
Chem. Fer	8302.00	5067.20[a]
Significance	NS	***
CV (%)	14.26	4.62
LSD	2298.9	431.47
Mean	8071.79	4670.55

Significant at P ≤ 0.05**; LSD: Least significant difference; CV: Coefficient of variation. NS, Non significant; che fer:Chemical fertilizer; Bio fer: Biofertilizer; Cont:control: (without any fertilizer).

3.4. Biomass and Grain Yields of Soybean at Guto Gida

3.4.1. Grain Yield

Significantly higher grain yield (50.67Qt/ha) was obtained from the chemical N fertilizer than without any fertilizer which recorded (40.79Qt/ha) and numerically greater than with *bradyrhizobium biofertilizer* TAL-379 which recorded 48.66Qt/ha in table 4. Analysis of variance has shown that application of chemical N fertilizer had affected significantly (P<0.05) yields of soybean than the treatment with no fertilizer and *Bradirhizobial japonicum* (TAL379) biofertilizer at Guto Gida district. The treatment with chemical N fertilizer gave additional 2.16 Qt/ha weight of Soya bean yield over *Bradyrhizobium biofertilizer* at this district. This finding agree with [12] which said the application of biofertilizer did not significantly increase the yield and yield components of soybean by on the adverse soil conditions such as drought or soil acidity.

3.4.2. Biomass

Analysis of variance did not show any significant (P ≤ 0.05) difference between the treatments. Above-ground dry biomass parameter of soybean did not affected because of the difference between treatments. Even though the treatment difference couldn't cause significant (P ≤ 0.05) difference on the yield of above-ground dry biomass of soybean, there was numerical difference between the treatments at Guto Gida district. 79.36Qt/ha, 79.77Qt/ha and 83.02 Qt/ha were recorded with no fertilizer, *Bradirhizobial japonicum* (TAL-379) biofertilizer and chemical fertilizer respectively. The treatment with chemical N fertilizer gave additional 3.25 Qt/ha weight of Soya bean biomass yield over *Bradirhizobial japonicum* (TAL-379) biofertilizer at this district. This finding again agreed with [11]. Who reported similar findings on dry biomass that are insignificant difference with inoculations of rizobium biofertilizer on the adverse soil conditions such as drought or soil acidity.

Table 5. Results of Combined ANOVA for Effect of rhizobial inoculation and chemical fertilizer on plant height branch per plant and pod per Plant of soybean at Wayu Tuka district in 2019/20 and 2020/21 cropping seasons.

Treatments	Parameters		
	Plantheight	Branchnumber/plant	Podnumber/plant
Cont	65.4[b]	6.20[b]	55.47[b]
Bio-fer	71.61[a]	7.25[a]	71.00[a]
Chem. Fer	71.33[ab]	7.08[a]	68.23[a]
Significance	**	***	**
CV (%)	3.627	4.20	8.17
LSD	5.03	0.57	10.59
Mean	69.45	6.85	64.90

Significant at P ≤ 0.05**; LSD: Least significant difference; CV: Coefficient of variation. NS, Non significant; che fer: Chemical fertilizer; Bio fer: Biofertilizer; Cont:control: (without any fertilizer).

3.5. Plant Height, Branch Number and Pod Number at Wayu Tuka

The highest plant height, branch number and pod number (71.61m, 7.25 and 71.00) respectively were obtained from the application of *Bradirhizobial japonicum* (TAL-379) biofertilizer. With chemical fertilizer, (71.33m, 7.08, 68.23) of plant height, branch number and pod number respectively were recorded and with no fertilizer (65.4m, 6.2 and 55.47)

of plant height, branch number and pod number were recorded respectively at Wayu Tuka district in (table 5). The treatment with *Bradirhizobial japonicum* (TAL-379) biofertilizer show significant (P≤ 0.05) difference compared with no fertilizer on plant height, branch number and pod per plant. This results is in agreement with [13]. Who reported similar findings and concluded that the number of pods per plant, seeds per pod, branch per pod were significantly increased by application of Rhizobium inoculation.

Table 6. *Results of Combined ANOVA for Effect of rhizobial inoculation and chemical fertilizer on Biomass and yield of soybean at Wayu Tuka district in 2019/20 and 2020/21 cropping seasons.*

Treatments	Parameters	
	Biomass (Kg/ha)	Grainyield (Kg/ha)
Cont	7659.4[b]	3641.7[b]
Biofer	10709[a]	4923.0[a]
Chem. Fer	10661.7[a]	4827.5[a]
Significance	***	**
CV (%)	5.09	9.32
LSD	984.71	830.86
Mean	9676.75	4464.07

Significant at P ≤ 0.05**; LSD: Least significant difference; CV: Coefficient of variation. NS, Non significant; che fer: Chemical fertilizer; Bio fer: Biofertilizer; Cont:control: (without any fertilizer).

3.6. Biomass and Grain Yields of Soybean at Wayu Tuka

3.6.1. Biomass

Analysis of variance has shown that application of *Bradirhizobial japonicum* (TAL-379) biofertilizer had affected significantly (P<0.05) above-ground dry biomass parameter of soybean. Bradyrhizobium biofertilizer significantly enhanced the above-ground dry biomass yield (107Qt/ha) of soybean over non-fertilized (76.59Qt/ha) and chemical fertilizer (106.62Qt/ha) treatment at Wayu Tuka district. The treatment with *Bradirhizobial japonicum* (TAL-379) biofertilizer gave additional 0.47Qt/ha weight of biomass yield over chemical fertilizer at Wayu Tuka (in table 6). This finding agree with [14]. Who said an increased above-ground dry biomass was observed with treatment of rhizobial inoculation of TAL-379 strain.

3.6.2. Grain Yield

Significantly higher grain yield (49.23Qt/ha) was obtained from *Bradirhizobial japonicum* (TAL-379) biofertilizer than with no fertilizer which recorded (36.42Qt/ha) and with chemical N fertilizer which recorded (48.28Qt/ha) in table 5. Analysis of variance has shown that application of *Bradirhizobial japonicum* (TAL-379) biofertilizer had affected significantly (P<0.05) yields of soybean than the treatment with no fertilizer and chemical N fertilizer at Wayu Tuka district. The treatment with Bradyrhizobium biofertilizer gave additional 0.96Qt/ha weight of Soya bean yield over chemical fertilizer at Wayu Tuka.

4. Conclusion and Recommendations

Declining Soil fertility are the major soil chemical constraints which limit crop productivity in Ethiopia. In this study, application of *Bradirhizobial japonicum* (TAL-379) biofertilizer showed a considerable difference in plant height, branch number, pod number/plant, yields and yield components of soybean when compared with no fertilizer and chemical N fertilizer at Wayu Tuka district. The study has shown that to increase soybean production, application of 1Kg/ha TAL 379 strain of (Bradyhizobium japonicum) biofertilizer inoculants is recommended. Depending on the results of present study, it is important to promote the appropriate use of *Bradirhizobial japonicum* (TAL-379) biofertilizer through any possible means, to paramount technology of biofertilizer to western oromia farmers especially for farmers of Wayu tuka districts. The present study therefore recommends inoculating of soybean seeds with 1Kg/ha of *Bradirhizobial japonicum* (TAL-379) biofertilizer before planting to increase grain yield at Wayu Tuka district. However in this study *Bradirhizobial japonicum* (TAL-379) biofertilizer has shown significant effect at Wayu Tuka district, it did not brings the expected significant result at Guto Gida district. So another experiment has to be repeated at this district to find out the appropriate strain of *Bradirhizobial japonicum* biofertilizer that can significantly influence plant height, branch number, pod number yields and yield components of soybean at Guto Gida district.

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