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Effects of Nitrogen and Bio-fertilizers on Growth and Yield of Roselle (*Hibiscus sabdariffa* var sabdariffa L.)

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Abstract The crop producers are aware of the need to reduce the use of chemical means of production and conducive to the development of biological alternatives. Bio-fertilizers have some microorganisms which convert elements to available nutrient for plant's roots. A field experiment was conducted during 2010-2011 rainy season at two locations in North Kordofan State of Sudan. The aim of this study was to investigate the comparative efficiency of Nitrogen and Bio-fertilizers on growth and yield of roselle crop (*Hibiscus sabdariffa* var sabdariffa L.). The Ten treatments consisted of zero fertilizer, 1/2N (23Kg Nitrogen), 1N (46Kg Nitrogen), 2N (92Kg Nitrogen), Azatobacteria, Azospirillum, Azatobacteria+Azospirillum, 1/2N+Azatobacteria, 1/2N+Azospirillum, 1/2N+ Azatobacteria+ Azospirillum. A randomized complete block design (RCBD) with three replicates was used. The results showed that nitrogen and bio-fertilizers had a significant effect on most of the growth and yield attributes measured. The Nitrogen fertilizer (1/2N) and Azatobacteria treatment had a thicker stem diameter, a highest number of leaves per plant, number of productive branches, shoot fresh and dry weight and highest number of calyces per plant. The treatments (1/2N+ Azatobacteria) and (Azatobacteria +Azospirillum) had the highest plant height, dry calyx weight, harvest index and final calyx yield (kg ha^{-1}).

Keywords Fertilizer efficiency, Hibiscus, Azatobacteria, Azospirillum

1. Introduction

Bio-fertilizers are live formulates of microorganisms (useful bacteria and fungi) that are ready to be used and improve the quality and the health of the soil and the plant species by increasing the nutrient availability for the soil and plants. Bio-fertilizers naturally activate microorganisms found in the soil natural fertility and protecting it against drought and soil diseases and therefore stimulate plant growth [1]. Bio-fertilizers were obtained using natural election of different type of beneficial living organism [2]. Using bio-fertilizers that contain different microbial strains has led to a decrease in the use of chemical fertilizers and has provided high quality products free of harmful agrochemicals for human safety [3]. Roselle (*Hibiscus sabdariffa* L) family Malvaceae, known commonly as "Karkade". It is known under different names in different countries viz roselle, razelle, sorrel, red sorrel, Jamaica sorrel, Indian sorrel, Guinea sorrel, sour -sour, and Queens land jelly plant [4]. It is an important crop in tropical and sub-tropical regions. The economical part of the plant is the fleshy calyx (sepals) surrounding the fruit (capsules). In Sudan fully developed fleshy calyx is peeled off from the fruit by hand and dried naturally under shade to give the dry (calyx), which is the consumable product. The plant, normally grown as annual plant, is 0.5 to 1.5 meters in height. It has a bushy shape with somewhat dense canopy of dark green leaves. The colour of the calyx plays an important role in determining the quality of the crop. The crimson red colour is the characteristic and most popular and desirable colour of roselle while other shades and colors exist, including the white or greenish white colour. It is an important cash crop in Western Sudan, particularly in Northern Kordofan State where the largest area of roselle is grown (Elrahad and Um-Rawaba locality). The crop is mostly produced in traditional growing conditions by small-farmers, depending on rainfall and natural soil fertility without using chemical fertilizers or insecticides [5]. Roselle has many industrial and domestic uses. It is used as a beverage in the Sudan, where the dried calyx is soaked in water to prepare a colorful cold drink. Traditionally the product has been used for medicinal purposes for relief of sour throat and for healing wounds as an anti-septic [6]. El

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Naim *et al.* [6] reported that, leaves is consumed as green vegetable in many parts of the world and the stem is used as a source of pulp for paper industry. Seeds used as a poultry feed and as an aphrodisiac coffee substitute [25]. The main production comes from Western Sudan States, and the most of the exported crop is grown in the Eastern Kordofan localities. Roselle is also scattered in the southern region and south Fung area and recently at Abu Naama in the rain-fed central clay plains of Sudan [7, 8]. The production of Roselle in Sudan is facing many problems, which resulted in: unstable total production. The main yield-limiting factor is the amount and distribution of rainfall. Another problem is the labour requirement for harvesting which amounts to about half the total cost of production. Moreover, the cultivars used for production are local types, which are characterized by low yield potential. Most of the traditional farmers sow the crop in a very wide space, which affects the total production as well as poor cultural practices, low soil fertility and inadequate weed control. The crop is considered as a possible future crop, because of its natural production without using any chemical (fertilizers or insecticides). The uses of bio fertilizers in agriculture play an important role of providing an economically viable level for achieving the ultimate goal to enhance productivity. On the other hand, the value of organic materials as a source of plant nutrients is greatly enhanced by composting. Composted materials are also more stable and pleasant to handle. In North Kordofan State no trials have yet been carried out to evaluate the response of roselle to biological fertilizers. This study was under taken with an aim to study the beneficial effects of biological and chemical fertilizers and their various combinations on growth and yield of rosselle (*Hibiscus sabdariffa* L) under rain-fed condition in North Kordofan state of Sudan in semi-arid environment.

2. Materials and Methods

2.1. The Experiment

A field experiment was conducted during season 2010-2011 under rain fed condition at two locations in North Kordofan state located between latitude 11° 15' and 16° 30' N and longititude 27° and 32° E, which were: Elobied Agricultural Research Station Farm and Khour Tagat (8 km East of Elobeid). The climate of the area is arid and semiarid zone. The soil is sandy with low fertility. Annual rainfall ranges between 350-500 mm. Average maximum daily temperatures varied between 30°C to 35°C most of the year [5, 9]. The aim is to study the comparative effects of nitrogen and bio-fertilizers on growth and yield of roselle (*Hibiscus sabdariffa* var sabdariffa L.).

The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. The plot size was 5×4 meters. The treatments consisted of ten levels of fertilizers (nitrogen and bio-fertilizers) as follows:

No fertilizer, 23kg nitrogen, *Azatobacteria*, *Azospirillum*, *Azatobacteria* + *Azospirillum*, 23Kg Nitrogen + *Azatobacteria*, 23kg Nitrogen + *Azospirillum*, 23Kg Nitrogen + *Azatobacteria* + *Azospirillum*, 46 Kg Nitrogen and 92 kg Nitrogen. Designated as C, ½N, A, B, AB, ½NA, ½NB, ½NAB, N, 2N respectively.

Azatobacteria and *Azospirillum* strains used in this study were obtained from the Bio-fertilization Department, Environment and Natural Resources Institute, National Centre for Research, Khartoum, Sudan.

Sowing dates on 16th of July for Elobeid Agricultural Research Station farm and Khour Tagat respectively. Seeds were sown on lines in spacing of 70 cm between rows and 60 cm within row, five seeds were placed in each hole. The plants were thinned to two plants per hole two weeks later.

2.2. Growth Attributes

A sample of four plants was taken at random from inner rows in each experimental unit to measure the following growth attributes:

- Plant height: measured from the ground level to the tip of the plant.
- Stem diameter cm: Measured by using a Vernier (caliper) at second node.
- Number of leave per plant:
- Number of branches per plant: determined by counting reproductive branches at harvest.
- Number of node per plant: was determined by counting the number of differential nodes of the main stem.
- Shoot dry weight (g): by weighed dry at 85°C for 24 hours to constant weight.

2.3. Yield Attributes

A destructive sample of four plants (uprooting) was taken at random from the five inner rows of experiment plot to measure the following attribute:

- Number of calyces per plant (at 95% physiological maturity).
- Calyces yield per plant (g): The calyces of four plants were peeled off from the capsules by using simple hand tools. The calyces were dried under shade to constant weight. Then average calices yield per plant (g) was determined.
- Final calyces yield (kg ha^{-1}): Calculated by using the following formula:

$$\text{Calyces yield (kg / ha)} = \frac{\text{Calyces weight (kg) of plot}}{\text{Harvested plot area (m2)}} \times 10000$$

- Harvest index was determined by using the following formula:-

$$\text{Harvest index} = \frac{\text{Economical Yield (calyces per plant)}}{\text{Biological yield (shoot dry weight)}} \times 100$$

2.4. Statistical Analysis

The collected data were analyzed for the estimation of the statistical parameters according to Sigma stat procedure for a randomized complete block design (computer program). For comparison between means, they were separated using Duncan Multiple Range Test (DMRT) at 0.05 level of significance, according to the procedure described by Gomez and Gomez [10].

3. Results and Discussion

3.1. Growth Attributes

Table 1 represents the effect of nitrogen and bio-fertilizers on stem diameter and plant height. The fertilizers had significantly effect on plant height. The AB treatment (*Azatobacter* +*Azospirillum*) had the highest plant height. Rajyalakshmi [26] found that inoculation of foxtail millet (variety Lepakshi) with three strains of *Azospirillum* lipoferum either alone or in combination with nitrogen fertilizer (40 kg N ha^{-1}) increased the plant height, dry weight of shoot and root, and total N content of shoot, root and grain. Kamal (2008) found significantly increased plant height. Hassan *et al*. [29] reported that the bio-fertilizer rhizobactr application increased the vegetative growth (plant height, number of leaves, leaf area and dry weight). Zaki *et al*. [27] mentioned that broccoli plants treated with bio-fertilizer showed highest vegetative growth attributes: plant height, leaf number, fresh and dry weight of leaves and total yield.

Table 1. Effect of nitrogen and bio-fertilizers on stem diameter (cm) and plant height (cm) of Roselle

Treatments	Location I		Location II	
	Stem diameter (cm)	Plant height (cm)	Stem diameter (cm)	Plant height (cm)
C	1.3^{a}	116.1^{c}	1.4^{a}	101.2^{c}
1/2N	1.3^{a}	114.5^{d}	1.4^{a}	101.6^{c}
A	1.3^{a}	121.3^{b}	1.4^{a}	107.0^{a}
2N	1.4^{a}	114.9^{d}	1.4^{a}	107.1^{a}
A+B	1.4^{a}	128.9^{a}	1.5^{a}	107.8^{a}
1/2N+A	1.4^{a}	126.1^{a}	1.5^{a}	108.6^{a}
B	1.3^{a}	119.5^{b}	1.4^{a}	108.0^{a}
1/2N+B	1.4^{a}	118.4^{b}	1.5^{a}	104.1^{b}
1N	1.4^{a}	114.1^{d}	1.5^{a}	103.6^{b}
1/2+AB	1.4^{a}	116.9^{c}	1.5^{a}	111.6^{c}
SE ±	0.10	5.98	0.15	11.1
CV%	11.35	6.15	17.99	19.05

Means in each column having the same latter's are not significantly different at 0.05 level of probability, according to the Duncan s Multiple Range Test.

There were no significant differences among the fertilizers treatments for the stem diameter (Table 1) Generally in the second location the fertilizer treatments almost had a thicker stem diameter than in the first location. Hassan *et al*. [29] reported that the bio-fertilizer rhizobactr application increased the vegetative growth.

Table 2 shows the effect of bio-fertilizers and chemical fertilizers on number of leaves per plant. Analysis of variance showed that the effect of bio and chemical fertilizers were not significantly different. The bio-fertilizers and nitrogen had no significant on the number of leaves per plant in this experiment. Hassan *et al*. [29] reported that the bio-fertilizer rhizobactr application increased vegetative growth (plant height, number of leaves per plant, leaf area and dry weight). The beneficial impact of N fixing bacteria to be direct improved of plant growth promotion by the production of plant growth regulators [11].

Table 2. Effect of nitrogen and bio-fertilizers on number of leaves per plant and number of nodes per plant of Roselle

Treatments	Location I		Location II	
	No. of leaves	No. of Nodes	No. of leaves	No. of Nodes
C	34.6^{a}	33.5^{b}	40.8^{d}	25.8^{d}
1/2N	33.6^{a}	34.5^{ab}	71.1^{b}	26.9^{c}
A	35.4^{a}	37.6^{a}	70.0^{b}	25.4^{d}
2N	33.2^{a}	35.0^{a}	63.8^{c}	25.7^{c}
A+B	34.7^{a}	34.8^{a}	74.7^{b}	34.1^{b}
1/2N+A	34.8^{a}	36.3^{a}	80.8^{ab}	36.6^{a}
B	32.6^{a}	34.1^{ab}	78.5^{b}	25.6^{e}
1/2N+B	35.7^{a}	33.6^{ab}	84.0^{a}	27.7^{c}
1N	33.5^{a}	35.3^{a}	84.5^{a}	32.5^{b}
1/2N+AB	33.9^{a}	35.3^{a}	75.2^{b}	28.4^{c}
SE ±	1.75	1.7211	11.25	3.44
CV%	8.71	8.61	26.91	22.16

Means in each column having the same latter's are not significantly different at 0.05 level of probability, according to the Duncan s Multiple Range Test.

Results of the effects of bio-fertilizers and chemical fertilizers on number of nodes per plant at maturity are shown on Table 2. There was significant difference among treatments in number of nodes. The significant differences among the number of nodes per plant may be refer to similar effect of Nitrogen and Bio-fertilizers. The bio-fertilizers increased the number of nodes per plant. The treatment (A) had the highest number of nodes per plant.

Results of number of reproductive branches per plant are shown in Table 3. Statistical analysis exhibited that fertilizers had no significant effect on means number of productive branches per plant. A+B treatment gave a lesser number of branches per plant in the first location. In the second location the fertilizers had highest branches than non-fertilizer treatment. In this study all the treatment had no effect on number of productive branches per plant. Venkateswarlu and Rao [11] found that inoculation with *Azospirillum brasilence corrig* increases in growth and dry matter were obtained by for pearl millet following inoculation with data presented in Table 3 show the effect of bio fertilizer and Nitrogen fertilizer on shoot dry weight (g/plant). Statistical analysis indicated that shoot dry weight was non significantly different among fertilizers treatments.

Table 3. Effect of nitrogen and bio-fertilizers on number of number of branches per plant and shoot dry weight (g/plant) of Roselle

Treatments	Location I		Location II	
	No - of Branches/ plant	Shoot dry weight (g)	No of Branches/ plant	Shoot Dry Weight (g)
C	1.3^{e}	40.3^{e}	1.0^{d}	21.6^{f}
1/2N	2.5^{a}	43.3^{d}	3.2^{a}	26.6^{e}
A	1.6^{d}	46.3^{c}	2.4^{b}	44.5^{b}
2N	2.5^{a}	50.0^{b}	1.8^{c}	26.6^{e}
A+B	1.3^{e}	56.5^{a}	1.1^{c}	45.4^{ab}
1/2N+A	1.9^{c}	60.5^{a}	3.3^{a}	52.5^{a}
B	1.6^{d}	49.3^{b}	g)3.0^{a}	32.5^{c}
1/2N+B	2.0^{b}	41.8^{e}	1.5c	41.2^{b}
1N	2.0^{b}	53.5^{ab}	2.9^{b}	44.0^{b}
1/2N+AB	2.7^{a}	50.8^{b}	3.5^{a}	42.5^{b}
SE ±	0.17	7.03	0.97	9.61
CV	54.89	24.72	23.82	44.06

Means in each column having the same latter's are not significantly different at 0.05 level of probability, according to the Duncan s Multiple Range Test

The treatment 2N had the highest shoot dry weight (60.0g/plant) in the first location. In the second location the 1/2N+A. treatment had a highest weight compared to others. Generally, the first location had higher shoot weight than the second location. Narula *et al.* [12] found that inoculation with *Azotobacter* sp. increased wheat and cotton yield, dry weight, and plant nitrogen. Similarly, nitrogen concentration in wheat grain and root tissue may increase due to *Azotobacter* bio-inoculants [13]. Inoculation of *Azotobacter chroococcum* Beijerinck onto *Brassica napus* cv. ISN-129 produced an increase in grain yield and total dry matter production when no external nitrogen was applied [15, 16]. Inoculation of *Azospirillum* to cereals and non-cereal species results in increases in shoot dry weight and in the amount of nitrogen in the shoots [17-19].

Similarly, significant increases in growth and dry matter were obtained by Venkateswarlu and Rao [11] for pearl millet following inoculation with *Azospirillum br*asilence corrig. This may be due to the ability of bio-fertilizers to transport major nutrients like N and P besides secreting plant growth promoting substances such as IAA, gibberellins and abscisic acid. An organic acid obtained from organic manures has led to increase in soil acidity and consequently convert insoluble forms of phosphorus into soluble ones [20], [21].

3.2. Yield Attributes

The Table 4 presented the effected of nitrogen and bio-fertilizers on number of calycies per plant. The treatment had a significant different on number of calyces per plant in the both locations. The bio-fertlizers and nitrogen treatments had highest number of calycies per plant compared to non-fertilizer treatment. A, A+B and 1/2N+A. treatments had a highest number of calyces per plant. Narula *et al.* [12] found that inoculation with Azotobacter and Azospirillum increased growth and yield parameters in cotton.

The results of the effects of fertilizers on dry calyces weight (calyx yield, g/ plant) are presented in Table 4. As could be seen from the statistical analysis of data a significant differences were observed among treatments in weight of dry sepals.

The 1/2N+A (half doses nitrogen and zotobacter) treatment had a highest calyces yield (g/plant) compared to others. Zaki *et al*. [27] mentioned that broccoli plants treated with bio-fertilizer showed highest vegetative growth attributes: plant height, number of leaves per plant, fresh and dry weight of shoot and total yield.

Table 4. Effect of nitrogen and bio-fertilizers on number of calyces per plant and dry calyx (g/plant) per plant of Roselle

Treatments	Location I		Location II	
	No. of calyces/plant	Dry calyx (g/plant)	No. of Calyces/plant	Dry Calyx (g/plant)
C	10.7^{e}	4.0^{e}	9.5^{e}	1.2^{f}
1/2N	12.0^{d}	7.6^{c}	20.5^{c}	4.1^{c}
A	12.8^{d}	7.5^{c}	20.7^{c}	4.7^{b}
2N	17.9^{c}	8.0^{b}	19.4^{d}	3.7^{d}
A+B	21.1^{b}	8.7^{b}	20.7^{c}	4.3^{c}
1/2N+A	23.0^{a}	9.2^{a}	25.2^{a}	8.2^{a}
B	11.5^{e}	6.8^{d}	25.9^{a}	3.0^{e}
1/2N+B	21.9^{b}	8.8^{b}	20.5^{c}	4.1^{c}
1N	17.0^{c}	8.5^{b}	20.9^{c}	3.4^{d}
1/2N+AB	17.9^{c}	7.2^{d}	24.0^{b}	5.0^{b}
SE ±	1.96	1.31	3.09	0.72
CV%	25.67	27.85	25.83	29.75

Means in each column having the same latter's are not significantly different at 0.05 level of probability, according to the Duncan s Multiple Range Test.

Table 5 represented the effect of bio fertilizers and nitrogen on harvest index. The statistical analysis revealed significant differences between treatments in harvest index in two locations. The A+B had highest harvest index (21.27) in the first location. However in second location the 1N treatment had a highest harvest in index (15.40). Azotobactermix Azospirillum had overall means (21.3%) in Elobied location, and lowest means (12.1%) in Taggat location. Zaki *et al*. [27] mentioned that broccoli plants treated with bio-fertilizer showed highest vegetative growth attributes: plant height, leaf number, fresh and dry weight of leaves and total yield.

Table 5 shows the effect of bio-fertilizers and chemi-fertilizerse on calyx's yields. The bio-chemifertilizers treatment had significant effect on calyces yield. The 1/2N+ A treatment was the best for maximizing yield in both locations. Chemi-bio-fertilizers strains inoculation proved to be effective in increasing the yield in this study (half doses nitrogen (23Kg) with Azotobacter treatment in taggat location). Narula *et al.* [12] found that inoculation with

Azotobacter sp. increased wheat and cotton yield, dry weight, and plant nitrogen. This might be due to more availability of nutrients from compost and beneficial effects accrued due to Azotobacter and phosphate solubilizing bacteria (PSB) inoculation which provide nitrogen and phosphorus to plant growth. It may also be due to production of amino acids, vitamins and growth promoting substances like indole acetic acid and gibberellic acid secreted by these introduced beneficial microorganisms which resulted in enhanced nutrient uptake, translocation and synthesis of photosynthate assimilates which resulted increased plant growth characters and in obtaining economically profitable yield [22, 23] Growth and yield were significantly higher when the bio-fertilizers were inoculated with combined treatment (Azotobacter and Azospirillum) compared to individual inoculation and control. This could be due to the collective effect of bio-fertilizers. Plants inoculated with Azospirillum and half nitrogen dose fixed more nitrogen and produced more grain yield than singly inoculated plants [28]. Similar growth increase was noticed in black pepper with combined inoculation of bio-fertilizers (Azospirillumm, Azotobacter and Phosphobacteria) [24].

Table 5. Effect of nitrogen and bio-fertilizers on calyces yield (kg ha^{-1}) and Harvest index (HI) of Roselle

Treatments	Location I		Location II	
	Calyces yield (kg ha^{-1})	Harvest Index	Calyces Yield (kg ha^{-1})	Harvest Index
C	422.67e	10.2d	98.6e	4.8f
1/2N	582.2c	14.8b	318.9c	12.8b
A	522.9c	14.3b	382.6bc	12.7b
2N	641.7b	12.2c	300.5c	12.3
A+B	698.4ab	21.3a	571.7a	14.7a
1/2N+A	737.9a	16.5b	658.9a	13.6a
B	485.3d	12.2c	244.0d	8.9e
1/2N+B	711.5a	17.5ab	329.6c	8.0e
1N	684.8b	14.8b	274.4d	13.4b
1/2N+AB	582.9c	12.6c	406.4b	10.8c
SE ±	107.09	2.05	60.92	2.81
CV%	29.12	23.42	31.17	45.15

Means in each column having the same latter's are not significantly different at 0.05 level of probability, according to the Duncan s Multiple Range Test.

4. Conclusions

The findings of this study have clearly showed that combined application of nitrogen and bio-fertilizers has resulted in obtaining highest plant growth, crop yields and dry matter production. It is concluded that plant height and biological yield have been affected significantly by co-inoculation followed by single inoculation because this bio-fertilizer can enhance absorbed of nitrogen by plant. Thus, it can be said that for obtaining maximum calyces yield from roselle, soil should be inoculated with Azotobacter with half dose of nitrogen and Azospirillum. We suggest that some other study should be carried out on the efficiency of bio-fertilizers.

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