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The response of Swiss chard (*Beta vulgaris* L.) to nitrogen levels and intra-row spacing in Debre Berhan Central Ethiopia

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ABSTRACT

Purpose: Swiss chard (*Beta vulgaris* L.) is highly appreciated for its nutritional properties, year round availability, low cost and wide use. In central highlands of Ethiopia, Swiss chard has been producing in urban and peri-urban areas but limited information is available about its nutrition and planting distance. **Research method:** An experiment was designed to study the effects of 50, 100 and 150 kg ha^{-1} and control (0 kg ha^{-1}), and different spacing with row including 20 cm, 30 cm and 40 cm distance on growth, yield and nutritional composition of Swiss chard in Debre Berhan. Treatments were set in randomized complete block design with three replications making 45 treatment combinations. **Findings:** Results indicated that total plant fresh weight, total plant dry weight, above ground fresh weight, above ground dry weight, root fresh weight, root dry weight, plant height, leaf number per plant were significantly affect by the interaction effect of plant spacing and N levels. Leaf area index was significantly affected by intra-row spacing but not by N rate and their interaction. Remarkable difference was recorded on total nitrogen, nitrate contents and vitamin C of leaves due to varied N levels. Generally, growth attributes, yield performance and nutritional composition of Swiss chard obtained best at the combination of 100 kg N ha^{-1} N and 40 cm plant spacing. **Research limitation:** Intra-Row needs further study above the 40 cm planting distance. **Originality/Value:** combination of 100 kg N ha^{-1} and 40 cm in-row spacing can be used for Swiss chard production in Debre Berhan central highlands of Ethiopia.

INTRODUCTION

Swiss chard (*Beta vulgaris* L.*)* is a biennial leafy vegetable belonging to Chenopodiaceae family. It is highly appreciated in many parts of the world for its nutritional properties, year round availability, low cost and wide use in many traditional dishes as well as more robust and easier to grow than spinach and celery (Gao et al**.**, 2009). In Ethiopia, it has been producing around urban and peri-urban areas compared to lettuce and cabbage and little research effort was made before on this vegetable. However, this vegetable has nutritional advantages that can contribute significant positive impact on improving food security especially on reducing malnutrition.

Swiss chard is very low in saturated fat and cholesterol contents and is a very good source of dietary fiber, vitamins A, C, E, K, B2, B6, thiamin, folate, calcium, iron, magnesium, potassium, copper, phosphorus, zinc and manganese (Knežević et al., 2014). Ninfali and Angelino (2013) reported that bioactive molecules from Swiss chard extracts have anti-diabetic, anti-inflammatory, anti-oxidant and anti-cancer activities. In addition, it has heart protection flavonoids and siringic acid, due to the high content of potassium, it helps the release of water from the body and it is an ideal food that keeps the blood pressure under control. Moreover, it is rich in biotine, an important vitamin for the hair (Mihai et al., 2015).

Fertilizer use is the most practical and effective agronomic practice that improves yield and nutritional quality of many vegetable crops for human consumption (Anjana et al., 2007). Content of mineral elements, total quality and yield are influenced by the amount, frequency and method of fertilization (Miceli & Miceli, 2014). Particularly, N play a fundamental role in vegetative growth and hence yield and quality of Swiss chard. For instance, vitamin C and sugar contents in the aboveground parts of spinach and Swiss chard are increased under the application of high rates of N (Dzida & Pitura, 2008). Likewise, high levels of N cause compositional changes (reduced ascorbic acid, sugar and acidity) and altered ratios of essential amino acids; as well reduced yields, low N levels generally result in less protein content in harvested vegetables, and inferior other nutritional and visual qualities (Zeka et al., 2014).

However, an application of excess N fertilizer has negative impact on consumer, environment and the crop itself. According to Hoque et al. (2010), about 80 % of dietary nitrates are derived from vegetable consumption where if taken by humans it will result in so many chronic diseases. Especially, Swiss chard has great tendency to accumulate more nitrate and over 60% of its total nitrate is accumulated in petioles (Santamaria et al., 1999). Therefore, proper N fertilizer application at the farm level is very important to reduce the risk of human health and environmental pollution as well as maintain the product quality.

In addition to the fertilizer management, planting population per unit area is one of the main factors influencing crop yield. An increase in the number of plants per fixed area may reduce or increase the yield depending on the varieties, environment and other management practices. Optimizing plant density is very important to obtain good yield and quality product and plant spacing is dependent on many factors such as climactic condition and varietal difference. Farmers have been producing Swiss chard in Ethiopia especially in highland areas as well as in in urban and around urban areas under both irrigation and rain-fed conditions. However, little information on doses of N and intra-row spacing is scant. Hence, determining of planting spacing and amount of nitrogen is necessary for specific cultivar and environment. Therefore, the objective of this research was to determine the N rate and intra-row spacing for Swiss chard under Debre Berhan condition.

MATERIALS AND METHODS

Experimental site description

Research was conducted at Debre Berhan University research farm located at 09° 35' 45" to 90° 36' 45" north latitude and from 39° 31' 30" east longitude and found in the 125 km North East of Addis Ababa with an elevation 2845 m above sea level. Climatic data obtained from the Ethiopian national metrology agency indicates that, the area receives a mean annual rainfall of 927.10 mm and characterized by a unimodal rainfall pattern with a from 4.72 mm to 293.02 mm and peaks in August and December, respectively. The mean monthly minimum and maximum temperature range was from 2.4 to 8.9 °C and 18.3 to 21.8 °C, respectively.

Experimental treatment, design and procedures

The treatments consisted of four nitrogen rates (0, 50, 100 and 150 kg ha^{-1}) calculated from urea used as sources of nitrogen and three intra-row spacing (20, 30 and 40 cm) applied for Swiss chard plant variety '*Fordhook Giant*'. Treatments were arranged in a randomized complete block design (RCBD) at which each treatment was replicated three times with six rows in each plot. Plots and blocks were separated by 1m. Nitrogen fertilizer was applied three times in split method at transplanting time (35 days old seedling) and then 30 and 65 days after transplanting for efficient use. Other cultural practices were applied uniformly to all of plots throughout the experimental time.

Soil sampling and analysis

The representative soil samples were collected before transplanting from 0-30 cm in collecting in zigzag manner using a soil auger make once composite sample to analyze for the experimental site soil texture and soil chemical properties. Soil pH was measured at 1:2.5 soil, water suspension, using the pH meter. The textural class, soil cation exchange capacity (CEC), total N, Organic carbon and available phosphorus were analyzed based on standard procedures (Van Reeuwijk, 2002).

Collected crop Data

Total plant fresh and dry weight, above ground fresh and dry weight, underground fresh and dry weight, plant height, leaf number per plant, leaf area index, were determined using both destructive and nondestructive sampling method accordingly. Vitamin C content in fully mature leaves of Swiss chard was determined by Bajaj and Kaur (1981). Total N of the leaves was determined by micro-kjeldahl method and nitrate was determined as described by AOAC official methods of analysis (AOAC, 2002) by calorimetrically through reduction of nitrate to nitrite at maturity

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the statistical analysis system (SAS), Version 9.13 (SAS Institute, Cary, NC) as appropriate to the design of the experiment. The mean separation was done using least significant difference (LSD) at the 5% level of probability.

RESULTS AND DISCUSSION

Soil physical and chemical properties of the experimental site

Available P, total nitrogen, organic matter, pH and CEC of the soil were analyzed as shown in Table 1. The soil had textural class of clay loam with 39% clay, 32% silt and 29% sand, with pH 5.8 showing the nature of the soil as moderately acidic (Bruce & Rayment, 1982). The available P (13.30 ppm) lies in the moderate range (Holford & Cullis, 1985), however, the total N (0.15%) was low level (Charman & Roper, 2007).The value of 1.90% organic matter was rated as moderate (Metson, 1957). Similarly, the CEC of the soil was 25.30 c mol (+) kg^{-1} showing it in medium range; the amounts of exchangeable K, Na, Ca and Mg in the soil was found at 0.73, 0.2, 20.81 and 3.58 cmol (+) kg^{-1}, respectively (Table 1).

Table 1. Pre planting soil physic-chemical properties of the experimental site

Parameters	Value	Methods
Clay: Silt: Sand (%)	29: 39: 32	Hydrometer
Texture class	Clay loam	
pH (1:2.5 soil to water)	6.0	
Organic matter (%)	1.90	Walkley and Black
Total N (%)	0.1	Kjeldahl
C:N ratio	11	
Available P (ppm)	13.30	Olsen
CEC (cmol(+) kg^{-1})	27.5	
Base saturation (%)	92	
Exchangeable Na($cmol_c$ kg^{-1})	0.02	
Exchangeable K($cmol_c$ kg^{-1})	0.71	Ammonium acetate
Exchangeable Ca($cmol_c$ kg^{-1})	20.79	
Exchangeable Mg($cmol_c$ kg^{-1})	3.60	

Growth and yield responses of Swiss chard to N and intra-row spacing

Above ground fresh and dry weight

Analysis of variance revealed that above ground fresh weight significantly ($p<0.05$) affected by nitrogen rate, intra row spacing and their interaction. As shown in Table 2 the highest values of above ground fresh and dry weight of Swiss chard plants were recorded from treatment combinations of 40 cm in row spacing and 100 kg N ha^{-1} rate and the lowest values were in 20 cm intra row spacing and 0 kg N ha^{-1} (control treatment). The significant impact on dry and fresh weight of the Swiss chard in high rate might be due to the fact that N is a component of chlorophyll, nucleic acids, nucleotides, coenzymes, phytohormones and cytokinins and hence, its suboptimal amount inhibits emission of new cells and plant organs. Miceli and Miceli (2014) also reported similar findings. van Averbeke et al. (2007) reported that fresh and oven-dry mass of marketable leaves increased with an application rate of N in *Brassica rapa subsp. Chinensis*. Besides, lower intra-specific competition for light, water and nutrients in the greater plant spacing favors the further growth and development of plants (Echer et al., 2012) Generally, optimum N level and plant density support rapid cell division and enlargement that results in fast vegetative growth and consequently high fresh and dry weight accumulations.

Table 2. Effects of N and intra row spacing on above ground fresh weight (AFW), above ground dry weight (ADW), total plant fresh weight (TPFW), root fresh weight (RFW), root dry weight (RDW) petal dry weight and total plant dry weight (TPDW) of Swiss chard

Treatment		Parameters					
Intra-row (cm)	N (kg ha^{-1})	TPFW (g)	TPDW (g/plant)	FW (g/plant)	DW(g/plant)	RFW (g/plant)	RDW (g/plant)
20 cm	0	1320^{f} †	263.6^{e}	696^{d}	106.4d	278.5^{bcd}	74.44^{bcd}
	50	3373.5^{bc}	527.82^{abc}	5256^{bc}	667.1c	384.5^{ab}	102.77^{b}
	100	3178^{bcd}	518.82^{abc}	5117^{bc}	672.0c	347.5^{b}	92.88^{bc}
	150	2710^{cde}	415.85^{cde}	4132^{c}	633.1c	267.5^{bcd}	71.5bcd
30 cm	0	2744.5^{cde}	432.75^{bcde}	4276^{bc}	692.9bc	336^{bc}	89.81^{bcd}
	50	3301^{bcd}	511.85^{abcd}	5152^{bc}	795.2abc	342^{bc}	91.42^{bcd}
	100	2104^{ef}	329.6^{de}	3374^{c}	815.9abc	224.5^{cd}	60.0^{d}
	150	3977^{ab}	612.26^{ab}	6638^{ab}	1003.5ab	381.5^{ab}	101.97^{b}
40 cm	0	2795^{cde}	427.13^{cde}	4317^{bc}	692.9bc	339^{bc}	90.61^{bcd}
	50	2861.2^{cde}	443.2^{bcde}	493^{bc}	785.0abc	288^{bcd}	76.97^{bcd}
	100	4242.5^{a}	669.62^{a}	8707^{a}	1017.9^{a}	504^{a}	134.71^{a}
	150	2455de	383.56^{cde}	3812^{c}	591.3c	195^{d}	69.89^{cd}
	LSD	862.48	183.52	2396.2	314.72	122.6	31.93
	Seg. Level	***	**	*	*	*	*
	CV	14.095	18.36	24.31	21.78	17.04	14.212

† Means of the same main effect within columns followed by the same letter are not significantly different at 5% of probability Fisher's least significant difference (LSD)

Fresh and dry weight of root

Root dry weight and fresh weight had been significantly ($p<0.05$) affected by interaction effects of N and in-row spacing (Table 2). The highest root fresh and dry weight was obtained at 40 cm in-row spacing with 100 kg N ha^{-1}. However, the lowest root fresh weight was obtained from 150 kg N ha^{-1} and 40 cm supporting the fact that N deficient plants have poor root development. Petropoulos et al. (2008) also reported that root weight per plant increased with N application in Parsley. Similarly, Sharma et al. (2013) conducted an experiment on the influence of plant density and nutrient management on growth, yield and quality of radish, found that maximum root fresh and dry weight recorded in wider spacing, which might be due to individual plant that utilizes more N nutrient applied. Optimum plant density ensures wise use of resources and increases the accumulation of photo-assimilate under optimum soil fertility especially nitrogen where needed by plants in substantial amount.

Total plant fresh weight

Interaction effects of N rate and in-row plant spacing had shown significant ($p<0.05$) effects on total fresh weight of Swiss chard. The highest and lowest total plant fresh weight had shown in treatment combinations of 40 cm x 100 kg N ha^{-1} and 20 cm x control treatment (unfertilized plot), respectively (Table 2). Hasan et al. (2017) also reported similar findings in lettuce plant. Echer et al. (2012) showed that application of N promoted a linear increase in fresh weight of total and marketable shoots of Swiss chard. Similarly, Kołota and Chohura, (2015) found that irrespective of plant population, enhancement of N rate resulted in an increment of cabbage yield in which indicate that plant population only at high rate of N affected the total yield of cabbage. This could be due to the high competition among plants in case of narrow spacing (20 cm) as well as a lack of N (0 kg N ha^{-1}). This might be resulted in a small number of leaves, narrow leaf expansion and poor vegetative growth as N influences cell division and main components of the leaf chlorophyll. In the same way, Echer et al. (2012) also justify that narrow spacing between plants results in higher density, favored disease proliferation, infecting the lower leaves of Swiss chard plants which in turn resulted in lower fresh weight.

Total plant dry weight

The effects of N and in-row spacing on Swiss chard total plant dry weight is illustrated in Table 2. Results revealed that significant ($p<0.05$) variation in total plant dry weight was shown due to interaction effects of N and in-row spacing. The highest value of plant dry weight was obtained from 40 cm in-row spacing with 100 kg N ha^{-1} whereas the lowest value was obtained from plots treated with 150 kg N ha^{-1} and 40 cm planting distance (Table 2). The highest plant dry weight obtained from the widest planting distance with relatively high N rate could be due to less competition for resources such as moisture, sun light and fertilizer. Results obtained by Hasan et al. (2017) in lettuce were also similar with the present study. This also justified that N has various effects on plant physiological processes such as photosynthesis, transpiration rate and stomatal conductance. Similar to the current results, van Averbeke et al. (2007) reported a reducing trend for fresh and oven dry mass of marketable yield decline when the application rate exceeded the 250 to 300 kg N ha^{-1}. In line with these findings, Hasan et al. (2017) underlined that optimum level of N and plant spacing ensured the maximum vegetative growth and the allocation of optimum number of plants that leads to produce the highest yield per hectare.

Plant height

Results of variance analysis showed that interaction of intra-row spacing and nitrogen rate had significant ($p<0.05$) effect on plant height (Table 3). The tallest plants were observed in the narrow plant spacing (20 cm) that received 150 kg N ha^{-1} (Table 3). Whereas, the shortest plants of Swiss chard were observed at 40 cm combined with unfertilized plot. Hasan et al. (2017) also reported similar results. Generally, slender plants were observed under narrow spacing with low nitrogen rate. Miceli and Miceli (2014) observed that plant height at harvest increased linearly by increasing the N level up to 100 kg N ha^{-1}. Similarly, Dzida et al. (2012) also shown that plants which had received higher doses of different N forms of fertilizers resulted in higher plant size by 3 cm than the lowest rates. Plants deficient in nitrogen also develops thin and spindly stems. Nitrogen fertilizer ensured favorable condition for the elongation of leafy vegetable plants with optimum vegetative growth. Length of stems may occur when plants compete for light at dense plant population. Besides, when plants are exposed to low light radiation due to high population per unit area they compete for that via increasing their internodes towards light with less number of leaves.

Number of leaves per plant

The effects of in-row spacing and N rate was shown significant ($p<0.05$) variation in leaf number per plant. As shown in Figure 1, with decreasing in plant distance a decreasing number of leaves was observed and the lowest number of leaves (8.5) was recorded from the control treatment (0 kg N ha^{-1}) combined with 20 cm distance between plants. Conversely, the highest number of leaves per plant (28.38) was obtained from 150 kg N ha^{-1} combined with wider in-row spacing (40 cm). Miceli and Miceli (2014) in Swiss chard and Hasan et al. (2017) reported similar findings in lettuce plants. The lowest numbers of leaves per plant were recorded at 20 cm in-row spacing and 150 kg N ha^{-1}indicating that at high N level may not be granted for the new leaf formation and high yield if the spacing is not optimum as competition for resources is high. Similarly, increasing the amount of N had increased the number of leaves in Parsley in two trials of consecutive years (Petropoulos et al., 2008). As shown in Figure 1, the number of leaves was decreased with increment of internode length, which intern reduces the leaf fresh weight per plant and leaf dry weight (Fig. 1). Maboko and Plooy (2013) also reported that more number of leaves per plant were observed in wider plant spacing in lettuce under the soilless production system. This also reduces competition of individual plants in higher spaces and higher rate of N hastens new leaves production, which intern increases yield in Swiss chard.

Leaf area index (LAI)

Significant variation had shown on leaf area index when plants were planted in the different spacing (Fig. 2). As shown in Figure 2, the small LAI was shown from plants arranged at spacing of 20 cm. Much larger leaves were recorded from the higher spacing as compared to closer spacing.

Plants arranged with 30 cm and 40 cm planting distance was wider than plants with 20 cm spacing by 53.5% and 27.9%, respectively. This might be due to relatively less competition for nutrient, moisture available and other resources in higher spacing. Densely planted crop impede the proper growth and development of the plants as poor expansion of leaf area reduce photosynthetic capacity of the plant and in turn reduce the carbohydrates supply and storage of the plant.

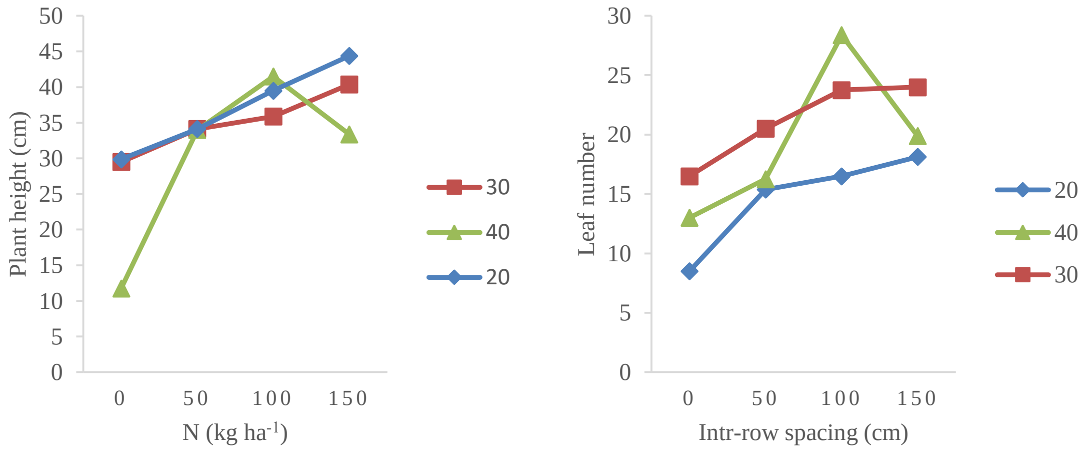

Fig. 1. Interaction effects of N and Intra-row spacing (cm) on plant height (cm) and leaf number of Swiss chard

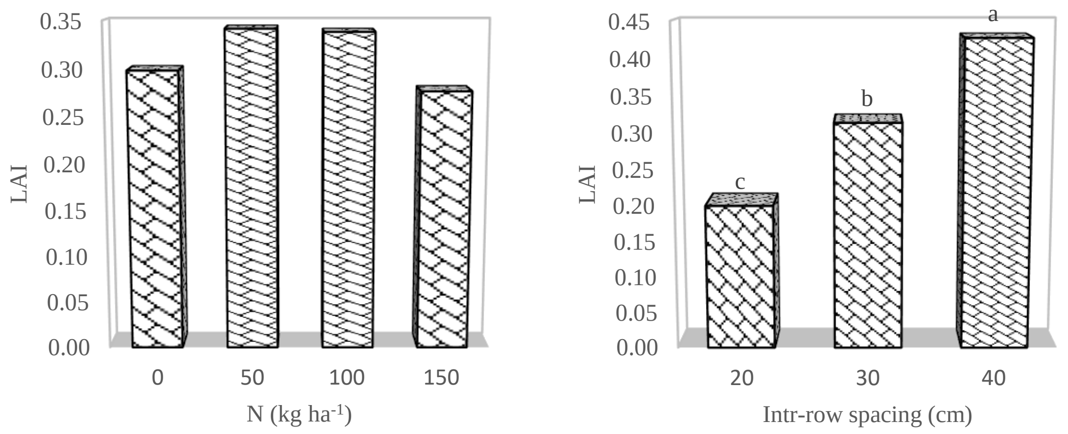

Fig. 2. Effects of N rate and within-row spacing on leaf area index of Swiss chard

Effects of N rate and intra-row spacing on N and vitamin C content of Swiss chard

Total nitrogen of leaf and nitrate content

Results reveled that different rates of N had significant ($p<0.05$) effect on total N and nitrate content of Swiss chard leaves. The effects of in-row spacing and its interaction with nitrogen rate was statistically inconsistent ($p>0.05$). Total N and nitrate increased with increasing N level, but it was not consistent with in-row (planting distance) (Fig. 3). Similar results of total N and nitrate content was obtained from treatment with 100 and 150 kg N ha^{-1} (Fig. 3).

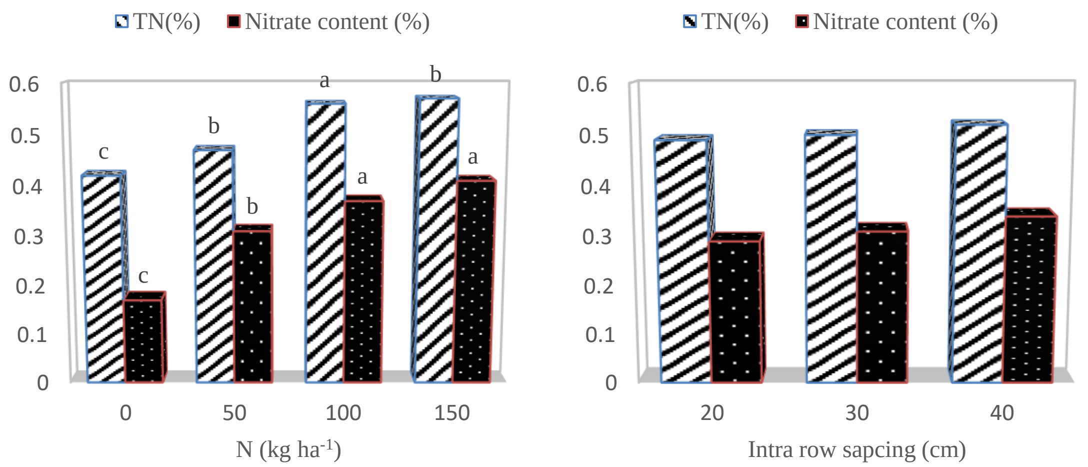

Fig. 3. Effects of N rate and intra-row spacing on leaf nitrate and total nitrogen content of Swiss chard

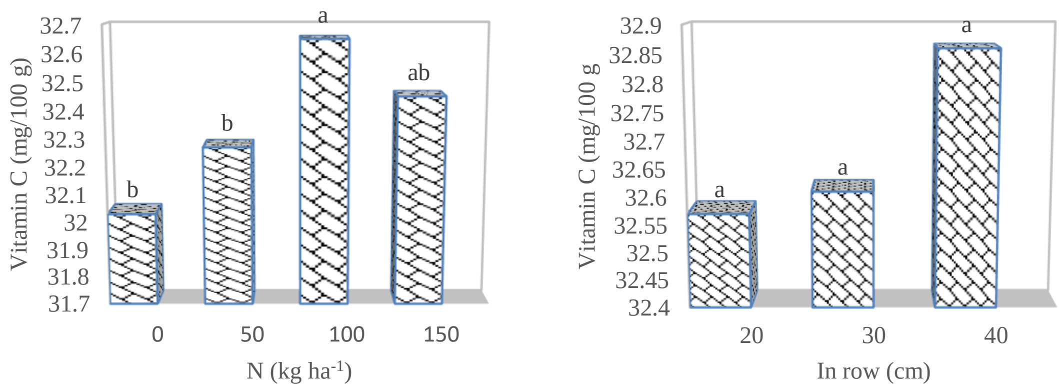

Fig. 4. Effects of N rate and within-row spacing on vitamin C content of Swiss chard leaves

Similarly, Dzida et al. (2012) reported that different rates and form of N increased total N content of leaf. With increasing the N dose from zero to 100 kg N ha^{-1} an increase in nitrate content was recorded (Fig. 2). However, statistically similar content of nitrate was measured on Swiss chard plants treated with 100 and 150 kg N ha^{-1}. In accordance with the current findings, Miceli and Miceli (2014) on Swiss chard, Hasan et al. (2017) in cosmos caudatus, Konstantopoulou et al. (2010) on lettuce, Biesiada et al. (2010) in Red cabbage and Zeka et al. (2014) on spinach also shown an increase in nitrate content with an increment of N fertilization. Besides, Dzida et al. (2012) underlined that not only the N fertilization rate but also plant species and variety, harvest date, and the growth medium affects the content of nitrate in plant material. As nitrate is harmful to animals and humans, application of N to fresh vegetables needs care. Rembialkowska (2007) also point out that readily soluble chemical fertilizers which are absorbed rapidly into the plant tend to lead to higher nitrate/nitrite levels and may result in formation of nitrosamines which have been associated with chronic diseases such as Leukaemia and gastrointestinal cancers. However, the effects of in-row spacing of total N and nitrate content of Swiss chard plants were not remarkable. Conversely, Kołota and Chohura (2015) reported that high population density decreases NO_3^- content in cabbage.

Vitamin C

Different rate of N significantly ($p<0.05$) affected the content of vitamin C in Swiss chard leaves. However, the effects of different in-row spacing and interaction with different N rate of vitamin C were inconsistent ($p>0.05$). The highest value of vitamin C was recorded in 150 and 100 kg N ha^{-1} treatments and lowest value was at 50 and 0 kg N ha^{-1} (Fig. 4). In the current results intra row spacing did not affect the vitamin C content of Swiss chard that was in agreement with Riad et al. (2009) in cabbage.

Vitamin C contents of Swiss chard leaves were affected by different rates of N application rather than planting distance. This could be due to the direct effects of N as components of plant cell and involved in major plant physiological process. In accordance with the current result, Dzida and Pitura (2008) showed that vitamin C and sugars content in the above ground parts of spinach and Swiss chard increases under the application of high rates of N fertilizers with a range of 43.8 and 59.8 mg/100gm of fresh weight. Similarly, Vitamin C content increased with increment of N dose were observed by Biesiada et al. (2010) in Red cabbage. Vitamin C content increased with increase in N dose. Dzida et al. (2012) also reported that vitamin C was shown variation under different doses of N fertilizer. Similarly, Zeka et al. (2009) shown that vitamin C content increased as rate of N increased up to 240 kg N ha^{-1} in spinach. Conversely, vitamin C content of lettuce and Swiss chard was decrease with increment of N application rates as reported by Konstantopoulou et al. (2010) and Kolota and Czerniak (2010) respectively. Even, Kolota and Chohura (2015) found that the vitamin C content of cabbage was not affected by different rates of N fertilizer. This all confirmed that N has significant effects on leaf composition of Swiss chard. However, the inconsistent reports in the literature could be due to variation in variety and other environments as well as management practices.

CONCLUSION

The study suggested that a prominent effect of both N rate and intra-row spacing were observed on growth, yield and composition of Swiss chard. Plant height, number of leaves, fresh weight of root, total plant fresh weight, root dry weight and total plant dry weight was significantly influence by N levels and different intra-row spacing. Vitamin C, nitrate and total nitrogen contents significantly affected at high N doses (150 kg N ha^{-1} and 100 kg N ha^{-1}). Our research proposed that combination of 100 kg N ha^{-1} and 40 cm intra could be possible to use under Debre Berhan condition and other areas with similar agro ecology.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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