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Response of Kale (Brassica Oleracea L) Crop to Cyanobacterial Biofertilizer in Ziway Area, Ethiopia

Eshetu Gebre[1] Tulu Degefu[2] Endalkachew Wolde-Meskel[3] Alemu Lelago[4] Jessica Davis[5]
1.Ministry of Environment, Forest and Climate Change, P.O. Box 12760, Addis Ababa, Ethiopia
2.Department of Biology, Hawassa University, P.O. Box 05, Hawassa, Ethiopia
3.International Livestock Research Institute, P.O. Box 5689, Addis Ababa, Ethiopia
4.Department of Chemistry , Wolita Sodo University, P.O. Box 138, Sodo, Ethiopia
5.Department of Plant Science, Colorado State University, USA

Abstract
Gradual soil fertility depletion is resulted in declining agricultural production and productivity generally in Ethiopia. It is obviously understood that commercial fertilizers have helped to improve yield of crops. Use of this inputs among smallholder farmers are very low in the study area due to higher costs, accessibility and crop nutritional quality reduction. This experiment was conducted to evaluate the effect of cyanobacterial biofertilizer on yield and nutritional quality of kale (Brassica Oleracea L.) crop in Ziway. Five treatments: liquid cyanobacterial, dry cyanobacteria, urea, cattle manure and control were laid out in RCBD with three replications. All necessary data from each experimental treatment were collected and subjected to analysis of variance (ANOVA).The results showed that plant height, leaf number and leaf nitrogen have increased when liquid cyanobacterial biofertilizer was applied by 13.97cm, 2.68, and 3.07% over the control, respectively whereas the application of dry cyanobacterial biofertilizer has increased the plant shoot fresh weight, shoot dry weight, leaf area, Leaf phosphorus and plant beta carotene by 341.8 g, 26.8g, ,2089.1 cm, ^{2} 2.43 g, 0.187 mg kg-1 and 0.9 µg/g over the control, respectively. Therefore, the use of cyanobacterial as a biofertilizer should be recommended as an alternative source of inorganic N fertilizer to increase yield and nutritional quality of kale crop for medium and small-sized farms in the study area.
Keywords: Anabaena spp, Biofertilizer, β-carotene content, Cyanobacteria, Kale crop and N-fixing

INTRODUCTION

Soil fertility depletion and nutrient reduction have gradually increased and have become serious threats to agricultural productivity in Ethiopia, particularly in the study area. In most agricultural practices, nitrogen and phosphorus is probably more often deficient than other essential nutrient elements in the soils (EARO, 2001; Fassil and Yamoah, 2009). 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. Despite the fact that Organic wastes and bio-fertilizers are alternative sources to meet the nutrient requirement of crops and to bridge the future gaps. Bio-fertilizer, organic manuring and bio-control have emerged as a promising component of integrating nutrient supply system in agriculture.

Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing crop growth and yield as a natural biofertilizer (Song *et al*., 2005). Cyanobacteria are now regarded to have enormous potential in serving humanity in many ways. Cyanobacteria (Blue-Green Algae) are one of the major components of the nitrogen fixing biomass. The agricultural importance of cyanobacteria in crop cultivation is directly related with their ability to fix atmospheric nitrogen and other positive effects on plants and soil. After water, nitrogen is the second limiting factor for plant growth and development. The use Cyanobacterial as of biofertilizer is a viable alternative to improve crop production and nutritional quality. 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).

Kale (Brassica Oleracea L.) is one of the curly-leafed cabbages, it is considered as one of the most important vegetable crops in many regions of the world. It is planted in many countries for utilizing its edible leaves (vegetative parts) and it is common food in our country. This crop requires a high rate of nitrogen for growth and development. In many countries, farmers applied different type of nitrogen fertilizer to increase kale yield and nutritional quality. Nitrogen is the most important nutrient to increase the production of vegetable crop because of their yield is depends on availability of Nitrogen amount in the soil. 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 crop nutritional quality reduction. 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, fertilizer quality and soil fertility depletion. Among these, soil fertility and fertilizer quality problem is critical. Therefore, this experiment was conducted to investigate the influence of different application methods of Cyanobacteria as biofertilizer on yield and nutritional quality of kale when compare to Urea and Cattle manure.

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 1645m.a.s.l. Its annual rainfall amounts to 450-850 mm and the maximum and minimum temperature of 27 and 16^{o}c, respectively. Ziway is situated 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).

Source and mass production of Cyanobacteria biofertilizer

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 yield and nutritional quality of kale crop 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 100kgN-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 this experiment. All agronomic practices including weeding, harvesting was done as per the recommendation for the kale crop.

Plant Sampling and Analysis

All necessary data from each experimental treatment was recorded. These include, plant height, leaf number, plant growth rate and leaf area, fresh weight, dry weight, The leaves and stem parts were separately dried in an oven at 70^{o}C for 48 hours, until constant weight were achieved. Total leaf area was measured using the leaf area meter at the end of this study after the harvesting process. In addition to these, Samples of kale leaves from each plot were taken at maturity stage. The samples were oven dried until constant weight and grounded (with 1mm sieve) separately for analysis of leaf nitrogen (N) and Phosphorus (P) contents. Total N and P analysis were made by colorimetric and kieldahl method (Nelson and sommers, 1980), respectively. The plant leaf beta carotene concentrations were determined by using spectrophotometer methods at Ethiopian health and nutritional research institute (EHNRI). The *β*-carotene in the sample was extracted according to the method described by Tee *et al*. (1996) with slight modifications. The sample (10 g) was added to 40 ml of 99.8% ethanol and 10 ml of 100% (w/v) potassium hydroxide, and homogenized for 3 min using a blender. The resulting extract was diluted to 10 ml with n-hexane. All samples were carried out in triplicates. The extract was purified by using silica gel.

Statistical Analysis

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

RESULTS AND DISCUSSIONS

Influence of Cyanobacteria Biofertilizer on Kale Production and quality

Plant Height

Results from analysis of variance indicated that significant ($p<0.05$) variations was recorded on plant height of kale due to different sources of nitrogen fertilizer. Comparing to the different sources of the nitrogen fertilizer, liquid Cyanobacteria was resulted in significantly higher plant height of kale by 3.63 cm, 7.23 cm, 9.9 cm and 13.97 cm than dried Cyanobacteria, urea, cattle manure and control, respectively followed by dried Cyanobacteria (Table 1) but statistically at par with that of dried Cyanobacteria . Kale crops that received urea and cattle manure have shown statistically similar height but they have got significantly higher height than the control treatment. Similar to the current results Hegazi et al. (2010) observed that the performance of bean plants, in terms of plant height, number of leaves/plant, leaf area/ plant, fresh and dry weight/ plant, was enhanced by Cyanobacteria biofertilizer application in both two successive seasons. Besides this, Nanjappan-Karthikeyan et al. (2007) stated that Cyanobacteria biofertilizer have growth promoting activity as inoculants of wheat. The shortest kale plants were obtained in the control treatment indicating that soil of the experimental area requires additional nitrogen fertilizer sources. However, the kale were best responded to application of liquid cyanobacteria followed by dry Cyanobacteria fertilizer sources. Perhaps this could be as results of accelerated nitrogen fixation by the help of bacterial activity and better root system that in turn help the kale plant to effectively take up essential nutrients and water from the soil.

Shoot fresh weight

The shoot fresh weight of Kale was significantly ($p<0.05$) affected by different nitrogen sources fertilizer. The highest shoot fresh weight (807.6g) was recorded on Kale crop treated with dried Cyanobacteria biofertilizer but it was only shown remarkable difference as compared to control (465.8g) otherwise it was statistically the same with the other treatments(Table 1).The significant improvement in shoot fresh weight with the application of dry cyanobacterial biofertilizer was as a result of the ability of cyanobacteria biofertilizer to raise the fertility status of the soil thereby enhancing vegetative growth (Cordell, 2009). In line with current data Brady and Weil (2002) observed that pretreatment of *Viciafaba* seeds with the extract of *Anabaena variables* induced an increase in germination percentage, root growth and seedling dry weight as compared with untreated seeds. In agreement with the result of this study, Jagannathet al. (2002) reported that the effect of blue green algae as potent biofertilizer on chickpea and they found that it enhanced all the morphological characters and biomass of the chickpea. Hegazi et al. (2010) reported that increases performance of bean attributed to the nitrogenase as well as nitrate reductase activities of the alga associated with the surface of plants; or the amino acids and peptides produced in the algal filtrate and/or other compounds that stimulate growth of crop plants.

Number of leaves

Number of leafs were significantly influenced ($p<0.001$) by application of different nitrogen fertilizer sources (Table 1). The highest leaf numbers was recorded from the application of liquid cyanobacteria (9.8) followed by plants treated with dried cyanobacteria (9.5) and the small numbers of leafs (7.11) was recorded from unfertilized plot; the rest of N sources performed between the two. But no significance difference between the dried cyanobacteria and the liquid cyanobacteria biofertilizer application and no significance difference between the application of cattle manure and control were observed. The increasing of the kale leaf number by application of cyanobacteria biofertilizer might be due to cyanobacteria produce the bioactive compounds for leaf formation. Mulat (2013) reported that the application of the dry cyanobacteria on kale (*Brassica carinata L.*) grown on Andosolsand Alfisolshad increased the leaf number of the plant, which is in agreement with our findings. Similarly, Makiso (2013) reported that the application of dried cyanobacteria increased the leaf number of the pepper over the control in Ziway and Yirgalem soil. Also, Metting and Pyne (1986) reported that cyanobacteria produce a variety of bioactive compounds including growth phytoregulators that could be used in the in vitro production of vegetables, fruits fungi and ornamental flowers.

Leaf area

Results from the analysis of variance indicated that leaf area was significantly ($p<0.05$) affected by different sources of nitrogen fertilizers. The highest leaf area (5801.9$cm^{2)}$ was recorded in kale plants treated with dry cyanobacteria followed by plants treated with liquid cyanobacteria (4939.4cm^2) (Table 1). The lowest leaf area was obtained in control treatment. The increases in leaf area of the kale crop might be due to cyanobacteria supply plant nutrients, improvement of soil environment and biological processes of the soil (Hashem, 2001). Moreover, Amal et al. (2010**)** reported that the performance of bean plants, in terms of leaf area per plant was enhanced by cyanobacteria biofertilizer application as compared to the control treatment. During co-cultivation of cyanobacteria and plants, the growth of plants and an increase nitrogen fixation may be boosted (Rai, 2000), but at the same time plants may benefit from the presence of cyanobacteria biofertilizer by using the nitrogenous compounds synthesized during nitrogen fixation, which are either liberated during growth or made available to the crop on autolysis of the cyanobacteria.

Shoot dry weight

The result of this study showed that there was significant ($p<0.05$) difference in shoot dry weight among kale crops treated with different fertilizer sources. The highest shoot dry weight of kale (69.92g) was recorded by application of the dried cyanobacteria. Based on the results shown in (Table 1), only the control treatment (kale crop which did not receive any of the fertilizer sources) got statistically the lowest shoot dry weight. Glass (1989) reported that increased fresh and dry weights can be explained by the increase in nutrient uptake and growth enhancement induced by algal biofertilizer applied to the soil and the highest value of dry shoot weight of tomato seedlings resulted from application cyanobacteria *Anabaena spp*. Similarly, Brady (2002) also reported that application of cyanobacteria biofertilizers to tomato plants increased the different vegetative growth parameters measured and mineral accumulation in the roots and shoots.

The result of the present study also support the finding of Amal *et al.* (2010) who reported significant increase in shoot dry weight of common bean by the application of dried cyanobacterial biofertilizer because of its fast decomposition. Similarly, Mulat (2013) reported that the application of dried cyanobacteria biofertilizer increased the shoot dry weight of kale crop over the control in Ziway and Yirgalem soil.

Table 1**:** shoot fresh weight**,** Shoot dry weight, leaf area, leaf number and plant height of kale crops as influenced by different sources of nitrogen fertilizer at Ziway area

Treatments	LN	SFW (g)	SDW(g)	LA(Cm^2)	PH (cm)
Control	7.11^c	465.8^b	43.12^b	3712.8^c	49.76^b
Cyanodry	9.5^a	807.6^a	69.92^a	5801.9^a	61.1^a
Cynoliquid	9.79^a	719.7^a	63.78^a	4939.4^{ab}	63.73^a
Urea	8.4^b	796.5^a	65.16^a	4389.3^b	56.5^{ab}
Cattle manure	7.26^c	631.8^{ab}	60.30^{ab}	3981.8^{bc}	53.83^{ab}
$LSD_{(0.05)}$	0.437	233.71	20.59	1188.8	3.08
$CV_{(\%)}$	2.88	18.14	18.09	14.31	2.89

LN= Number of leaves SFW= Shoot fresh weight, SD W= Shoot dry weight PH=plant height, LA= Leave area

Means followed by different letters within a column are significantly different at 5% probability level

Beta carotene content

Beta carotene were significantly ($p<0.05$) affected by different source of N fertilizer (Table 2). Analysis of variance indicated that the highest β-carotene content was recorded on Kale plant treated with dry cyanobacteria biofertilizer but not significantly different from those treated with liquid cyanobacteria biofertilizer. Statistically, β-carotene content obtained from kale plant treated with other fertilizers including urea, cattle manure was similar with β-carotene content of the control plant. This is due to cyanobacteria have their potential to produce a diverse range of chemicals and biologically active compounds, such as vitamins, carotenoid pigments, proteins, lipids and polysaccharides . Similarly, Muluneh (2013) reported that application of cyanobacteria biofertilizers increased the lettuce β-carotene content concentration. The significant difference of beta carotene content observed in this experiment was in agreement with finding reported earlier.

Plant Leaf Nitrogen

In order to compare the effects of the different sources of nitrogen fertilizer on the leaf nitrogen content of kale crop, the leaf nitrogen content of the plant was measured and presented in (Table 2). The result showed that, significantly higher concentration of Nitrogen (9.10%) was obtained with Kale crops fertilized with liquid cyanobacteria biofertilizer followed by dried cyanobacteria biofertilizer. But, the lowest nitrogen content of leaf was shown with control treatment followed by cattle manure. Urea was resulted in lower leaf N content but it was significantly higher than the value of N content found in leaf of kale crop treated with cattle manure and control (kale which didn't receive any of the N sources) (Table 2). In accordance to the current result, Alla *et al.* (1993**)** reported that the significant increase in plant nitrogen under liquid and/or dried inoculate (dried) of cyanobacteria biofertilizer and suggested that it could be attributed to nitrogenase activity (nitrogen fixation) in the soil. In line with the resent finding Ranal *et al*. (2012) reported that application of cyanobacteria biofertilizer increased the N and P concentration of wheat grain by 23.8% and 5.7 %, respectively over the control. Similarly, Girma (2013) reported that application of cyanobacteria biofertilizers increased the maize N concentration. In addition, the increase % N and P content of wheat, barley and common bean crops due to cyanobacteria application has been reported earlier by other worker (Aref *et al*., 2009). Moreover, Mulat (2013) reported that use of dried cyanobacteria biofertilizer increased leaf N and P contents of kale crop by 6.47 and 0.58%, respectively. This might be, due to the availability of N and P nutrients in the soil.

Plant phosphorous

Significant ($p \leq 0.001$) difference in available phosphorous contents in kale crop leaf was observed due to application of different N fertilizer sources (Table 2)). The highest available phosphorous mean (0.577 mg kg^{-1}) was recorded for kale plant treated with dry cyanobacteria biofertilizer application followed by liquid cyanobacteria (0.494 mg kg^{-1}). The lowest available P content (0.39 mg kg^{-1}) was obtained from kale plant grown on unfertilized plot. However, there were no significance difference as such on the amount of available phosphorus obtained from kale plants that received urea and the control. In conformity with the results obtained from this study, availability of P in soil was also increased due to the use of dry cyanobacteria over the control. These findings were observed by Strik and Staden (2003) who explained that incorporation of fresh or dry *Azolla* into soil increased significantly the soil organic matter and N, which in turn upon its decomposition by the soil microorganisms had released the macro and micronutrients into soil, leading to increase the availability of P and K in soil. Also, under salt stress condition, cyanobacteria added to the soil either as free living and/or as *Azolla* symbiont lead to add to the soil organic matter, which is consequently increased the soil biological activity in terms of increasing the soil total bacterial count, CO_2 evolution, dehydrogenase activity and nitrogenase activity (Singh *et al*., 2008). In addition, cyanobacteria may be capable of fixing elemental nitrogen to ammonia and solubilizing insoluble phosphate reserves of soils and thus, these organisms were capable of providing N and P to the crop (Brady and Weil, 2008).

Table 2: Beta carotene content, leaf nitrogen and leaf phosphorous content of kale plants as influenced by different sources of nitrogen fertilizer at Ziway area

Treatments	β-carotene content *μg/g*	Leaf N %	Leaf p *(mg kg-^{1})*
Control	2.17^{b}	6.03^{e}	0.39^{d}
Cyanodry	3.07^{a}	8.67^{b}	0.577^{a}
Cynoliquid	2.48^{ab}	9.10^{a}	0.494^{b}
Urea	2.03^{b}	7.37^{c}	0.43^{cd}
CM	2.21^{b}	6.53^{d}	0.453^{bc}
LSD $_{(5\%)}$	0.65	0.38	0.062
CV $_{(\%)}$	14.34	2.66	7.042

Conclusion

Soil fertility depletion gradually increased and agricultural productivity is decline generally in Ethiopia. It is obviously understood that commercial fertilizers have helped to improve yield of crops. Use of this inputs among smallholder farmers are very low in the study area due to high costs, accessibility and crop nutritional quality reduction. Hence, to maintain soil fertility, to increase crop yield and quality use of alternative source of nitrogen fertilizer is important. The use Cyanobacterial as of biofertilizer is a viable alternative for soil fertility improvement to enhance crop production and nutritional quality. The finding of this study suggested that using cyanobacteria as biofertilizer improved yield and nutritional quality of kale crop by increasing beta carotene. By using cyanobacteria based biofertilizer, the hidden hunger resulting from lack of vitamins in food could be alleviated. Finally, cyanobacteria should be recommended to be used as biofertilizer by substituting costly inorganic nitrogen fertilizer in study area. It is important to conduct the research on the Cyanobacterial biofertilizer mass production to reach the smallholder farmers.

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