

# Effect of Cyanobacteria Application as Biofertilizer on Growth, Yield and Yield Components of Romaine Lettuce (*Lactuca sativa*L.) on Soils of Ethiopia

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## Abstract

Nitrogen is an important element for plant growth and its availability in sufficient amount boosts production per unit area, increases the total supply of food and contributes to the quality of food. However, resource poor farmers in the tropics especially in Ethiopia are not able to use enough amount of inorganic N fertilizer for crop production due to high cost, and hence looking for alternative means of improving available nitrogen in the soil is crucial. Therefore, this study was conducted to assess the potential of cyanobacteria biofertilizer for growth of lettuce (*Lactuca sativa* L.). A factorial combinations of two soil types with contrasting reaction (Ziway pH of 8.0 and Yirgalem pH of 5.7) and five different N sources (dried and liquid cyanobacteria, urea, compost and a negative control) were laid out in a complete randomized design with three replications in the green house. The ANOVA revealed that the maximum value on all yield and yield parameters of the lettuce crop were obtained by application of the dried cyanobacteria. The dried cyanobacteria increased the number of leaf, leaf area, leaf length, fresh weight of the leaf, leaf dry weight and the root dry weight of the lettuce by 159.5, 112.4, 80.8, 48, 137.5 and 110%, respectively, over the control. Similarly, as compared to the control treatments, incorporation of the dried cyanobacteria biofertilizer to the soil increased the lettuce plant tissue P, Zn and Fe concentration by 38.54, 18.95 and 105.57%, respectively. Also, the lettuce tissue N concentration increased by 33.3% over the control due to the application of the liquid cyanobacteria while this was increased by 6.25% for dried cyanobacteria application.

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Similarly, the soil chemical properties and fertility parameters significantly changed by the application of the cyanobacteria biofertilizer treatments. Thus, as compared to the control, total soil N increased by 0.27%, by applying the dried cyanobacteria. However, this study should be verified under field condition to consolidate our findings in the greenhouse.

**Keywords:** biofertilizer; Cyanobacteria; lettuce; N-fixing

## 1. Introduction

Lettuce (*Lactuca sativa* L.) is one of the *compositae* plants. It is considered as one of the most important vegetable crops in many regions of the world. It planted in many countries for its edible leaves (vegetative parts). Nutritionally, a 100 g of Lettuce contains 95g water, 1 g of protein, 3 g carbohydrate, 22 mg calcium and 25 mg phosphorus [1]. Investigation by Stevens (1974) in USA, ranked lettuce 26<sup>th</sup> among vegetables and fruits in terms of nutritive value and 4<sup>th</sup> in terms of consumption rate highlighting the ever-increasing importance of this crop.

Lettuce needs an abundant supply of soil nitrogen to attain maximum yield and quality required by the fresh market. But the deficiency of soil nutrient is now considered as one of the major constraints to successful upland lettuce production in all worlds [2]. As organic farming eliminates agrochemicals and reduces other external inputs to improve the environment as well as farm economics, organic farming can play a vital role in the maximum profitable production of lettuce with sustaining soil fertility [3].

At present, the issue of soil productivity has become a global concern as soil fertility is diminishing gradually for many reasons including soil erosion, nutrient mining, accumulation of salts and other toxic elements. Intensification of agriculture emphasizes heavy use of chemical fertilizers, which leads to adverse environmental effects. Many efforts are being exercised to combat these, the unfavorable consequences of chemical farming [4].for this reason the study was conducted with the following objective of to determine the growth performances of lettuce to the applications of cyanobacteria biofertilizer, compost and urea.

## 2. Material and methods

The study was carried out at green house at College of Agriculture, Hawassa University, Ethiopia during 2012, using two soil samples with measurable differences in pH. The soil samples were collected from two different areas that represent two contrasting soil reactions, from Ziway (pH =8.0) and Yirgalem (pH=5.7)(Table -1). Hawassa is located at latitude of 07<sup>o</sup>03'18.6"N and the longitude of 38<sup>o</sup>30'15.6" E with the elevation of 1620 masl. It has an average annual rainfall of 1046 mm and average maximum and minimum temperature of 13.3 and 27.5°C, respectively[5].

From the two sites (Ziway and Yirgalem), soil samples were collected for pot experiments from the upper 0-20 cm depth from 30 different random spots with 5 meter interval by zigzag manner and the composite sample was prepared for analysis of the physical and chemical properties of the soils and to conduct the pot experiment in the green house.

Ziway is characterized as semi-arid agro ecological zone with an elevation of 1640 masl. Its annual rainfall amounts to 450-850 mm and the maximum and minimum temperature of 27<sup>o</sup>C and 16<sup>o</sup>C, respectively. Ziway is found at latitude of 07<sup>o</sup> 58' 6.7"N and longitude 38<sup>o</sup> 23' 20.9" E. Yirgalem is located at latitude and longitude of 06<sup>o</sup> 44' 57.5" N and 38<sup>o</sup> 23' 26" E , respectively, and with the altitude of 1742 masl. The soil type of the Ziway and Yirgalem areas represent tropical Andosols and Alfisols [6].

### 2.1 Laboratory Analyses

All Laboratory analyses were done following the procedures in laboratory manual prepared by [7] Sahlemedhin and Taye (2000). The soil samples were air-dried and ground to pass a 2-mm sieve and 0.5 mm sieve (for total N) before analysis. Soil texture was determined by Bouyoucos hydrometer method.

**Table1:** Physico chemical characteristics of the surface soils of Yirgalem and Ziway prior to treatments application

Soil characteristics	Yirgalem soil	Ziway soil
Clay (%)	42	28
Silt (%)	28	41
Sand (%)	30	31
Textural class	Clay	Clay loam
pH in water (1:25)	5.7	8.0
EC(mmhoscm <sup>-1</sup> )	0.02	0.24
Organic carbon (%)	1.62	2.67
Total N (%)	0.15	0.19
Na (C mol(+))kg <sup>-1</sup> )	0.09	0.45
K(C mol(+))kg <sup>-1</sup> )	1.34	2.1
Ca(C mol(+))kg <sup>-1</sup> )	7.2	16.0
Mg(C mol(+))kg <sup>-1</sup> )	4.55	6.00
CEC(C mol(+))kg <sup>-1</sup> )	20.08	43.52
Av. P(mg kg <sup>-1</sup> )	1.45	14.05
Fe (mg kg <sup>-1</sup> )	18.0	4.0
Mn (mg kg <sup>-1</sup> )	52.5	25.54
Zn(mg kg <sup>-1</sup> )	6.42	3.43
Cu(mg kg <sup>-1</sup> )	1.08	0.67
FC (%)	38	29
PWP (%)	35	17

The pH and electrical conductivity of the soils were measured in water (1: 2.5 soil: water ratio). Organic carbon content of the soil was determined following the wet combustion method of Walkley and Black while Total

nitrogen by (wet digestion) procedure of Kjeldahl method. The available phosphorus content of the soil was determined by Bray II method. Exchangeable cations and the cation exchange capacity (CEC) of the soil were determined following the 1 N ammonium acetate (pH 7) method. The exchangeable K and Na in the extract were measured by flame photometer. Ca and Mg were measured using EDTA titration method. The available potassium was determined by Morgan's extraction solution and potassium in the extract was measured by flame photometer. Exchangeable acidity of the soil was determined by leaching exchangeable hydrogen and aluminum ions from the soil samples by 1 N potassium chloride solution. Micronutrients (Fe, Zn, Mn and Cu) were determined with Di-ethylene tri-amine penta-acetic acid (DTPA) method.

## **2.2 Source of Cyanobacteria strains and the lettuce seed**

*Anabaena* spp. of Cyanobacterial strains E3 was used for the study. The E3 strain was isolated from soil sample from pigeon pea field of Ziway. This strain was isolated at Colorado State University U.S.A and obtained from soil microbiology laboratory of Hawassa University College of agriculture. The seeds of the Romaine lettuce (*Lactuca sativa* L.) "Variety Summer Crisp" was obtained from Deberzeit Genesis farm.

## **2.3 Cultivation of Cyanobacteria in the Hoop house for pot experiment**

Mass cultivation of the selected cyanobacterial strain (E3) was carried out in a hoop house, constructed from transparent polyethylene sheets. The cultivation was made in a large pond, 6 m×2m and 15 cm depth which is inoculated in 1:10 ratio (cyanobacteria culture and media). The pond was aerated using the compressor operated every other hour every day until 6pm. In addition to aeration using the compressor, the culture pond was stirred manually by paddle wheels for 10 minutes to prevent stagnation of the culture. Every day, the depth of the cyanobacterial culture media in the pond was measured to replace the amount of water evaporated by adding from the same water source. No temperature regulation or additional illumination was provided to the culture. After 21 days of culturing, supernatant were separated and dried. Thus, the dried and pounded powder was used as solid fertilizer (cyanopith) while the liquid was used as liquid fertilizer to grow lettuce, the experimental plants.

## **2.4 Pot experiment**

The second phase of the experiment was about measuring the impact of different N-fertilizers on production of the lettuce vegetable in two soils with contrasting soil reaction measurements of 5.7 and 8.0 pH (collected from Yirgalem and Ziway, respectively).

The pots were filled with 10 kg of soil and the three germinated seeds of lettuce were planted to the pot and in each pot different amounts of N sources were added i.e. 16.45g dried cyanobacteria, 18.5 liters of the liquid cyanobacteria, 1.085g of urea and 22.7 g of compost were added to each pot according to the treatments. All the nitrogen fertilizers were added at one time to achieve the recommended rate of 100kg N ha<sup>-1</sup> or 217kg ha<sup>-1</sup> of urea in addition 0.1 g of TSP (100kg ha<sup>-1</sup>) added to each pot. The liquid cyanobacteria biofertilizer was applied by split application methods to control the water logging condition and to keep the soil on optimum field capacity. These factorial combination of five different N sources treatments and two soil types arranged in a

complete randomized design and replicated three times making a total 30 experimental unites.

Data on each experimental plant was recorded from each pot. These include, leaf length, leaf number, and leaf area, fresh weight of the leaf, dry weight of leaf, The dry weight of leaves were determined by drying in an oven at 70°C for 48 hours, until constant weight were achieve and then ground for analysis. Sub-samples were taken from each sample for further analysis. Total leaf area was measured using the leaf area meter at the end of the study after the harvesting process. The above-ground part was separated and was weighed separately for fresh yield.

Data were statistically analyzed using the PROC ANOVA function of SAS and means were compared using LSD at a probability level of 5%.

### **3. Results and descussion**

#### ***3.1 Leaf length***

Leaf length of the lettuce plant showed statistically significant differences between the N sources ( $P \leq 0.01$ ) and the soil types ( $P \leq 0.05$ ), but there was no interaction effect because of the two factors (Table 2). Lettuce leaf length ranged from 13.00 - 23.5 cm. The highest leaf length (23.5cm plant<sup>-1</sup>) was recorded from the applications of dried cyanobacteria while the lowest leaf length (13cm plant<sup>-1</sup>) was recorded from the control treatment.

The dried cyanobacteria biofertilizer significantly increased leaf length of lettuce over the control and this might be due to the release of enough nitrogenous compounds for lettuce growth, mostly nitrates and ammonium, which can be readily taken up by vascular plants [8]. Absorbed nitrogen in turn increases leaf length through stem elongation brought about by cell division and expansion [9].

#### ***3.2 Leaf Fresh weight***

Nitrogen sources had highly significant ( $P \leq 0.001$ ) effect on leaf fresh weight of lettuce. The highest leaf fresh weight of lettuce (239.16g plant<sup>-1</sup>) was recorded from dried cyanobacteria application while lowest value of this parameter (161.50 g plant<sup>-1</sup>) was observed from the control. The later was statistically at par with that obtained from the liquid cyanobacteria (224.333 g plant<sup>-1</sup>) and urea (219.3plant<sup>-1</sup>) (Table 2).

The reason for the highest leaf fresh weight of the lettuce under dried cyanobacteria application could be linked to cyanopith as a biofertilizer improving the growth of lettuce by providing essential nutrients, which result in maximum cell growth, a phenomenon that influenced the growth of plant and this result is in line with [10]. [11] also reported that plants supplied with cyanobacteria biofertilizer had a higher grain yield compared to unfertilized ones. It is well known that nitrogen deficiency reduces plant growth and consequently the size and storage capacity of the plant.

### **3.3 Leaf numbers**

Number of leaves plant<sup>-1</sup> was significantly influenced ( $P \leq 0.01$ ) by application of different N sources but soil type and the interaction between the soil type and the N source were not significant (Table 2). The highest number of leaves (NL) (9.5) was recorded from the application of dried cyanobacteria and the smallest (3.6) was recorded from unfertilized pot (control). All the rest N sources performed between the two. However, there was no significant difference between the dried and the liquid cyanobacteria applications as there was no significance difference between the application of compost and the control.

The higher leaf number obtained from cyanobacteria applied pot might be attributed to efficiency of cyanobacteria biofertilizer and its capacity to supply plant nutrients, thus improving the soil fertility, biological process in soil, liberation of growth promoting substances and vitamins [12].

### **3.4 Leaf dry Weight**

Nitrogen source had significant ( $P \leq 0.01$ ) effect on dry weight of lettuce leaves. The highest leaf dry weight of lettuce (11.79 g plant<sup>-1</sup>) was recorded by application of the dried cyanobacteria (Table 2). The highest leaf dry weight of lettuce under the dried cyanobacteria application could be attributed to the release of synthesized nitrogenous compounds through microbial decomposition, which enhance leaf area development as well as photosynthetic activity of the leaf and therefore increase leaf dry weight [13]. In contrast, the lowest leaf dry weight (4.55 g plant<sup>-1</sup>) obtained from the control can be explained by lower N content, which retarded leaf area development resulting in lesser radiation interception, and consequently lower efficiency in converting solar radiation and thereby reduce the leaf dry weight [14].

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### 3.7 Leaf area

The leaf area of the lettuce was significantly ( $P \leq 0.01$ ) influenced by N sources. Among the four N sources fertilizers, the highest value (2046.33 cm<sup>2</sup> plant<sup>-1</sup>) was recorded from the dried cyanobacteria application and the lowest leaf areas were recorded on the control (963.33 cm<sup>2</sup> plant<sup>-1</sup>) (Table 2). Soil type and its interaction with N sources did not show significant effect on leaf area of the lettuce.

The increases in leaf area of the lettuce due to the application of cyanobacteria might be due to the fact that cyanobacteria supply plant nutrients, improve soil environment and biological processes in the soil, thus improving growth and photosynthesis of the plant [12].

**Table 2:** Lettuce growth parameters: leaf length (LL), leaf fresh weight (LFW), number of (NL), leaf dry weight (LDW), leaf area (LA) and root dry weight (RDW) as influenced by N source and soil type

Parameters						
fertilizers	Leaf length	Leaf fresh weight	Number of leaves	Leaf dry Weight(g)	Leaf area(cm <sup>2</sup> )	Root dry weight(g)
Cyano dry	23.5 <sup>a</sup>	239.17 <sup>a</sup>	9.5 <sup>a</sup>	11.79 <sup>a</sup>	2046.33 <sup>a</sup>	1.92 <sup>a</sup>
Cyano liquid	21.66 <sup>b</sup>	224.33 <sup>b</sup>	9.33 <sup>a</sup>	10.01 <sup>b</sup>	1744.83 <sup>b</sup>	1.75 <sup>b</sup>
Urea	19.33 <sup>c</sup>	219.33 <sup>b</sup>	6.33 <sup>b</sup>	8.55 <sup>c</sup>	1607.50 <sup>c</sup>	1.42 <sup>c</sup>
Compost	17.5 <sup>d</sup>	194.29 <sup>c</sup>	3.83 <sup>c</sup>	6.63 <sup>d</sup>	1302.50 <sup>d</sup>	1.18 <sup>d</sup>
Control	13.00 <sup>e</sup>	161.50 <sup>d</sup>	3.66 <sup>c</sup>	4.55 <sup>e</sup>	963.33 <sup>e</sup>	0.91 <sup>e</sup>
LSD 5%	1.68	7.18	1.51	1.43	64.369	0.08
Soil type						
Ziway	20.13 <sup>a</sup>	208.8	6.73	8.96 <sup>a</sup>	1555.00 <sup>a</sup>	1.49 <sup>a</sup>
Yirgalem	17.86 <sup>b</sup>	206.6	6.33	7.78 <sup>b</sup>	1510.80 <sup>b</sup>	1.37 <sup>b</sup>
LSD 5%	1.06	NS	NS	0.91	3 9.01	0.05
CV (%)	7.29	2.85	19.11	14.18	3.31	4.45
Interaction	NS	NS	NS	NS	NS	NS

Means followed the same letters in the same column are not significantly different at  $p \leq 0.05$  probability

### **3.8 Root dry weight**

Root dry weight of lettuce was significantly ( $P \leq 0.01$ ) influenced by N sources. The higher root dry weight was recorded by the application of dried cyanobacteria ( $1.92 \text{ g plant}^{-1}$ ) and the lowest root dry weight was recorded on unfertilized treatment ( $0.91 \text{ g plant}^{-1}$ ) (Table 2).

This effect of cyanobacteria biofertilizer on root biomass could be due to the fact that cyanobacteria produce growth-promoting substances, such as cytokinins as observed by [15]. Also [16] reported that rice seeds presoaked with cyanobacterial culture extracts showed enhanced germination, promotion of the growth of roots and shoots, and an increase in the weight and protein content of grains.

The mean root dry weight of the lettuce is significantly ( $P \leq 0.05$ ) influenced by soil type. The Ziway soil produced the highest root dry weight ( $1.49 \text{ g plant}^{-1}$ ) as compared to the Yirgalem soil ( $1.37 \text{ g plant}^{-1}$ ) (Table 2). The interaction of the main effect of N sources and soil type had no significance influence on root dry weight. The lower root dry weight on Yirgalem soil could be due to the fact that Yirgalem soil has acidic properties, which influence the available P for root growth [17].

## **4. Conclusion**

In general the results of this study have indicated that application of the dried cyanobacteria strain E3 as biofertilizer increase growth performance of the lettuce, thus could enhanced growth and yield of lettuce without using the costly chemical fertilizers. Hence based on the result of this study, dried cyanobacteria might be recommended to be used as biofertilizer by substituting and/or supplementing the costly inorganic fertilizer. However, the results presented here should be confirmed through conducting similar studies under field experiments (which involves various soil conditions and agro climates) prior to dissemination to the farmers.

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