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Comparison of cyanobacterial bio-fertilizer with urea on three crops and two soils of Ethiopia

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Although chemical fertilizers have long been used to meet the high demand of nitrogen (N), the most common limiting nutrient to plant growth, the frequent use of this fertilizer gradually deteriorates soil fertility in addition to its high cost, lower supply and agronomic use efficiency in Ethiopia. Nevertheless, N-fixing cyanobacterial biofertilizers are eco-friendly, and currently considered important to support the developing organic agriculture. Therefore, this study was conducted to evaluate the potential of cyanobacterial biofertilizer for the growth and yield of three commonly growing crops in Ethiopia: maize, kale, and pepper under Alfisol and Andosol, and to investigate the potential contribution of cyanobacteria biofertilizer in selected soil fertility parameters. Three independent factorial experiments were conducted simultaneously in the greenhouse. Each experiment included a factorial combination of four nitrogen fertilizer sources applied at recommendation rate for each crop (control, urea, dried cyanobacteria, and liquid cyanobacteria,) and two soil types with acidic and alkaline pH (Alfisols and Andosols, respectively) arranged in a complete randomized design (CRD) with three replications. Application of dried and liquid cyanobacterial biofertilizer treatments significantly improves the soil N, soil organic carbon (SOC) and available P, Fe and Zn. Cyanobacteria treatments were also found to reduce or maintain the mean soil pH. Accordingly, maximum values of all the vegetative growth attributes of kale, and maize were obtained from the application of two comparable-fertilizer treatments: air-dried cyanobacteria and urea while for pepper crops only dried cyanobacteria. Concentrations of N, P, Zn, and Fe in leaves of kale, pepper, and maize were also significantly increased by air-dried cyanobacterial biofertilizer. Overall, dried cyanobacteria improved the growth and yield of the three crops, and the fertility of the soils. Therefore, the use of dry cyanobacterial biofertilizer could be recommended as a supplementary N source to inorganic fertilizer for kale, pepper and maize production in both study sites.

Key words: Alfisols, andosols, biofertilizers, cyanobacteria, N-fixing.

INTRODUCTION

Nutrient depletion is one of the major causes that contribute to decline in soil productivity in Ethiopia; soils

under subsistence agriculture have been mined of nutrients for years without replenishment with fertilizer inputs in the country. Hence, the two essential plant nutrients, N and P are the most limiting nutrients nearly in all agricultural soils of Ethiopia (Paulos, 2001; Wassie et al., 2006). On average, N and P depletion rates in Ethiopian soil exceed 40 and 6 kg ha^{-1} yr^{-1}, respectively (Stoorvogel and Smaling, 1990; Smaling et al., 1997).

One way of improving soil fertility is the use of inorganic fertilizers. Adugna and Hiruy (1988) reported that majority of Ethiopian soils gave high response to applied nitrogen. Nevertheless, the use of this input among smallholder farmers is currently very low in the country. High fertilizer costs, marketing problems and poor infrastructures are some of the major reasons for low use of fertilizers in Ethiopia (Schneider and Anderson, 2010; Girma et al., 2016). Moreover, synthetic N fertilizers have lower agronomic use efficiency due to losses of applied N through volatilization, leaching and denitrification (Havlin et al., 2010); as a result, higher amount of chemical N fertilizers is applied to meet the crop demand. Excess use of chemical fertilizers may result in multi-nutrient deficiencies and nutrient imbalance in soil. Furthermore, it also generates several environmental problems including acidification of water (Choudhury and Kennedy, 2005). Therefore the use of other alternative options of soil fertility replenishment is indispensable to maintain soil fertility and productivity (Girma et al., 2016; Wassie et al*.,* 2006).

Biofertilizers, being essential components of organic farming, play key role in maintaining long term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micronutrients or converting insoluble phosphate present in the soil into forms available to plants, thereby increasing their use efficiency and availability (Sahu et al*.,* 2012). They are cost effective, ecofriendly and a renewable source of plant nutrients to supplement chemical fertilizers (Aref et al*.*, 2009). Thus, the possibility of using biofertilizers as an alternative or a complementary for mineral fertilization has been the focus of researchers (Prasanna et al*.,* 2012). Cyanobacteria as a biofertilizer can decrease the demand for mineral form of N fertilizers. They are photosynthetic prokaryotic microorganisms capable of fixing atmospheric N_2 using sunlight as the sole energy source. They are free-living as well as symbiotic. Some filamentous cyanobacteria exhibit cellular differentiation to produce heterocysts; highly specialized cells that fix atmospheric nitrogen (Hegazi et al*.,* 2010; Kulasooriya, 2011). The dominant nitrogen fixing cyanobacteria are *Anabaena, Nostoc, Aulosira, Calothrix* and *Plectonema* (Sahu et al*.,* 2012).

Cyanobacteria have been widely employed as inoculants for enhancing soil fertility and improving soil structure in addition to enhancing crop yield. They are a cheap source of N, which does not cause pollution and quite suitable for resource poor smallholder farmers (Kulasooriya, 2011). Beneficial effects of cyanobacteria inoculation were reported on rice, barley, oats, tomato, radish, cotton, sugarcane, chilli and lettuce (Thajuddin and Subramanian, 2005). In a similar scenario, Prasanna et al*.* (2009) reported that inoculation of *Calothrix* as a biofertilizer shows an increase of 21% in grain yield of rice over the recommended NPK. In addition, the possibility of reducing Fe and Zn malnutrition in developing countries through cyanobacteria biofertilizer has been reported (Rana et al*.,* 2012). Cyanobacteria also change the physical, chemical and biological properties of the soil. Inoculation of soil with *Nostoc muscorum* led to a pronounced effect on soil chemical properties, with total carbon increasing by 56 % and total N increasing by 120% of the initial (Rogers and Burns, 1994). Many cyanobacteria have also been shown to mobilize the insoluble phosphate in the soil, thereby increasing their availability to the crop plants, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH and increase the efficiency of fertilizer use in crop plants (Kaushik, 2004). Besides, many researchers demonstrated increase in availability of Fe and Zn content of the soil through cyanobacteria biofertilization (Belnap and Harper, 1995; Puste and Das, 2002).

Despite the fact that many experiments had been conducted on cyanobacteria biofertilizer, research findings were contradicting each other when it comes to the method of application. Moreover, scanty information was available in Ethiopia on the potential of this biofertilizer in the growth of maize, kale and pepper, under Alfisols and Andosols soils which represent the major soil portion in the study areas. The objectives of the present study were; therefore, to study the effect of cyanobacterial biofertilizer application on growth and yields of three commonly growing crops in the study areas: maize, kale and pepper and to investigate the potential contribution of cyanobacteria biofertilizer to selected soil fertility parameters. The expected result could identify the best cyanobacterial bio-fertilizer management practices for kale, maize and pepper growth and for soil fertility improvement at greenhouse level.

MATERIALS AND METHODS

Three independent experiments were carried out simultaneously on

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Table 1. Plant varieties, fertilizer application rates, and harvest age for greenhouse experiments on kale, hot pepper, and maize.

Variety	Kale	Hot pepper	Maize
	Yellow Dodolla	MarekoFana	Gibe II
N Rate (kg N/ha)	100	100	64
P Rate (kg P/ha)	30	40	20
Harvest Age (d)	50	58	45

Table 2. Physico-chemical characteristics of the surface of Alfisols and Andosols prior to treatment application.

Soil characteristics	Alfisols	Andosols
Textural class	Clay	Clay Loam
Organic carbon (%)	1.7	2.4
Cation Exchange Capacity (cmol(+) kg^{-1})	21.5	40.4
Field Capacity (%)	38	30
Permanent Wilting Point (%)	30	18
pH in water (1:2.5)	5.8	8.1
EC (mS cm^{-1})	0.11	0.27
Total N (%)	0.19	0.22
Av. P (mg kg^{-1})	8.0	15.0
Exch. K (cmol(+) kg^{-1})	1.3	2.1
Av. Zn (mg kg^{-1})	6.2	2.4
Av. Fe (mg kg^{-1})	10.7	1.6

kale (*Brassica carinata* L.), hot pepper (*Capsicum annuum* L.), and maize (*Zea mays* L.) in a greenhouse at Hawassa University, Hawassa, Ethiopia (Table 1). Each experiment included a factorial combination of four nitrogen fertilizer sources applied at recommended rate for each crop (control, urea, dried cyanobacteria, and liquid cyanobacteria,) and two soil types (Alfisols and Andosols) arranged in a complete randomized design (CRD) with three replications. The soils samples were collected from Ziway and Yirgalem Southern part of Ethiopia. The coordinates of the two locations were 07° 58' 6.7" N and 38° 23' 20.9" E and 06° 44' 57.5" N and 38° 23' 26" E, respectively. The soils at Ziway and Yirgalem area represented a tropical Andosols (pH 8.0) and typical tropical Alfisols (pH 5.7), respectively (Girma et al*.,* 2012).

Soils were collected from a depth of 0 to 20 cm, air dried, and sieved to pass through a 5 mm sieve. Triple super phosphate (TSP) was mixed with soil prior to sowing at recommended rates for each crop. Pots were 20 cm in diameter and 18 cm deep, and each pot was filled with 4 kg soil (12 cm deep). Five seeds were sown per pot and thinned to two plants after establishment. Weeds were removed weekly, and pots were watered up to field capacity every other day. Following each watering, any leachate captured on saucers was reapplied to the pots.

Soil samples were air dried and ground to pass through a 2 mm sieve for all analyses except soil organic carbon (SOC) and total nitrogen (TN), for which soil samples were further passed through a 0.5 mm sieve. The soil samples were analyzed for soil texture by the hydrometer method (Bouyoucos, 1962), OC by dichromate oxidation (Walkley and Black, 1934), cation exchange capacity (CEC) by the 1M ammonium acetate method at pH 7 (Chapman, 1965), moisture content at field capacity (-1/3 bar) and permanent wilting point (-15 bars) using pressure plate extraction (Klute, 1965), pH in a soil: water ratio of 1:2.5 (Van Reeuwijk, 1992), electrical conductivity (EC) in a 1:2.5 soil:water ratio soaked for one hour (Sertsu and Bekele, 2000), N by the micro Kjeldahl method (Bremner and Mulvaney, 1982), available P extracted with $NaHCO_3$ (Olsen et al.,1954), exchangeable K by NH_4OAc extraction (Chapman, 1965), and available Zn and Fe by DTPA extraction (Lindsay and Norvell, 1978) (Table 2).

Anabaena sp. strain E-3 was cultured from local soil samples using Allen-Arnon media (Allen and Arnon, 1955) and grown under cool white fluorescent lights (2500 lux) with bi-weekly transfers. Then large quantities were grown in Allen-Arnon media in aerated 1 × 2 m ponds with a 0.2 m depth (filled to 0.15 m and lined with transparent polyethylene sheeting) under plastic (transparent plastic painted white) inside a hoop house on the Hawassa University. The ponds were seeded with 20 L of Allen-Arnon based *Anabaena* sp. strain E-3 and filled up to 300 L with Allen-Arnon media. The daytime air temperature in the hoop house ranged from 27 to 38°C, and light intensity ranged from 5700 to 7700 lux. Cyanobacteria were harvested after 21 d of growth and utilized as either liquid (42 mg N L^{-1}) or air-dried (3.0% N) fertilizers.

All fertilizers were applied at the same N rates within each study based on crop-specific N recommendations from the government of Ethiopia (Table 1). An air-dried and ground to pass through a 2 mm sieve cyanobacterialbiomass was incorporated into pots nominated for this application one week prior to seed sowing for kale and hot pepper and 15 days before sowing for maize, and water was appliedup to field capacity to allow time for decomposition. The liquid cyanobacterial culture was split into three applications (the first third one to two weeksprior to sowing, and other splits thereafter in seven to 10 d intervals) to avoid overwatering and was uniformly poured into each pot. Other treatments received equal amounts of water in order to keep this variable constant across

treatments. Urea was applied prior to sowing for maize and half prior to sowing and half 20 d later for hot pepper and kale.

Measurements

Plant parameters were measured at end of the experiments (Table 2). Plant height was measured as the length from the soil surface to the apical bud of kale, to the uppermost growth of pepper (at the blooming stage) and maize (at the end of the experiment). The average height of the two plants in each pot was taken on the day of harvest. The leaf number was counted on the harvest date for kale and maize, and the number of primary branches was counted for hot pepper. The total leaf area was recorded from each plant using a leaf area meter (Li-cor 3100), and average per plant was calculated for each pot. Plant biomass was harvested, and root and shoot parts were separated and dried in an oven at 60 to 70°C for 48 h or more to a constant weight, and final weights were recorded.

Leaf samples were taken at harvest (all leaves for kale and maize, and most recently matured leaves of hot pepper), oven dried as described above and ground. N was analyzed by modified Kjeldahl procedure (Nelson, 1980). One gram of plant material (dried at 105°C for 24 h) was calculated in a muffle furnace at 450°C, dissolved in 20% nitric acid, and filtered. Extracts were analyzed for P, Zn, and Fe contents by colorimetric analysis (Wolf, 1982), andatomic absorption spectroscopy (Isaac and Kerber, 1971), respectively. After harvest, soil samples were taken from the entire 12 cm soil depth, air-dried, sieved, and analyzed for pH, SOC, Kjeldahl N, and available P, Zn, and Fe following standard laboratory procedures.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using general linear models (Proc GLM) of the statistical analysis system (SAS Institute, 2003). Whenever significant differences were detected in the F- test, the means were compared using the least significant difference (LSD) test at the 5% significance level. Correlation analysis was conducted between relevant parameters using Pearson's correlation test.

RESULTS AND DISCUSSION

Soil properties

Cyanobacterial bio-fertilizer reduced soil pH in all three trials (Table 3). This might be due to the fact that, as the cyanobacteria decompose, they release organic acids, and the nitrification process also releases H^+ ions, thus leading to the reduction in soil pH. The urea treatment was moderate in pH between the control and cyanobacteria treatments. Interestingly, in the maize study there was a significant interaction between fertilizer source and soil type for soil pH, SOC, and available P (Table 4). The pH reduction was 0.5 units in the alkaline soil (Andosols) but only 0.2 units in the acidic soil (Alfisols), resulting in soil pH levels of 7.5 and 5.5, respectively. The result was in agreement with the finding of Dasappa et al. (2004) who reported a reduction of soil pH from 8.4 to 7.0 in cyanobacteria treated pots on Mulberry. This was also in conformity with the study of Amal et al. (2010) who found that soil pH was slightly decreased by inoculation with cyanobacteria in the first season, while, the second season revealed a significant reduction in these parameters particularly when the combined application of seed coating and soil drench was applied with 75% N.

The cyanobacterial bio-fertilizer treatments, applied in either dry or liquid form, consistently increased SOC, which was expected (Table 4). The urea also affected SOC, although the impact was inconsistent, decreasing it in the kale experiment, increasing it in maize, and having no effect in pepper. This could be due to carbon fixation capacity of cyanobacteria as they are photoautotrophic in nature. The observed increase in soil organic carbon was comparable with the finding of Maqubela et al. (2009). The observed increases in soil SOC were comparable to those reported by Dasappa (2004) and Christopher et al. (2009) in a similar study.

Although urea and the cyanobacterial bio-fertilizer treatments were applied at the same N rate, the total N concentration in the soil was higher at the end of the experiments in the cyanobacterial treatments as compared to urea, with the exception of the liquid cyanobacteria applied to hot pepper (Table 3). This could be due to higher volatilization of NH_3 and N_2O from urea or increased N fixation by the cyanobacteria; however, continued N fixation was doubtful in the dry cyanobacterial treatment due to the drying and grinding process that was used to prepare this fertilizer. Christopher et al. (2009) forwarded the reason for higher concentration of total N in the soil after cyanobacteria inoculation that the slow release and lower lose of nutrients in the case of application of this biofertilizer. The increase in total soil N due to the applied nitrogen-fixing biofertilizer was also noted by Kemka et al. (2007).

In addition, soil available P, Zn, and Fe were all increased in the cyanobacterial bio-fertilizer treatments, while the urea treatment was equivalent to the control (Table 3). This is not surprising since P, Zn, and Fe are all supplied in the Allen-Arnon media to optimize the growth of the cyanobacteria. In the maize study, the impact on available P was only significant in the alkaline soil (Andosols) (Table 4). The possible reason for this is that cyanobacterial biofertilizer has the ability to dissolve and complex with those ions (Fe and Zn), making them more available in the soil (Kemka et al., 2007). Similarly, Aref et al. (2009) and Hegazi et al. (2010) also reported a significant increase in P availability of alkaline soil due to the application of cyanobacteria biofertilizers.

Plant growth parameters

The dry cyanobacteria application resulted in the greatest plant height and shoot dry weight for all three plant species tested (Table 5). The plant height and shoot weight in the urea treatment was equivalent to that of the dry cyanobacteria treatment in kale and maize, but the

Table 3. Impact of N fertilizer sources on soil pH, OC, Kjeldahl N, and available P, Zn, and Fe concentrations.

Fertilizer source	pH	OC	N	Available P	Available Zn	Available Fe
		--%--	--%--	--mg kg^{-1}--	--mg kg^{-1}--	--mg kg^{-1}--
				Kale		
Control	7.0^{a†}	2.2^{b}	0.15^{c}	29.7^{c}	5.8^{c}	2.8^{c}
Urea	6.8^{ab}	2.1^{c}	0.23^{b}	24.2^{c}	5.9^{c}	2.4^{c}
Dry Cyanobacteria	6.4^{c}	2.4^{a}	0.27^{a}	54.4^{a}	10.2^{a}	5.8^{a}
Liquid Cyanobacteria	6.5^{bc}	2.4^{a}	0.26^{a}	41.0^{a}	7.2^{b}	4.5^{b}
				Hot pepper		
Control	7.1^{a}	2.0^{c}	0.15^{c}	30.9^{c}	3.9^{c}	3.1^{b}
Urea	6.9^{ab}	1.9^{c}	0.25^{b}	29.0^{c}	3.8^{c}	2.8^{b}
Dry Cyanobacteria	6.6^{c}	5.1^{a}	0.33^{a}	74.4^{a}	8.7^{a}	6.4^{a}
Liquid Cyanobacteria	6.8^{b}	3.7^{b}	0.27^{b}	66.1^{b}	7.5^{b}	5.3^{a}
				Maize		
Control	6.8^{a}	2.3^{d}	0.20^{c}	8.7^{c}	4.8^{b}	10.8^{b}
Urea	6.7^{b}	2.5^{c}	0.22^{b}	8.8^{c}	4.8^{b}	11.1^{b}
Dry Cyanobacteria	6.5^{c}	2.8^{b}	0.24^{a}	11.0^{b}	6.1^{a}	12.2^{a}
Liquid Cyanobacteria	6.5^{c}	3.0^{a}	0.24^{a}	11.9^{a}	6.3^{a}	11.9^{a}

†Means followed by a common letter within crop and nutrient are not significantly different based on Least Significant Differences at p≤0.05.

Table 4. Interactions between fertilizer source and soil type in soil pH, OC, and available P in a maize greenhouse study.

Fertilizer Source	pH		OC		Available P	
Soil Type	**Alfisols**	**Andosols**	**Alfisols**	**Andosols**	**Alfisols**	**Andosols**
			----%----		----mg kg^{-1}----	
Control	5.7^{a†}	8.0^{a}	2.2^{d}	2.4^{c}	2.3^{a}	15.0^{c}
Urea	5.6^{ab}	7.8^{a}	2.3^{c}	2.6^{b}	2.5^{a}	15.1^{c}
Dry Cyanobacteria	5.5^{ab}	7.5^{b}	2.7^{b}	3.0^{a}	2.7^{a}	19.2^{b}
Liquid Cyanobacteria	5.5^{b}	7.5^{b}	2.9^{a}	3.0^{a}	3.0^{a}	20.9^{a}

†Means followed by a common letter within column are not significantly different based on Least Significant Differences at p≤0.05.

liquid cyanobacteria resulted in shorter plants with less mass (although they were greater than the control). The root dry weights showed a similar pattern to shoot dry weight in kale and pepper, but in maize, the urea and cyanobacterial treatments were not different in root dry weight. These results are in agreement with Amal et al. (2010) who found out that the morphological characters and performance of bean in terms of plant height, was enhanced by cyanobacteria application. Similarly, Bhuvaneshwari et al. (2011) reported that cyanospray applied, 0.5% cyanospray treated plants showed better results on all the morphological parameters such plant height and dry weight of shoot.

The leaf number and area were also significantly impacted by the fertilizer treatments (Table 5). In maize and pepper, the dry cyanobacteria resulted in the greatest leaf number and branch number, respectively, and urea and liquid cyanobacteria were intermediate between the control and dry cyanobacterial treatments. In kale, the leaf number in the urea treatment was equivalent to that of the dry cyanobacteria. The leaf area was consistently highest in the dry cyanobacterial treatment, with urea having an equivalent leaf area in kale and maize, but a significantly lower leaf area in pepper. The more leaf number and area per plant in kale and maize obtained in treatments receiving cyanobacteria was probably due to its better capacity to supply N and other nutrients to the plant during its growth (Mahmoud et al., 2007). This result was consistent with the finding of Amal et al. (2010) who reported the significant increase in

Table 5. Impact of N fertilizer sources on plant growth characteristics of kale, hot pepper, and maize.

Fertilizer source	Plant height (cm)	Leaf number†	Leaf area (cm^2 $plant^{-1}$)	Shoot dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)
			Kale		
Control	29.2[c‡]	9.2[c]	261[c]	2.0[c]	0.4[c]
Urea	38.5[a]	12.8[a]	629[a]	4.3[a]	0.9[a]
Dry Cyanobacteria	40.0[a]	12.3[a]	648[a]	4.4[a]	1.0[a]
Liquid Cyanobacteria	34.9[b]	11.4[b]	388[b]	3.2[b]	0.6[b]
			Hot pepper		
Control	13.3[c]	2.8[c]	161[c]	5.1[c]	0.9[c]
Urea	18.0[b]	5.5[b]	327[b]	8.3[b]	2.0[b]
Dry Cyanobacteria	22.7[a]	8.9[a]	405[a]	14.0[a]	3.0[a]
Liquid Cyanobacteria	18.8[b]	5.8[b]	332[b]	9.5[b]	2.2[b]
			Maize		
Control	32.4[c]	3.8[c]	269[c]	1.2[c]	0.9[b]
Urea	41.5[ab]	5.1[b]	526[a]	2.6[a]	1.6[a]
Dry Cyanobacteria	45.0[a]	5.8[a]	540[a]	2.8[a]	1.5[a]
Liquid Cyanobacteria	39.9[b]	5.0[b]	408[b]	2.3[b]	1.6[a]

†Branch number is reported for hot pepper.
‡Means followed by a common letter within crop and nutrient are not significantly different based on Least Significant Differences at $p \leq 0.05$.

number of leaves and area of common bean by the application of dried and fresh cyanobacterial bio-fertilizer. In addition, Bhuvaneshwari et al. (2011) find out that incorporation of Cyanobacterial Bio-fertilizer increased number of leaves and area of Sunflower. Krishna et al. (2012) also revealed that cyanobacterial bio-fertilizer of cyanopith and cyanospray applications have significantly increased the leaf width of *Aloe vera* crop when compared to control.

In general, the plants receiving dry cyanobacteria grew better than those fertilized with liquid cyanobacteria. This may be due to the drying and grinding process resulting in quicker N mineralization from the dry cyanobacteria. In the liquid cyanobacterial treatments, green growth was visible around the pot edges, demonstrating that the liquid cyanobacterial fertilizer did not completely die and release its nutrients for plant uptake, but continued to live throughout the experimental duration. The dry cyanobacterial bio-fertilizer, generally, increased plant growth compared to urea, as well, even though they were applied at the same N rate. This may be due to the presence of other plant nutrients in the cyanobacterial bio-fertilizer. We had applied TSP at equivalent rates across the fertilizer treatments to avoid interference due to differential P levels, and the soils did not seem to require any additional nutrients (Table 2). Alternatively, this difference could be due to higher N volatilization losses from the urea treatment; unfortunately, this parameter was not measured.

Plant nutrient concentrations

All fertilizer treatments increased plant N concentrations as compared to the control (Table 6). The dry cyanobacterial bio-fertilizer resulted in the highest plant N concentrations in all three crops, although urea resulted in equivalent plant N levels in kale and maize. Nitrogen concentrations in plants receiving liquid cyanobacteria were higher than control but usually lower than urea.

Plant N was highly significantly ($p<0.001$) correlated with soil N in kale and hot pepper, but this correlation was not significant in maize. Both soil N and plant N concentrations were significantly correlated with plant height, leaf number, leaf area, shoot dry weight, and root dry weight for all three crops (Table 7). In general, the correlations were stronger with plant N than with soil N. Both the dry and liquid cyanobacterial bio-fertilizers increased plant P, Zn, and Fe concentrations in kale, pepper, and maize (Table 6). This could be due partly to the presence of these nutrients in the Allen-Arnon nutrient media used to produce the cyanobacterial bio-fertilizer. Moreover, cyanobacteria are known to increase the availability of P in the rhizosphere, which facilitate its transport to the root and provide P to the crop, and consequently increase tissue P concentration (Prasanna et al., 2012). Dried cyanobacteria increased N status of crops over the control; this may positively influence the mobility and root uptake of Zn and Fe from the soil (Cakmak et al., 2010). The expression level of Zn and Fe

Table 6. Impact of N fertilizer sources on N, P, Zn, and Fe concentrations in leaves of kale, hot pepper, and maize.

Fertilizer source	N	P	Zn	Fe
	--%--	--%--	--mg kg^{-1}--	--mg kg^{-1}--
		Kale		
Control	4.16^{c†}	0.29^{c}	38^{d}	92^{c}
Urea	6.14^{a}	0.56^{a}	83^{b}	126^{b}
Dry Cyanobacteria	6.47^{a}	0.58^{a}	104^{a}	154^{a}
Liquid Cyanobacteria	5.46^{b}	0.42^{b}	62^{c}	120^{b}
		Hot pepper		
Control	2.39^{c}	0.37^{b}	53^{b}	88^{d}
Urea	4.33^{b}	0.41^{b}	60^{b}	102^{c}
Dry Cyanobacteria	5.25^{a}	0.60^{a}	156^{a}	166^{a}
Liquid Cyanobacteria	4.55^{b}	0.56^{a}	140^{a}	142^{b}
		Maize		
Control	2.16^{c}	0.24^{c}	35^{d}	89^{c}
Urea	4.14^{a}	0.54^{a}	59^{c}	123^{b}
Dry Cyanobacteria	4.47^{a}	0.52^{a}	101^{a}	151^{a}
Liquid Cyanobacteria	3.46^{b}	0.37^{b}	80^{b}	117^{b}

†Means followed by a common letter within crop and nutrient are not significantly different based on Least Significant Differences at $p \leq 0.05$.

Table 7. Correlation coefficients among soil and plant characteristics in kale, hot pepper, and maize greenhouse trials.

Correlations	Kale	Hot pepper	Maize
Soil pH vs. Soil N	0.03	0.11	0.72***
Soil pH vs. Soil P	0.17	-0.02	0.93***
Soil pH vs. Soil Zn	-0.85***	-0.78***	-0.92***
Soil pH vs. Soil Fe	-0.90***	-0.86***	-0.99***
Soil pH vs. Plant N	-0.18	-0.14	-0.04
Soil pH vs. Plant P	-0.09	-0.07	-0.06
Soil pH vs. Plant Zn	-0.37	-0.73***	-0.34
Soil pH vs. Plant Fe	-0.43*	-0.68***	-0.41*
Soil N vs. Plant N	0.69***	0.93***	0.36
Soil P vs. Plant P	0.10	0.92***	0.04
Soil Zn vs. Plant Zn	0.56**	0.98***	0.45*
Soil Fe vs. Plant Fe	0.62**	0.87***	0.42*
Soil N vs. Plant Height	0.64***	0.54**	0.68***
Soil N vs. Leaf Number‡	0.63***	0.47*	0.53**
Soil N vs. Leaf Area	0.46*	0.42*	0.42*
Soil N vs. Shoot Dry Weight	0.62**	0.43*	0.83***
Soil N vs. Root Dry Weight	0.48*	0.45*	0.61**
Plant N vs. Plant Height	0.76***	0.72***	0.66***
Plant N vs. Leaf Number	0.85***	0.65***	0.88***
Plant N vs. Leaf Area	0.82***	0.65***	0.91***
Plant N vs. Shoot Dry Weight	0.86***	0.62***	0.71***
Plant N vs. Root Dry Weight	0.84***	0.69***	0.66***

†*,**,*** significantly different at $p \leq 0.05$, 0.01, and 0.001 respectively.
‡Branch number was utilized in place of leaf number for hot pepper.

transporter proteins located on the root cell membrane increased by the plant N status and these proteins enhance uptake and accumulation Zn and Fe in the plant tissue (Rana et al., 2012).

However, the urea fertilizer also increased plant P, Zn, and Fe concentrations in kale and maize and Fe in hot pepper. This was apparently due to the soil pH reduction caused by urea application. Since the cyanobacterial bio-fertilizers also reduced soil pH, this reduction may also contribute to increased plant P, Zn, and Fe measured in those treatments. Soil pH was highly significantly ($p<0.001$) negatively correlated with available soil Zn and Fe for all three crops, as expected (Table 7). Soil pH was also significantly negatively correlated with plant Fe concentrations for all three crops, but pH was only significantly correlated with plant Zn for hot pepper.

The relationship between soil pH and P availability is more complicated since P availability is optimum in near neutral pH. Therefore, we would expect that in acid soil, there would be a positive correlation between pH and available P, but the opposite would be true in alkaline soils. Soil pH was only significantly correlated with available soil P in maize and was not significantly correlated with plant P in any crop (Table 7). Soil P and plant P concentrations were only significantly correlated in hot pepper.

Conclusion

Application of dried cyanobacteria led to increase in growth and yield of maize pepper and kale, and fertility of both soils. Dried cyanobacterial bio-fertilizer also improved nutritional qualities of the three crops by increasing micronutrient (Zn and Fe) concentration especially in the edible parts of kale and pepper. This will have a paramount importance in alleviating the problem of zinc deficiency among pregnant women and children in Ethiopia. Hence, production and selling of dry cyanobacteria biofertilizer locally could have a positive impact on food and nutritional security in Ethiopia.

Moreover, cyanobacterial bio-fertilizer also consistently increased soil organic carbon sequestration and improved organic carbon stock in the soil, and this has important soil quality implications for Ethiopia's degraded soils. Overall, the use of dry cyanobacteria will reduce application of urea in agricultural land. Reducing imported urea and supplementing with locally-produced cyanobacterial bio-fertilizer could reduce CO_2 emissions from fertilizer production and transportation while also enhancing carbon sequestration.

In addition, volatilization losses of NH_3 and N_2O have yet to be measured and compared to commonly used fertilizers. Collecting these data may help to explain the increased soil N values in these greenhouse studies. Therefore, additional research should be carried out to evaluate the impact of cyanobacterial bio-fertilizer on soil pH, soil quality, carbon sequestration, and NH_3 and greenhouse gas emissions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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