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Calyx Yield and Nitrogen Use Efficiency of Roselle *Hibiscus sabdariffa* L as Affected by Variety and Levels of Nitrogen Fertilizer

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Abstract

Roselle (Hibiscus sabdariffa L.) is an important beverage and leafy vegetable plant. Constraints such as poor soil fertility and a lack of improved varieties have an impact on its growth and yield characteristics. A field trial was conducted to evaluate Roselle's calyx yield response and nitrogen use efficiency. In this study, two roselle varieties (WG-Hibiscus-Jamaica and WG-Hibiscus-Sudan) and six levels of nitrogen fertilizer (Kg ha^{-1} (0, 23, 46, 69, 92, 105) were used in a factorial combination arrangement with three replications in a randomized complete block design. Data on calyx yield, growth variables, and nitrogen use efficiency parameters were recorded. WG-Hibiscus-Jamaica exhibited higher plant height (196.2cm) and branch number plant^{-1} (34.9) from the application of 115kg ha^{-1} N, while lower values were recorded on an unfertilized plot of both varieties. Variety WG-Hibiscus-Sudan exhibited the highest number of calyx plant^{-1} (50.3), fresh calyx yield ha^{-1} (5934 kg), and dry calyx yield ha-1 (1866 kg) from the application of 92 kg ha^{-1} N, while lower values were recorded from the unfertilized plot of the WG-Hibiscus-Jamaica variety. A higher nitrogen efficiency ratio (42.5 kg kg^{-1}) was recorded from WG-Hibiscus-Sudan while a lower value was recorded from WG-Hibiscus-Jamaica. Higher (12.5kg) and lower (0.7kg) agronomic efficiency were recorded from the application of 92 and 115 kg ha^{-1} N respectively. WG-Hibiscus-Sudan was effective but not reactive, while WG-Hibiscus-Jamaica was not effective but reactive. In conclusion, for maximum commercial produce, calyx yield and profitability, applying 69 kg ha^{-1} N on WG-Hibiscus-Sudan and 92 kg ha^{-1} on WG-Hibiscus-Jamaica was found best.

Keywords: Calyx Yield; Nitrogen; Roselle use efficiency; Variety

Introduction

Roselle Hibiscus sabdarifa L is an annual herbaceous shrub grown in tropical and subtropical countries belonging to the family Malvaceae[1]. More than 300 species are grown all over the world and wider genome diversity is found in Sub-Saharan Africa[2]. According to Osseo-Asare , roselle is originated in West Africa and distributed to other parts of the world by African slaves. It is known by different names in different countries such as Jamaican Sorrel, karakade, roselle, bissap, zobbo, and Queen's land jelly plant [3]. It is a tetraploid (2n = 4x = 72) which has a more related chromosome number with a diploid fibre crop (2n = 2x = 36) kenaf [4]. Roselle mainly adapts in areas with semi-humid to a subtropical climate in the altitudinal range of 600-2000 m above sea level and annual rainfall between 400-500 mm with an annual temperature ranging between 18-35°C. It grows and performs well in well-drained, fertile soil with high organic matter and a pH of 4.5 [5].

Roselle is an economically important beverage, leafy vegetable, and medicinal plant used in several local dishes [6]. Its swollen fleshy calyx (sepals) is an economically important part that is harvested by hand, dried, and sold completely into the herbal tea and beverage industry for food preparation in sauces, jams, juices, jellies, and syrups as a flavoring and coloring agent for food and drinks; and also leaves as a leafy vegetable; seed as a valuable food resource on account of its protein, calories, and stem as a substantial amount of fiber [7]. The chemical composition of the calyx is approximately 15–30% organic acids, including citric, malic, tartaric, oxalic, stearic, and hibiscus acid, which is most likely to contribute to the tartness of the herb and its teas. Its health importance in traditional medicine around the world is mainly attributed to the water extract of the roselle calyx (mainly as a mild laxative, antipyretic, and diuretic) [8]. It is used in folk medicine for treating hypertension, pyrexia, liver damage, and cancer, as well as for its lipid-lowering and renal effects [9].

Apart from its nutritional and health importance, in developing countries, roselle plays an important role in income generation and subsistence among rural farmers, is relatively easy to grow, and can be grown as part of the multi-cropping system [10]. Demand has steadily increased over the past decades. Approximately 15,000 metric tons enter the world's international trade each year through countries like Germany and the United States for consumption [11]. However, growth and yield of roselle are affected by various factors, such as the growing environment and sowing time [12], application of organic and inorganic fertilizer [13], variety and supplementary irrigation management, growing soil type and nutrient status, insect pest, weeds, and pathogens and post-harvest handling. It has an overall growing period of 4-6 months from seed sowing to harvesting of the calyx, depending upon the growing environment and variety.

Application of organic and inorganic fertilizers on roselle was reported as important, and its performance increased with an increasing level of nitrogen application[4]. Nitrogen is an essential nutrient required in the greatest amount for plant growth, development, and metabolism in terrestrial ecosystems, and plants acquire nitrogen in a variety of different chemical forms, ranging from inorganic sources such as nitrate (NO_3^-) and ammonium (NH_4^+) to different amino

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acids and more complex organic N. More importantly, the application of nitrogen significantly increases the growth and yield attributes of roselle. Its dry calyx yield ranges from 388.48 to 611.91 kg ha^{-1} for 0-100kg Nha^{-1} in Benin. Moreover, 1200 kg ha^{-1} dried calyx yield was reported in Guinea.

However, in Ethiopia, its yield is very low compared to reports from other countries. Its fresh calyx yield ha^{-1} (kg) ranges between 1855.8 and 2597.98 and dry calyx yield ha^{-1} (kg) ranges from 184.2 to 257.8 for variety WG-Hibiscus-Jamaica, whereas fresh calyx yield ha^{-1} (kg) ranges between 1464.4 and 1594.7 and dry calyx yield ha^{-1} (kg) between 148.6 and 161.7 for variety WG-Hibiscus-Sudan. These data suggest the economic yield of roselle is far below (about more than 50% lower) that obtained in other countries, despite the potential of the varieties. Thus, despite the need for improving the economic yield of roselle by appropriate nitrogen fertilization as one agronomic strategy, according to recent information in Ethiopia, there are no reports on optimum nitrogen fertilization on roselle varieties. With these rationales, the main aim of this study was [1] to evaluate the calyx yield response of roselle varieties to different levels of nitrogen fertilizer application and [2] the nitrogen use efficiency of roselle varieties at different levels of nitrogen application.Materials and Methods

Description of study site

The field experiment was conducted at the Hawassa Green Mark Herb PLc. experimental site during 2018–2019 under supplementary irrigation. The site was one of the implementing research sites for aromatic and medicinal plant research in collaboration with Green Mark Herbs, a private herb producer and exporter, and was used for this study. Geographically, the site is located at 7^0 05' North latitude and 39^0 29' East longitudes in the Sidama Regional State, south Ethiopia, at an altitude of 1652 meters above sea level (m.a.s.l.) and receives a mean annual rainfall of 964 mm with a minimum and maximum temperature of 13°C and 27 °C, respectively. The soil textural class of the experimental site was sandy loam (andosol) with a pH of 7.84 (slightly basic) (Table 1).

Treatments and experimental design

Two introduced roselle varieties, namely, WG-Hibiscus-Jamaica and WG-Hibiscus-Sudan, released in 2014 for calyx production in low and mid-altitude Ethiopia, were used for this study. The combinations of six levels of nitrogen (0, 23, 46, 69, 92, and 115 kg ha^{-1} in the form of urea) and two roselle varieties formed twelve treatment combinations arranged in a randomized complete block design (RCBD) and replicated three times. The recommended level of phosphorus fertilizer (20 kg ha^{-1} (TSP: 46% P_20_5) was used as a source of phosphorus, and urea containing 46% N was used as a source of nitrogen and applied at three growth stages: seedling (25 days after an emergency), vegetative (75 days after an emergency), and flower bud initiation (115 days after an emergency). Both fertilizers were applied in-band applications during the sowing time for phosphorus and at different growth stages for nitrogen. The spacing between plots and blocks was 1 m and 1.5 m, respectively. Each plot has a size of (3.6 m width x 3.6m length) or an area of 12.96 m^2 and accommodates six rows with an inter- and intra-row spacing of 60 $cm^{2.}$

Experimental procedures

The experimental site was ploughed, disked, harrowed, and leveled manually, and blocking was arranged perpendicularly to the soil fertility and slope gradient. After blocking, plots were arranged with adjusted spacing. The experiment area was cleaned, and a composite soil sample from a depth of 30 cm was collected from the experiment site to characterize the physicochemical properties of the study site. All the treatments were randomly assigned to each experimental unit. Four seeds were sown per hole, with 60 cm between plants and rows. Thinning was performed after one month, and one plant per hole was maintained. Urea was applied to the dressing at three growth stages (seedling, vegetative, and flower bud initiation stages). Irrigation, weeding, and hoeing, as well as other cultural practices, were made as required. After harvesting, soil samples were taken using an auger from each of the treatment plots. Soil samples collected both before sowing and after harvest were dried in the air, pulverized to pass through a 1mm sieve, and subjected to analysis of the major soil physicochemical properties.

Soil samples were taken before sowing and analyzed for pH, organic carbon (OC), total nitrogen, available phosphorus, and CEC (cation exchange capacity), while after harvest samples were analyzed only for soil pH and total nitrogen; soil texture was determined by the hydrometer method; pH was determined by using a pH meter; CEC was determined by using the 1N ammonium acetate method as described by Vance et al.; available phosphorus was estimated by using the Olsen extraction method as described by Olsen (32); total nitrogen was analyzed by the Kjeldahl digestion procedure as described by Bremner; Plant tissue sampling was done at physiological maturity from six randomly selected plants that were harvested from central rows and partitioned into shoots and calyx. Then, shoot and calyx samples were separately air-dried and ground to pass a 1-mm sieve. Nitrogen (N) in shoots and calyx sub-samples was determined by using the method and guide to laboratory establishment for plant nutrient analysis suggested by Blumenthal et al.

Data collection

Data on growth and yield variables were collected from six plants

Table 1: Physicochemical properties of the soil sample before planting.

Physiochemical properties	Unit	Test methods used	Values	Remark
pH	Soil: water	pH meter	7.8	Slightly basic
Soil Organic matter	%	Volumetric method	3.52	
Organic carbon content	%		2.02	Low
Total nitrogen	%	Kjeldahl technique	0.070	Very low
Available phosphorus	Ppm	Olsen	76.27	High
EC (µs/cm)	ds/cm		111.6	Saline
CEC	(Meq/100g)	1N ammonium acetate	30.48	High
Sand	%		56	
Clay	%		14	
Silt	%		30	
Textural class			Sandy loam	

in a central row after 130 days after sowing, as described below.

Plant height (cm): was recorded as the total plant height from base to top, including all flowering nodes at the harvesting stage;

Number of branches plant $^{-1}$: this was determined by counting the number of primary productive branches when the plants were at the harvesting stage;

Number of calyx per plant: The capsules of six randomly selected plants from the central rows of each plot were harvested carefully when they matured, and the average capsules were determined:

Calyx yield plant^{-1} (g) (fresh): The fully developed calyx of six randomly selected plants was peeled off from the capsules by using hand tools when they reached horticultural maturity and was measured immediately after peeling. Then the average fresh calyx yield per plant was determined.

Calyx yield per hectare (kg) (fresh): a ratio of total harvested fresh calyx yield per plot to an area of harvested plot multiplied by 10,000 m^2;

Calyx yield plant^{-1} (g) (dry): The peeled, fully developed calyx was dried by sunlight to a constant weight, and then the average dry calyx yield per plant was determined;

Calyx yield plant^{-1} (g) (dry): a ratio of total harvested dry calyx yield per plot to an area of harvested plot multiplied by 10,000 m^2; **Harvest index (%), which** is a ratio of economic yield to biological yield (above ground biomass yield), was determined by using the following formula:-

$$\text{Harvest index} = \frac{\text{Economical yield (calyx plant}-1\text{)(kg)}}{\text{Biological yield Dry matter (Kg)}} \times 100$$

Analysis of nitrogen use efficiency

Nitrogen use efficiency was determined after determining the plant tissue analysis for total N concentration and estimating the N uptake. N-uptake by leaves, calyx, and the seed was estimated as follows: N uptake = concentrations (%) x dry matter

Nitrogen use efficiency was determined after determining the plant tissue analysis for total N concentration and estimating the N uptake. To differentiate genotypes into efficient and inefficient nutrient utilizers. Nitrogen Efficiency Ratio (NER) = yield (kg)/nitrogen in plant tissue (kg); Agronomic Efficiency (AE) was determined to evaluate the economic production obtained per unit of nitrogen applied and was calculated as described by (36). Physiological Efficiency (PE) was calculated to estimate the biological yield obtained per unit of nitrogen uptake per increase in seed yield (kg/N).

Physiological efficiency (PE kg/kg) =

$$\frac{\text{The biological yield of N fertilized plot (kg)} - \text{Biological yield of N unfertlized plot (kg)}}{\text{Uptake in N fertilized plot (kg)} - \text{Uptake in N unfertilized plot (kg)}}$$

Apparent recovery efficiency (ARE) was calculated to determine a percent increase in the uptake of nitrogen in the fertilized plots as compared to nitrogen in the unfertilized plots, as described by (36). Apparent Recovery Efficiency (ARE %) =

$$\frac{\text{Uptake in N fertilized plot (kg)} - \text{Uptake in N unfertilized plot (kg)}}{\text{Amount of nitrogen applied (kg)}} \times 100$$

Utilization efficiency (UE): was estimated product physiological efficiency and apparent recovery efficiency, and it was estimated as described by.

$$\text{UE kg/kg} = \text{Physiological Effeciency (PE)} \times \text{Apparent Recovery Effeciency (ARE \%)}$$

Data analysis

A Shapiro-Wilk test was made to test the normality of the data, and analysis was done using the general linear model procedure by statistical software (SAS) version 9.1.3. Data The least significant difference (LSD) test was used to compare treatment means at a 5% probability level.

Results and Discussions

Yield response of Roselle varieties to levels of nitrogen

The collected yield variable analysis results show that the yield attributes of roselle were significantly *($p<0.05$)* affected due to varieties and different levels of nitrogen application. Higher and lower yield attributes were recorded from the application of 92 kg ha^{-1} N and unfertilized plots, respectively. Percent increase for a number of calyces' plants^{-1} (26.3%), fresh calyx yield ha^{-1} (18.5%), dry calyx yield ha^{-1} (18.3%), and economical yield (10.8%) from the application of 92 kg ha^{-1} N on WG-Hibiscus-Jamaica variety, and percent increase for a number of calyx plants^{-1} (22.3%), fresh calyx yield ha^{-1} (22.5%), dry calyx yield ha^{-1} (20.3%), and economical yield (22.8%) from the application of 92 kg ha^{-1}. This possibility might be that nitrogen stimulates growth, accumulates more biomass, and sets up a potential increase in yield. But the extent of a percent increase in biological yield was greater than the economic yield, which might be due to the increase in the amount of nitrogen and possibly more accumulation in vegetative growth, which promotes more growth in biological yield than economic yield. A similar finding was reported by application of nitrogen increases biomass yields as well as protein yield and concentration in plant tissue.

Higher economical yields were recorded from a lower level of nitrogen application, while lower values for these attributes were recorded from the higher level of nitrogen application as compared to the moderate level of nitrogen. This might be because a higher amount of nitrogen application promotes more vegetative growth because more accumulation of biomass growth results from an increase in biological yield rather than economic yield. This finding is in agreement with who found that higher biological yield was recorded from the higher level of nitrogen application, while higher economic yield was recorded from the application of a lower level of nitrogen. However, a higher and lower value for all yield attributes was recorded for the varieties WG-Hibiscus-Sudan and WG-Hibiscus-Jamaica, respectively. This might be due to the genetics of the variety, which might lead to it responding differently to nitrogen levels. This finding was in agreement with who found that different yield attributes were recorded between varieties in a study done on the response of cotton to different levels of nitrogen application (Table 2).

As the level of nitrogen increased, there was a slightly increasing and then decreasing trend for the harvest index The possible reason might be that an increase in the level of nitrogen promotes more vegetative growth and an increase in biomass yield rather than accumulation in the economic yield. This finding was in agreement with who found that the seed yield and harvest index increase with increasing levels of nitrogen up to 100 kg/ha and then decrease with a higher level of nitrogen application. The study done on the effect of nitrogen on agronomic yield, spad units, and nitrate content in Roselle under dry weather conditions. This finding is also in agreement with who found that increasing the level of nitrogen up to 100 kg ha^{-1} increases fresh and dry calyx yield at wider spacing.

The possible reason for low fresh calyx yields from the higher level of nitrogen is that the application might be promoting vegetative

Table 2: Yield response Roselle varieties to the different levels of nitrogen application at Hawassa, South Ethiopia.

Variety	Level of Nitrogen (kg ha^{-1})	Number of calyxes plant $^{-1}$	Fresh Calyx Yield (kg ha^{-1})	Dry Calyx Yield (kg ha^{-1})	Seed Yield Plant^{-1} (g)	1000-Seed Weight (g)	Biological yield (kg ha^{-1})	Economical yield (kg ha^{-1})	Harvest index (%)
WG -Hibiscus-Jamaica	0	15.1 ^{d}	4329^{d}	1319^{c}	7.9^{dc}	20.7^{f}	15046^{c}	4552^{b}	30.3^{c}
	23	15.6 ^{d}	5067^{bC}	1583^{b}	8.3 ^{dc}	27.7^{de}	15664^{c}	5324 ^{b}	34.0^{c}
	46	16.9 ^{d}	4747^{bcd}	1580.5^{b}	9.7^{bcd}	26.7^{de}	18519^{b}	5015 ^{b}	27.1^{c}
	69	17.1 ^{d}	4645^{dc}	1618^{b}	11^{bcd}	25.6^{e}	18519^{b}	4938 ^{b}	26.7^{c}
	92	17.2 ^{d}	5403^{ab}	1616^{b}	7.9 ^{dc}	25.7e	22377^{a}	5556^{bc}	24.8^{c}
	115	19.3^{d}	5309^{ab}	1610^{b}	6.9 ^{d}	28de	20833^{a}	5478^{b}	26.3^{c}
WG -Hibiscus-Sudan	0	34.6^{c}	4605^{dc}	1677.7^{ab}	10.8^{bcd}	32^{bc}	6250^{e}	4861 ^{b}	77.8^{a}
	23	39.2^{bc}	4944^{bcd}	1730.5^{ab}	12.4^{abc}	25.3^{e}	5324^{e}	5247^{b}	98.6^{a}
	46	41.9^{b}	4925^{bcd}	1751a^{b}	14.6 ^{ab}	25.6^{cde}	6250^{e}	5324^{b}	85.2^{a}
	69	43.8^{b}	5046^{bc}	1705.5^{ab}	16.5 ^{ab}	47.3^{a}	6327^{e}	5478^{b}	86.6^{a}
	92	50.3^{a}	5934^{a}	1866.6^{a}	12.9^{abc}	33.3^{b}	7639^{e}	6250^{a}	81.8^{a}
	115	40.6^{bc}	4982^{bcd}	1580.5^{b}	10.1^{bcd}	29.7^{cd}	11188^{d}	5247 ^{b}	46.9^{b}
Mean		29.3	4994.5	1636.5	10.7	29.2	12828	5273	53.8
LSD (0.05)		6.2	659.73	233	5	3.6	1900	613	23.3
CV (%)		12.6	7.8	8.4	27	7.3	21.2	7.4	8.4

growth by increasing the number of primary and secondary branches, which makes them only set calyx on the top of the respective branch. In general, higher and lower responses for yield attributes were recorded from WG-Hibiscus-Sudan and WG-Hibiscus-Jamaica, respectively, at a different level of nitrogen application. This difference in their extent of response might be controlled by their growing environment's interaction as well as root morphology. A similar finding was reported in Nigeria.

Nitrogen use efficiency of Roselle varieties at different levels of nitrogen

Study results show the nitrogen use efficiency of roselle was significantly (*p<0.05*) affected by the level of nitrogen and varieties (Figure 2). Higher values for efficiency parameters except apparent recovery efficiency were recorded from the WG-Hibiscus-Sudan variety, while lower values were recorded from the WG-Hibiscus-Jamaica variety. As the level of nitrogen increased, there was a significant decrease in the nitrogen efficiency ratio and agronomic efficiency. It is possible that nutrients accumulated in the above-ground part of the plant or the nutrients recovered within the entire soil-crop root system might be determined by the genetic makeup of the cultivar and lower absorption and utilization of absorbed nutrients due to the growing environment, which consequently results in a reduced nitrogen efficiency ratio and agronomic efficiency. Similar findings were reported by study on soil and input management options for increasing nutrient use efficiency. Conversely, as the level of nitrogen increased, a significant increase was recorded in physiological efficiency and apparent recovery efficiency.

Higher values were recorded for physiological and apparent recovery efficiency from the application of 115 kg ha^{-1} N, which was statistically at par with 92 and 69 kg ha^{-1} N, while lower values were recorded from the application of 23 kg ha^{-1} N. A possible reason for those differences in the efficiency of nitrogen between varieties might be a genetic capability that makes the ability of varieties within species to absorb nutrients at a higher rate, which makes plants responsible for efficient nutrient use at a low nutrient concentration of the growth medium. A higher nitrogen efficiency ratio was recorded from an unfertilized plot of WG-Hibiscus-Sudan, while a lower value was recorded from the application of 115 kg ha^{-1} N on variety WG-Hibiscus-Jamaica. This might be the potential reactiveness of roselle varieties to different levels of nitrogen. Similarly, higher agronomic efficiency was recorded from the application of 23 kg ha^{-1} N on WG-Hibiscus-Jamaica, while a lower value was recorded from the application of 115 kg ha-1 N on WG-Hibiscus-Sudan. Similar findings were reported who found that the highest agronomic efficiency was observed in soil with fertile soil and moderate N content, while the lowest was observed in soil with high soil fertility and N content (Figures 1 & 2).

Comparatively higher values for physiological and apparent recovery efficiency were recorded from WG-Hibiscus-Sudan, while lower values were recorded from WG-Hibiscus-Jamaica. The WG-Hibiscus-Sudan variety is non-responsive and efficient, while the WG-Hibiscus-variety is responsive and inefficient because the latter type of variety had a lower yield at a lower level of nitrogen application and a slight increase in efficiency ratio declined as the level of nitrogen increased. On the contrary, a higher efficiency value was recorded from the lower level of nitrogen application in the WG-Hibiscus-Sudan Variety and declined as the level of nitrogen increased. This possibly refers to the better efficiency of the WG-Hibiscus-Sudan variety as compared to the WG-Hibiscus-Jamaica variety (Figure 2).

In conclusion: The highest and lowest yield attributes and harvest index were recorded on the WG-Hibiscus-Sudan variety from the application of 92 kg ha^{-1} N and the WG-Hibiscus-Jamaica variety, respectively. Similarly, higher nitrogen use efficiency except agronomic efficiency was recorded from WG-Hibiscus-Sudan, while lower except agronomic efficiency was recorded from the WG-Hibiscus-Jamaica variety, for which higher agronomic efficiency was recorded. Among evaluated varieties at a different level of nitrogen application, the WG-Hibiscus-Sudan variety was observed as efficient but non-responding, and the WG-Hibiscus-Jamaica variety was observed as an inefficient and responding variety because for the former variety, a higher nitrogen efficiency ratio was recorded at a lower level of nitrogen application as compared to the later variety. In this study, for optimum calyx yield and profitability, the application of 62 kg ha^{-1} on WG-Hibiscus-Sudan and 92 kg ha^{-1} on WG-Hibiscus-Jamaica was found to be economical and efficient. Thus, further study should be carried out on the response and efficiency of roselle with different levels of nitrogen, and phosphorus at different locations to effectively estimate the efficiency, response, and yield of roselle varieties.

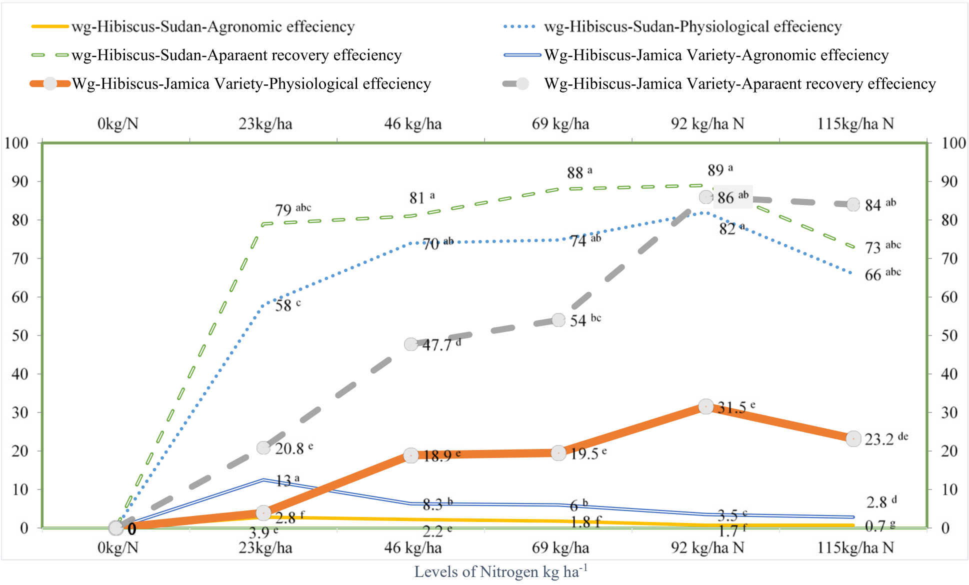

Figure 1: Nitrogen use efficiency of Roselle varieties at different levels of nitrogen application at Hawassa, South Ethiopia.

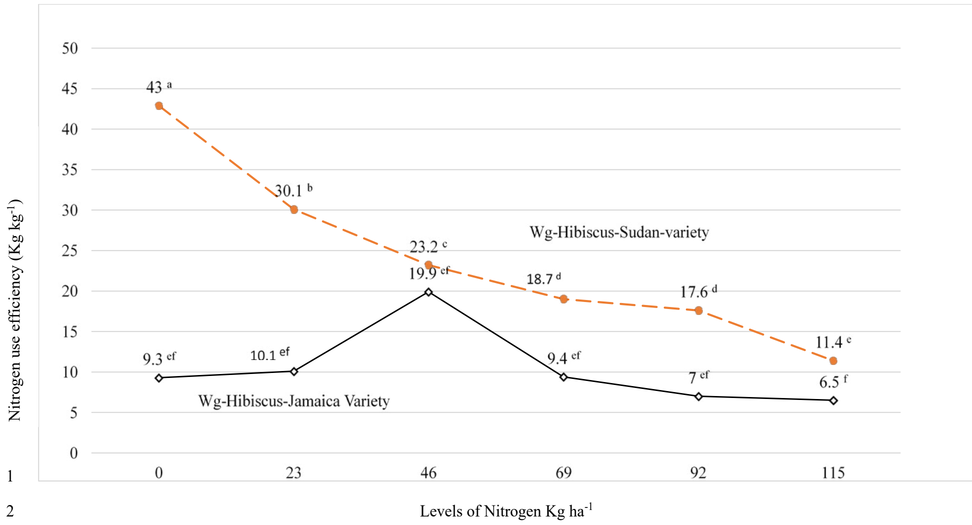

Figure 2: Nitrogen efficiency ratio of Roselle as affected by different levels of nitrogen.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Proposal writing, field and laboratory data collection, advising, and research report write-up

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