

Effect of Cyanobacterial Biofertilizer on Soil Quality in Kale (Brassica Oleracea L) Crop Growing Field at Ziway Area, Ethiopia

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Abstract
The agricultural soil of Ethiopia is generally low in nitrogen (N) and phosphorus (P) which can affect crop production. Basically, commercial fertilizers could improve the soil fertility and crop yield. However, farmers are unwilling to use sources of inorganic fertilizers because of higher costs, accessibility and also in the long run, they affect the soil biota. This experiment was conducted to evaluate the effect of cyanobacterial biofertilizer on soil quality on kale (Brassica Oleracea L.) crop growing soil in Ziway. Five treatments: liquid cyanobacterial, dry cyanobacteria, urea, cattle manure and control were laid out in RCBD with three replications. Soil data were collected before and after harvesting the kale crop for selected soil physicochemical properties laboratory analysis and the data subjected to analysis of variance (ANOVA). The results showed that, soil total N, Organic Carbon (OC), available P, available Fe, and available Zn contents have increased by 1.2%, 0.49%, 21.1mg kg^{-1}, 3.54 mg kg^{-1}, and 5.31mg kg^{-1}, respectively over the control when dry Cyanobacteria biofertilizer was applied. Similarly, the incorporation of dry and liquid cyanobacteria biofertilizer was significantly decreased the soil pH to 6.6 and 6.9 over the control 7.75, respectively. Therefore, the use of cyanobacteria as a biofertilizer should be recommended as an alternative source of inorganic N fertilizer to improve the soil quality and reclaim alkaline soil for kale crop yield improvement for medium and small-sized farms in the study area.
Keywords: Anabaena species, Biofertilizer, Cyanobacteria, Soil Quality, Kale crop and N-fixing

INTRODUCTION

Ethiopia is the second most populous and the fastest growing non-oil economy countries in Africa (IFDC, 2012). A growing human population requires additional food demands that obtained from conventional agriculture, which has also led to an increasing dependence on chemical Fertilizers and pesticides. However, Ethiopian agriculture has been characterized by low productivity. Several natural and anthropogenic factors have been responsible for the low agricultural productivity, among which decline in soil fertility mainly caused by absence of efficient, sustainable and site specific soil fertility management practices (Abush *et al*, 201, Sanchez et al., 2000) is considered as serious limitation (IFPRI, 2010). To meet the food, fiber, fuel, fodder and other needs of the growing population, the productivity of agricultural land and soil health needs to be improved.

One of the major constraints to crop production faced by smallholder subsistence farmers is the inadequate supply of nutrients (Quinones et al., 1998). The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water (Saadatnia and Riahi, 2009). Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food, long term sustainability, and concerns on environmental pollution associated with indiscriminate use of agrochemicals (Krishna et al., 2012). Plants like all other living things need food for their growth and development, and they require 16 essential elements. They are classified into two categories which are macronutrient and micronutrient depending on the quantity required (Al-Khiat, 2006). In the soil, the mineral nutrients are dissolved in water and absorbed through a plant's root.

However, the amounts of nutrients in soil are always unpredictable and not enough for plants growth. The use Cyanobacterial as of biofertilizer is a viable alternative to improve soil quality and crop production. In addition, cyanobacteria as biofertilizer have the advantages of lower cost, reduced production of greenhouse gasses, such as oxides of nitrogen and carbon dioxide by 30%, which results in less pollution of the environment (Pisciotta et al*.,* 2010). Basically, inorganic fertilizers can improve soil fertility and yield of kale crop. Use of this inputs among smallholder farmers are very low in our country due to high costs, accessibility and also in long run, they affect the soil biota and soil productivity. In addition, the production and quality of the kale crop is affected by many factors, such as cultivation selection, planting date, weather condition, soil type, water holding capacity and soil fertility depletion. Among these, soil fertility problem is critical. Therefore, this experiment was conducted to investigate the influence of different application methods of Cyanobacteria as biofertilizer in soil quality on kale crop growing field when compare to Urea and Cattle manure.

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MATERIALS AND METHODS

Description of the Study Area

This study was conducted in Ziway area of East Shewa Zone of Oromia Regional State during main cropping season (2014) at the Hawassa University experimental station. Ziway is characterized as semi-arid agro ecological zone with an elevation of 1645masl. Its annual rainfall amounts to 450-850 mm and the maximum and minimum temperature of 27^{o}c and 16^{o}c, respectively. Ziway is located at altitude of 07^{o}58'6.7" N and longitude 38^{o}23'20.9" E. The area is suitable for kale vegetable production and intensive horticulture crop production is common (Girma et al., 2012).

Soil sample collection and laboratory analysis

Composite soil samples were collected from a depth of 0-20cm before planting and after harvesting of the kale crop from the experimental site for analysis of physical and chemical properties of the soil. The collected soil samples were air-dried, ground and passed through a 2 mm mesh size for all analyses except soil organic carbon (OC) and total nitrogen (TN), for which soil samples were further passed through 0.5 mm sieve. All laboratory analyses were conducted according to Sahlemedhin and Taye (2000). Soil texture was determined by Bouyoucos hydrometer method. Available phosphorus was determined by the Olsen method (Olsen et al. 1954). Soil OC was determined by dichromate oxidation method (Walkley and Black, 1934). The pH and electrical conductivity of the soils were measured in water (1: 2.5 soils: water ratio).Total N of soil samples was analyzed by micro Kjeldahl method (Nelson and Sommers, 1980). Exchangeable cations and the Cation Exchange Capacity (CEC) of the soil were determined following the 1 N ammonium acetate (pH 7) method. K in the extract was measured by flame photometer. The micronutrients iron (Fe) and zinc (Zn) extracted with DTPA extraction method were measured by atomic absorption spectrophotometer (Lindsay and Norvell, 1978).

Source and mass production of Cyanobacteria

Anabaena species of cyanobacteria strains E3 (the name taken from pigeon pea used exclusively for Ethiopia) was used for the study to produce optimum amount of fertilizer for experimental filed application. 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. Mass cultivation of the selected Cyanobacteria (E3) biofertilizer was carried out in the hoop house at Hawassa University College of agriculture which was constructed from transparent polyethylene sheets. The Pond size was 6mx2mx15cm depth which is inoculated in 1:10 ratio (Cyanobacteria culture and media) for mass production. Allen-Arnon medium was prepared (Allen and Arnon, 1955) using tap water. Air was supplied to the cultures using compressor for six hours during day time only. The mass cultured Cyanobacteria depth was measured in each day to replace the evaporated amount of water by adding the same water source during mass production time. Factors that affect the mass production of Cyanobacteria such as pH, temperature, water temperature, Optical Density (OD) and Electric Conductivity (EC) were measured during their multiplication time. They were harvested with the interval of 21 days. Cultured Cyanobacteria was filtered and used as a dry bio-fertilizer and the supernatant was applied as a liquid.

Experimental treatments and Design

A field experiment was conducted during the 2014 main cropping season to determine the effect of Cyanobacteria biofertilizer application on soil quality at Ziway area. The treatment included: Control, Cyanobacteria liquid, Cyanobacteria dry, UREA and cattle manure. The experiment was laid out in randomized Complete Block Design (RCBD) with three replications, thus making 15 experimental units. The plot size was 2.16m^2 (1.2mx1.8m) which has 4 rows by using 30cm and 15 cm spacing between row and plant, respectively. The gap between block and plots was 1.5m and 0.8m, respectively. The gross area of experimental site was 6.6 m x12.2 m (80.52 m^2). A 100 kg N-fertilizer/ha was applied for all the treatments. The treatment are urea (46.9g/plot) was applied in split twice at a time of planting and twenty five days after planting to control leaching and volatilization effects of nitrogen. The dry Cyanobacteria (493.15 g/plot) and the decomposed cattle manure (1674.4g/plot) was incorporated in the soil one week before sowing, respectively and the liquid Cyanobacteria (456.08L/plot) was applied by drenching method based on the field capacity to control water-logging. Phosphorous was applied equally for all treatments (P was applied at sowing in the form of Triple Supper Phosphate (46% P/ha). The seed of the kale *(Brassica oleracea* L) were obtained from Worabe agricultural research center for the experiment. All agronomic practices including weeding, harvesting was done as per the recommendation for the crop.

Statistical Analysis

All data were subjected to Analysis of Variance (ANOVA) using Statistical Analysis Software (SAS version 9.1, 2000). Mean separations were done using LSD at 5% level of probability ($p < 0.05$) which was used in all statistical tests.

RESULTS AND DISCUSSIONS

Physical and chemical properties of the soil before planting in the study area

The analytical data for particle size distribution, soil pH, total N, available P, OC, Fe, Zn, and CEC of study area soil before planting are presented in Table 1.The percentage of clay, silt and sand confirmed that the textural class of the soil is Clay loam.The soil pH was found to be 7.79, indicating that the soil in the study area was moderately alkaline in reaction as per of rating of EthioSIS (2014) for Ethiopian soils. The total N content of the soil in the experimental site was recorded to be 0.24%, which is found in very low range according to Havlin et al. (1999). This calls for additional application of either organic or inorganic nitrogen fertilizer sources to boost the crop yields. The result of this study lines with Eylachew (2000) Mohammed (2003) and Alemu et al (2016) who reported that total N was the deficient nutrient element in soils of Ethiopia and thus called for the application of N fertilizers and the need for sound management of soil Organic Matter (OM). According to Marx (1996), available P in the soil of the experimental area is low (Table 1), which indicated the need for phosphorus application to enhance crop productivity. Exchangeable K in soil of the study area was found to be 2.65 C mol (+) kg^{-1}. According to Landon (1991), the soil of the study area was sufficient in Exchangeable K. Landon (1991) classified soils as very low, low, medium, high and very high in CEC when it is from <5, 5-15, 15-25, 25-40 and >40, respectively. Therefore, the CEC of the soil of experimental area can be grouped under high range (38.5). Considering the organic carbon (OC), Berhanu (1980) reported that soils are high, moderate, low and very low in organic carbon when they are in the range of > 5.2, 2.6-5.2, 0.7-2.6 and < 0.7mg/Kg, respectively. Therefore, the soil of the experimental area has low organic carbon content which calls for application of more organic carbon to enhance its nutrient supply and water holding capacity. As per the categorization of Esu (1991), the available Fe content in soil at high (> 5 $mgkg^{-1}$) and very low (< 1 $mgkg^{-1}$). On the other hand, available Zn in soil at high (5-20 $mgkg^{-1}$) and medium (1-5 $mgkg^{-1}$) range according to Tahere et al,.(2005) classification. In the soil of study area the status of available Fe and Zn failed in very low and medium range, respectively. This indicated that, the existence of Fe nutrient deficiency in the study area (Table 1) that may be the one the yield limiting nutrient. This may be due to high soil pH that can precipitate out Fe.

Table1: Physicochemical characteristics of soils at experimental site prior to treatments application

Parameters	Value
pH	7.79
K(C mol(+)kg^{-1})	2.65
Available P(mg kg^{-1})	17.40
OC (%)	2.50
TN (%)	0.24
Av. Fe (mg kg^{-1})	0.55
Av. Zn (mg kg^{-1})	2.01
Texture (%)	Clay loam
Sand	36.00
Silt	33.00
Clay	31.00

TN: total nitrogen, K: exchangeable potassium, OC: organic carbon, CEC: cation exchange capacity, Av. Fe: available iron and Av. Zn: available zinc.

Effects of Different Nitrogen Fertilizer Source Application on Soil Properties after Harvesting

Soil total nitrogen

There was highly significant (p<0.001) difference in the soil total nitrogen content among the experimental plots which received the different nitrogen sources as fertilizer. Soil total nitrogen was statistically similar in plots treated with dry cyanobacteria and cattle manure biofertilizer (Table 2). Plot which received liquid cyanobacteria biofertilizer were also shown higher soil total nitrogen than plots treated with urea and the control plot. Cyanobacteria can fix about 25 kg N/ ha/ season (Hegazi et al., 2010).This study showed the same result with that of Singh and Bisoyi (1989) who reported that cyanobacteria increases soil fertility by enhancing the available N and P levels**.** Similarly**,** the current results were in accordance with Omar (2001) and (Hegazi et al., 2010) who reported that available nitrogen (N), phosphorus (P) and potassium (K) in the soil significantly increased due to the cyanobacteria inoculation. The current result also agreed on the fact that majorities of cyanobacteria are capable of fixing atmospheric nitrogen and is effectively used as biofertilizers (Vaishampayan et al., 2001).

Available soil phosphorus

Analysis of variance result indicated that the application of different sources of nitrogen fertilizer had highly significant (p<0.001) effect on available phosphorus content of the soil. The highest value of available phosphorous (35.13mg/Kg) was recorded in soil fertilized by dry cyanobacteria biofertilizer followed by liquid cyanobacteria biofertilizer (Table 2). The lowest were obtained from soil treated with no fertilizer source (control) followed by cattle manure. Cyanobacteria is also known to increase soil fertility by enhancing the available N and P levels and exhibited an economical view that it can compensate about 50% of the recommended doses of N, P, K (Mahmoud et al., 2007). According to Hegazi et al., (2010), more extractable phosphates in soils with cyanobacterial cover were observed than in nearby soils without cover indicating that the ability of cyanobacteria to mobilize insoluble forms of inorganic phosphates. Phosphorus is the most limiting nutrients for food production in the sub-humid and humid tropical highlands of East Africa (Sanchez et al., 2000).

Soil organic carbon

There appeared to be highly significant ($p<0.001$) differences in soil organic carbon among soils treated with different fertilizer sources. The highest organic carbon was obtained in soil treated with liquid and dry cyanobacteria biofertilizer (Table 2). Similarly, soil organic carbon recorded from soils treated with urea, cattle manure and with no any fertilizer application (control) was statistically in par. The observed higher concentration of organic carbon in the soil treated with cyanobacteria based fertilizer was in accordance with the finding of Rogers and Burns (1994), who reported further significant increase in organic carbon of the soils when received cyanobacteria as fertilizer.

Soil pH

Results of the analysis of variance indicated that highly significant variation was observed in soil pH due to the different sources of nitrogen fertilizer (Table 2).The highest pH was recorded in soils treated with cattle manure (7.71). The pH values before planting was found to be higher than the pH values obtained after harvest in all the nitrogen fertilizer sources (Table 1 and Table 2). This data was in harmony with (Havlin et al. 2010) who observed that the use of nitrogenous fertilizers cause acidification of soils. In addition, Singh (1961) reported that alkaline soils could be reclaimed by using cyanobacteria biofertilizer that neutralize the pH of these soils. It is well documented that cyanobacteria have the capacity to reclaim soil salinity (Hashem et al., 1995).The reduction in soil pH due to cyanobacteria application could be explained by the ability of cyanobacteria to execrate extracellular compounds, like polysaccharides, peptides, lipids and organic compounds leading to decrease in the soil pH (EL-Ayouy et al., 2004). The result was consistence with the study of Dasappa et al. (2006) who reported a reduction of soil pH from 8.4 to 7.0 in cyanobacteria treated soil. Similarly, Mulat (2013) reported that the soil pH reduced by application cyanobacteria.

Soil Fe and Zn

Different nitrogen fertilizer sources had a significant ($p< 0.001$) effect on available of Fe and Zn contents in the soil. Significantly higher concentrations of soil Fe (4.02 mg kg^{-1}) and Zn (7.35 mg kg^{-1}) were found under dried cyanobacteria biofertilizer followed by liquid cyanobacteria biofertilizer application (Table 2). The least soil available of Fe (0.51 mg kg^{-1}) and Zn (2.04 mg kg^{-1}) values were found from control soils. This might be the cyanobacterial biofertilizer has the ability to dissolve and complex with those ions (Fe and Zn), making them more available in the soil. Cordell (2009) also showed the increment of micronutrients (Fe and Zn) in the soil because of cyanobacteria inoculation. The concentration of Fe and Zn in the soil is affected by higher pH value of the soil (Brady and Weil, 2008). In line with the present finding Mulat (2013) who reported that the application of the cyanobacteria increases the zinc and iron concentration of the soil.

Table 3: Soil Total Nitrogen, Soil Phosphorus, Soil Organic Carbon, Soil Iron, Soil Zinc and Soil pH as Influenced by Different Sources of nitrogen Fertilizer at Ziway Area

Treatments	%TN	%OC	Av. P (mg kg^{-1})	Av. Fe (mg kg^{-1})	Av. Zn (mg kg^{-1})	pH
Control (no N)	0.46^{d}	2.77^{c}	14.03^{e}	0.51^{d}	2.04^{d}	7.75^{a}
Dried Cyano	1.66^{a}	3.73^{a}	35.13a	4.02^{a}	7.35^{a}	6.6^{b}
Liquid Cyano	1.43^{b}	3.51^{a}	23.7b	3.34^{b}	4.44^{b}	6.9^{b}
Urea	1.02^{c}	2.65^{cd}	19.73^{c}	1.38^{c}	2.94^{c}	7.69^{a}
Cattle Manure	1.6^{a}	3.12^{bc}	17.03^{d}	1.33^{cd}	2.24^{cd}	7.71^{a}
LSD (5%)	**0.16**	**0.204**	**0.55**	**0.83**	**1.085**	**0.28**
CV (%)	**6.98**	**4.43**	**1.34**	**17.44**	**11.89**	**2.01**

Conclusion

The soil of the study area is generally low in total nitrogen (N), phosphorus (P), iron (Fe), zinc (Zn) and organic carbon that can limit crop production. Because of these problems, the crop yield is unsatisfactory to meet growing population demand. It is obviously understood that commercial fertilizers have helped to improve yield of crops and address soil fertility problems. However, farmers are unwilling to use sources of inorganic fertilizers because of higher costs. Hence, to maintain soil fertility quality and to increase crop yield use of alternative source of organic fertilizer is important. The use cyanobacteria as biofertilizer is attractive because they act as soil fertility enhancements, when there is insufficient quantity of essential nutrient in the soil for crop growth and development. They can also used as strengtheners, phytostimulators, plant health improvers and have the potential to fix nitrogen fertilizer. The finding of this study suggested that, using cyanobacteria as biofertilizer improves soil quality. Hence, cyanobacteria should be recommended to be used as biofertilizer by substituting or integrated with expensive inorganic fertilizer in the study area. In addition to this, further investigation on cyanobacterial biofertilizer mass production to reach the smallholder farmers to benefit from the rich untapped resource is vital.

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