Nitrogen Application and Leaf Harvesting Improves Yield and Nutritional Quality of Beetroot

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SUMMARY. Beetroot (Beta vulgaris), commonly known as table beet, is used as a staple in the diet of many people through the consumption of the entire plant, leaf, and the root. The objective of this study was to assess the effects of nitrogen (N) application and leaf harvest percentage on the yield and quality of roots and leaves of beetroot. The treatment design was a randomized complete block design with five levels of N (0, 60, 90, 120, and 150 kg ha–1) combined with three leaf harvest percentages (0, 30, and 50) and replicated three times. The first leaf harvest was initiated 35 days after transplanting (DAT) by removing the older matured leaves and the second harvest occurred 80 DAT by removing all the leaves. The results showed increases in leaf and root yield with an increase in N application. Nitrogen application at 90 and 120 kg ha–1 increased fresh leaf weight, leaf number, and fresh and dry root weight, including root diameter and length with the exception of leaf area which was significantly higher at 120 kg ha–1 N. Magnesium and iron leaf content, and N root content were significantly improved by the application of 120 kg ha–1 N. Leaf harvest percentage did not have a significant effect on leaf yield or root leaf mineral content. However, dry root weight was significantly reduced by the 50% leaf harvest. Leaf harvest at 30% or 50% increased total protein content of the roots of beetroot, whereas an increase in N application decreased concentration of total proteins. Results demonstrate that leaf and root yield, as well as magnesium, zinc, and iron leaf content, increased with the application of 120 kg ha–1 N, whereas 30% leaf harvest did not negatively affect root yield.

Beetroot is a cool weather crop, produced for its leafy greens and roots. It is rich in active compounds such as carotenoids, glycine, betaine, betacyanines, folates, betanin, polyphenols, and flavonoids (de Zwart et al., 2003; Dias et al., 2009; Vali et al., 2007). Beetroots can be consumed together with other vegetables in the form of salads, or can be consumed commercially as a dye to color processed food. The roots of beetroot are also used as a nutrient supplement and as a canned product.

Nitrogen is one of the most important minerals in determining plant growth and crop yield. It is a component of proteins, enzymes, and vitamins in plants as well as many structural, genetic, and metabolic compounds (chlorophyll and amino acids) in plant cells required for biomass synthesis (Petek et al., 2012).

Most farmers apply N fertilizer to increase crop yields. Insufficient supply of N can decrease plant N content, reducing photosynthetic production (Boussadia et al., 2010) and thus, reduce plant growth and decrease quality of harvestable materials (Mikkelsen and Hartz, 2008). An increase in plant growth characteristics such as plant height, shoot dry matter, and leaf area index in most crops was reported to be due to N application (Najm et al., 2013). The optimal application of N fertilizers positively impacts production. However, oversupply of N does not always lead to increased yield, and it might actually result in reduced growth and yield. Excessive application of N fertilizers leads to delayed maturity and competition between sink (tubers) and source (leaves), and occasionally lower yields (Najm et al., 2013). Excessive N application does not only delay plant maturity, but limits the formation of storage organs, especially for crops where roots and tubers are harvested. This results in lower yields and can reduce the quality of storage organs in terms of flavor and physical characteristics due to low sugar content, lower acidity, and reduced firmness (Johnson, 2014). The nutritional value of the crop will also be negatively affected by causing high nitrate accumulation (Bahn, 2000).

Leaves are responsible for providing the plant with photosynthates during the entire growing season. However, leaf removal can negatively affect plant growth, yield, and quality of beetroot. Isutsa and Mallowa (2013) reported that pumpkin (Cucurbita moschata) leaf and fruit yields were affected by leaf-harvesting intensity. Excessive leaf removal will result in a reduction in the production of photo-assimilates required for plant growth (Isutsa and Mallowa, 2013; Kiozya et al., 2001), thus affecting the yield negatively.

There is a demand for beetroot leaves as well as roots for consumption due to their nutritional value (Dias et al., 2009; Vali et al., 2007).

Units

| To convert U.S. to SI, multiply by | U.S. unit | SI unit |
|----------------------------------|-----------|---------|
| 0.3048                           | ft        | m       |
| 0.929                            | ft²       | m²      |
| 3.7854                           | gal       | l       |
| 2.54                             | inch(es)  | cm      |
| 25.4                             | inch(es)  | mm      |
| 1.1209                           | lb/acre   | kg ha–1 |
| 1                                | ppm       | mg kg–1 |
| 6.8948                           | psi       | kPa     |

\[(\text{°F} - 32) \times \frac{5}{9} = \text{°C} \]

\[(\text{°C} \times 1.8) + 32 = \text{°F}\]
Beetroot leaves are generally harvested at the end of the growing season together with the roots. South Africa is also promoting the intake of nutritious leafy vegetables to alleviate nutritional deficiencies, thus reduce disease especially in rural communities (Faber et al., 2007). In spite of the importance of beetroot as a leaf and root crop, there is limited information on procedures for leaf harvesting that growers should follow to maximize yield and quality of beetroot, unlike for other crops, such as pumpkin (Isutsa and Mallowa, 2013) and sweetpotato [Ipomoea batatas (Kiozya et al., 2001)].

There is also limited information on how leaf harvest percentage will affect beetroot production and how N management may modify that effect. Beetroot has the ability to generate new leaves, and the percentage of how many leaves could be harvested early without reducing harvestable yields (leaves and roots) at the end of the growing season is not known. An understanding of the plant’s response to leaf harvest and N application is important in developing improved cultivation practices for increased beetroot yield and nutritional quality. Therefore, the aim of this study was to determine the effect of N application and leaf harvest percentage on the yield and nutritional quality of roots and leaves of beetroot.

Materials and methods

Trial location. The trial was conducted under a shade net at the Agricultural Research Council, Rooi- deplatz, South Africa (lat. 25.59°S, long. 28.35°E, 1200-m altitude). The average minimum and maximum temperatures, rainfall, and relative humidity during the experimental period are presented in Table 1. Beetroot seeds of cultivar Crimson Globe were sown in 200-cavity polystyrene trays filled with a seedling growing medium (Hygromix®; Hygrotech, Pretoria, South Africa) to produce transplants according to the methods described by Maboko and Du Plooy (2013).

Soil type was a sandy clay loam comprising 68.0% sand, 8.0% silt, and 24.0% clay in the top 30 cm. The chemical composition of the soil comprised 25.9 mg kg⁻¹ phosphorus (P), 267 mg kg⁻¹ potassium (K), 1313 mg kg⁻¹ calcium (Ca), 437 mg kg⁻¹ magnesium (Mg), and 54.9 mg kg⁻¹ sodium (Na), with a soil pH of 7.5. Soil samples were analyzed at the Agricultural Research Council–Soil, Climate and Water Laboratory (Pretoria, South Africa).

Treatments and cultivation practices. The experiment was laid out as a 5 × 3 factorial with five N levels of 0, 60, 90 (control), 120, and 150 kg ha⁻¹, and three leaf harvests of 0% (no leaf harvesting), 30%, and 50% removal at 35 DAT, whereas at 80 DAT all the leaves were removed. Nitrogen application of 90 kg ha⁻¹ was used as the positive control according to the soil analysis recommendations (Fertilizer Society of South Africa, 2007). The first leaf harvest was initiated 35 DAT and the final harvest was at 80 DAT. The treatment combinations were replicated three times in a randomized complete block design. Limestone ammonium nitrate (28% N) was used as a source of N. Fifty percent of the total N fertilizer was applied at transplanting and the other 50% was applied as top dressing 5 weeks after transplanting. P and K were not required, based on the soil analysis results.

Five-week-old seedlings were transplanted onto 20-cm-high and 1-m-wide raised beds with four rows per bed. Beds were 90 cm apart. Seedlings were planted in a plot size of 1.6 m², at a plant spacing of 20 cm between-row and 10 cm in-row. Plants were irrigated with drip irrigation (between 0.90 and 1.10 HR) at a delivery rate of 2 L h⁻¹ and at a constant pressure of 200 kPa and emitter spacing of 20 cm. Plants were irrigated three (three irrigation cycles) times per week, with an application rate of 0.6 to 1.2 L (12 to 24 L/plot) of water per irrigation cycle. The amount of irrigation was gradually increased as the plants developed.

Leaf and root yield. The first leaf harvest was initiated at 35 DAT by removing 0% (no leaves), 30%, and 50% of the plant’s leaves. Thereafter, all the plant leaves were harvested at 80 DAT at the termination of the experiment. A garden fork was used to lift the roots from the soil and the leaves were separated from the roots. At harvest, fresh leaf weight, leaf number, leaf area, and dry leaf weight were measured from the 10 selected data plants per plot. A leaf area meter (LI-3100; LI-COR, Lincoln, NE) was used to measure the leaf area (square centimeters). Leaf samples were dried in an oven at 70 °C for 48 h for dry leaf weight determination. Roots of beetroot were harvested at the end of the experiment. Data collected on the roots were fresh weight, dry weight, diameter, and length.

Total soluble solids, pH, and electrical conductivity of root juice. Root firmness, total soluble solids (TSS), pH, and electrical conductivity (EC) of beetroot juice were determined. Firmness was determined using a fruit firmness tester (Wagner Fruit Test™ model FT, Wagner Instruments, Greenwich, Italy) with a 6-mm probe diameter. The roots of beetroot were cut into small pieces and placed in a laboratory blender to produce a puree. The puree was then filtered through a cheese cloth to produce beetroot juice. The pH and EC of the beetroot juice were measured using a pH and EC meter (Hanna Instruments, Woonsocket, RI). The TSS of the beetroot juice was determined using a refractometer (PAL-1; Atago, Tokyo, Japan).

Leaf and root mineral analysis. Ten plants per plot were selected to determine N, P, K, Ca, Mg, manganese (Mn), zinc (Zn), boron (B), copper (Cu), and iron (Fe) concentrations of beetroot leaves and roots. The leaves were oven-dried at 70 °C for 48 h and ground using a mill.

Table 1. Monthly air temperature, rainfall, and relative humidity during the growth period of beetroot under a shade net structure in Pretoria, South Africa, from Nov. 2014 to Jan. 2015.

| Month    | Rainfall (mm)* | Temp (°C)* | Relative humidity (%) |
|----------|---------------|------------|-----------------------|
|          | Minimum       | Maximum    | Minimum               | Maximum               |
| November | 3             | 30         | 15                    | 85                    | 27                    |
| December | 6             | 28         | 16                    | 90                    | 41                    |
| January  | 0             | 31         | 17                    | 89                    | 33                    |

*1 mm = 0.0394 inch; (1.8 × °C) + 32 = °F.
with a 1-mm sieve. Nitrogen was determined on dry milled material using an elemental analyzer (Carlo Erba NA 1500 C/N/S; Thermo Scientific, Milan, Italy) according to Jimenez and Ladha (1993). An aliquot of the digest solution was used for inductively coupled plasma optical emission spectrometry for determination of Ca, Mg, P, K, Fe, Zn, Mn, and Cu concentrations (Liberty Series II model; Varian, Mulgrave, Australia). All nutrient concentrations were expressed on a dry weight basis.

**Determination of total phenolic compounds, betalains, antioxidiant activity, and total protein content.** Freeze-dried roots of beetroot were used for the determination of the bioactive compounds. The evaluation was conducted at the Tshwane University of Technology, Fruit and Vegetable Technology Laboratory of the Department of Crop Sciences. Antioxidant scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Johannesburg, South Africa) according to Ribeiro et al. (2008). The content of phenolic compounds in the extracts was determined using the Folin–Ciocalteu colorimetric method reagent described by Prabhu and Barrett (2009). Betalain content was determined and quantified using the method described by Cardoso-Ugarte et al. (2014). The Broadford method was used for protein determination as described by Sawnney and Randhir (2006).

**Statistical analysis.** Data were subjected to analysis of variance using GenStat® version 11.1 (Payne et al., 2008). Means were separated using Fisher’s protected t test least significant difference (Snedecor and Cochran, 1980).

## Results and discussion

There were no significant interaction effects between N application rate and percentage leaf harvest on beetroot leaf and root yield, leaf and root mineral content, and the total protein. Therefore, only the main factors are discussed below.

**Leaf growth parameters and yield.** At 35 DAT, results showed no significant effect of N on fresh leaf weight, leaf area, and leaf number. The dry leaf weight was significantly higher at N application of 150 kg ha⁻¹ than 0 or 90 kg ha⁻¹; while between 60, 90, and 120 kg ha⁻¹ N there were no significant differences (Table 2). At 80 DAT, an increase in N application resulted in an increase in total fresh leaf weight (TFLW), although the yield at 150 kg ha⁻¹ N was not significantly different to the yields at 90 or 120 kg ha⁻¹ N. Total leaf area was greatest with 120 and 150 kg ha⁻¹ compared with 0–90 kg ha⁻¹. The total number of leaves per plant was significantly greater with 120 or 150 kg ha⁻¹ N compared with 0 or 60 kg ha⁻¹. Finally, dry leaf weight at 80 DAT was significantly greater at 120 and 150 kg ha⁻¹ N compared with 0 to 90 kg ha⁻¹ N. However, total leaf area and dry weight were significantly reduced by 90 kg ha⁻¹ N compared with 120 kg ha⁻¹ N. An increase in N application was reported to increase dry leaf weight of lettuce [Lactuca sativa (Tei et al., 2000)]. The improvement in yield could be explained by improved leaf area, which determines the percentage of incident radiation intercepted for energy supply in photosynthesis (Woodrow et al., 1990). Increased leaf area, as a result of an increase in the N application, is probably due to the increased availability of N for uptake, which, in the larger leaves, resulted in higher accumulation of photoassimilates, consequently increasing dry leaf weight (Table 2). Squire et al. (1987) reported that the main effect of N fertilizer was to increase the rate of leaf expansion, leading to increased interception of daily solar radiation by the leaf canopy. An increase in plant growth and yield as a result of increased N reconfirmed the role of N in improving the vigorous vegetative growth in leafy vegetables (Singh et al., 2015; Tei et al., 2000). Poor yield and retarded growth was a result of insufficient N supply. Lower growth parameters were obtained from plants that received zero N application. These findings are in agreement with the report on fodder beet [B. vulgaris (Khogali et al., 2011)].

It was expected for the first harvest (at 35 DAT) that the increase in leaf harvest percentage will increase leaf yield. However, the important results were on total leaf harvest at 80 DAT. At 80 DAT, leaf harvest percentage showed no significant effect

### Table 2. Effect of nitrogen and leaf harvest percentage on various beetroot leaf variables at 35 DAT and 80 DAT in Pretoria, South Africa, from Nov. 2014 to Jan. 2015.

| Nitrogen level (kg ha⁻¹) | Fresh leaf wt (g/plant) | Dry leaf wt (g/plant) | Leaf area (cm²/plant) | Leaves (no./plant) | Total fresh leaf wt (g/plant) | Total leaf area (cm²/plant) | Total leaves (no./plant) |
|--------------------------|-------------------------|-----------------------|----------------------|---------------------|-------------------------------|---------------------------|-------------------------|
| 0                       | 10.4                    | 0.77 b                | 195                  | 2.18                | 36.6 c                        | 44.3 bc                   | NS                      |
| 60                      | 16.1                    | 1.19 ab               | 194                  | 2.27                | 43.6 bc                       | 51.8 ab                   | 4.51 b                  |
| 90                      | 14.5                    | 1.07 b                | 182                  | 1.89                | 50.3 b                        | 59.0 a                    | 5.15 a                  |
| 120                     | 13.6                    | 1.21 ab               | 200                  | 2.15                | 59.0 a                        | 60.5 a                    | 5.30 a                  |
| 150                     | 23.2                    | 1.64 a                | 281                  | 2.34                | 65.0 a                        | 70.6 a                    | 5.79 a                  |
| LSD 0.05                | NS                      | 0.53                  | NS                   | NS                  | NS                            | NS                        | NS                      |

**Percentage of leaf harvest**

|                | 0%                     | 30%                   | 50%                   | LSD 0.05               |
|----------------|------------------------|-----------------------|-----------------------|------------------------|
| Percentage     | 0 c                    | 0.00 c                | 0.00 c                | 0.79 c                 |
| Dry leaf wt    | 0.00 c                 | 0.00 c                | 0.00 c                | 0.00 c                 |
| Leaf area      | 0.00 c                 | 0.00 c                | 0.00 c                | 0.00 c                 |
| Leaves (no./plant) | 0.00 c             | 0.00 c                | 0.00 c                | 0.00 c                 |

Note: 1 kg ha⁻¹ = 0.8922 lb/acre; 1 cm² = 0.1550 inch²; 1 g = 0.0353 oz; DAT = days after transplanting.

*Values in a column followed by the same letter are not significantly different (P ≤ 0.05), using Fisher’s protected t test; NS = not significant; LSD = least significant difference.

*The first leaf harvest was initiated 35 DAT by removing 0%, 30%, and 50% of the outer matured leaves and the second harvest occurred at 80 DAT by removing all the leaves.
on TFLW, total leaf area, and total dry leaf weight (Table 2). However, there was increase in TFLW, total leaf area, and total dry leaf weight when 30% of the plant’s leaves were harvested. When 30% of the plant’s leaves were harvested, there was a significantly higher number of leaves, compared with when 0% or 50% of the plant’s leaves were harvested (Table 2). This indicates that the minimal removal of leaves at 30% leaf harvest stimulated growth, thus leading to increased production of beetroot leaves. Using a leaf harvest of 50% might have adversely impacted the photosynthesis manufacturing process due to reduced leaf area (Dahniya et al., 1981), whereas 0% leaf harvest could have resulted in matured leaves not actively photosynthesizing. Beetroot leaves are a source of nutritious food that can be easily accessed at 35 DAT during the growing season by harvesting 30% of plant leaves.

**Root yield.** Yield parameters (at 80 DAT) such as fresh root weight, dry root weight, root diameter, and root length were significantly ($P \leq 0.05$) affected by N application (Table 3). Root yield increased over the entire range of N rates used in this experiment. The highest level of N (150 kg ha$^{-1}$) did not differ significantly from the 120 kg ha$^{-1}$ N application. Leilah et al. (2005) reported that root yield increase is caused by high N levels due to N playing a vital role in enhancing growth, chlorophyll formation, the photosynthesis process, and other variables contributing to yield improvement. The diameter of roots receiving 150 kg ha$^{-1}$ was significantly higher than at the 0 or 60 kg ha$^{-1}$ N, even though 90 and 120 kg ha$^{-1}$ did not differ significantly with 150 kg ha$^{-1}$ N. Roots were significantly longer at 150 kg ha$^{-1}$ than at 0, 60, and 90 kg ha$^{-1}$, although 90 kg ha$^{-1}$ N did not differ significantly with 120 kg ha$^{-1}$ N. Furthermore, these results support the studies conducted on fodder beet by Albayrak and Yüksel (2010), and on sugar beet ($B. vulgaris$) by Hellal et al. (2009) who reported increased root diameter and length with an increase in N application. TSS and root firmness were unaffected by the application of N. The results are in agreement with El-Sarag and Moselhy (2013) who found that even though different levels of N were applied, there were no significant differences in the TSS of sugar beet. The

**Table 3. Effect of nitrogen application and leaf harvest percentage on various root variables in Pretoria, South Africa, from Nov. 2014 to Jan. 2015.**

| Nitrogen level (kg ha$^{-1}$) | Fresh root wt (g/plant) | Dry root wt (g/plant) | Root diam (mm) | Root length (mm) | Total soluble solids (%) | Firmness (kg) |
|------------------------------|-------------------------|-----------------------|----------------|-----------------|------------------------|--------------|
| 0                            | 65.0 d$^*$               | 7.6 c                 | 49.4 c         | 42.0 c          | 12.3                   | 2.2          |
| 60                           | 88.0 c                  | 9.6 b                 | 54.6 b         | 46.0 b          | 12.7                   | 2.3          |
| 90                           | 96.6 bc                 | 11.2 ab               | 55.7 ab        | 46.3 b          | 12.0                   | 2.5          |
| 120                          | 111.9 ab                | 12.3 a                | 58.9 ab        | 49.3 ab         | 10.8                   | 2.4          |
| 150                          | 120.6 a                 | 12.8 a                | 59.6 a         | 51.0 a          | 12.3                   | 2.6          |
| LSD 0.05                     | 19.0                    | 1.8                   | 4.4            | 3.899           | NS                     | NS           |

Values in a column followed by the same letter are not significantly different ($P \leq 0.05$), using Fisher’s protected $t$-test; NS = not significant; LSD = least significant difference.

**Table 4. Effect of nitrogen application and leaf harvest on various minerals in dried beetroot leaves in Pretoria, South Africa, from Nov. 2014 to Jan. 2015.**

| Nitrogen level (kg ha$^{-1}$) | Total N (%) | K (%) | P (%) | Mg (%) | Fe (mg kg$^{-1}$) | Zn (mg kg$^{-1}$) |
|------------------------------|-------------|-------|-------|--------|------------------|------------------|
| 0                            | 2.80        | 3.66  | 0.30  | 1.01   | 1.680            | 22.57 b          |
| 60                           | 2.96        | 3.78  | 0.26  | 1.10   | 2.882            | 24.11 ab         |
| 90                           | 2.98        | 3.60  | 0.24  | 1.09   | 2.731            | 24.89 ab         |
| 120                          | 3.12        | 3.60  | 0.26  | 1.27   | 2.780            | 27.64 a          |
| 150                          | 3.18        | 4.02  | 0.27  | 1.23   | 2.440            | 27.32 a          |
| LSD 0.05                     | NS          | NS    | 0.03  | 0.13   | NS               | 3.600            |

Values in a column followed by the same letter are not significantly different ($P \leq 0.05$), using Fisher’s protected $t$-test; NS = not significant; LSD = least significant difference.

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results contradict the findings of Leilah et al. (2005) who stated that high N levels increase the moisture content in root tissue, resulting in lower TSS.

Leaf harvest percentage did not have a significant effect on fresh root weight, root diameter, root length, TSS, and root firmness (Table 3). Compared with harvesting 0% or 30% of the leaves at 35 DAT, harvesting 50% of the leaves at 35 DAT reduced dry root weight (Table 3). There was no significant difference in dry root weight between the 0% or 30% leaf harvests. Removal of 50% beetroot leaves might have disrupted the photosynthesis process and reduced photosynthates supply during the growth of the plant, leading to reduced dry root weight (Table 3). Harvesting 30% of leaves at 35 DAT could increase the consumption of leaves as vegetables for broadening the food base without negatively impacting on root yield.

**Mineral content in leaves.** The results in this experiment indicated no significant effect of treatments on N, K, and Fe content in the leaves of beetroot (Table 4). However, there was a tendency of an increase in N leaf content \( (P = 0.08) \) with an increase in N application. Increasing N was associated with lower uptake of P in the leaf tissue (Table 4). Magnesium leaf content was significantly higher at 120 kg ha\(^{-1}\) N. Zinc leaf content was associated with increased N application, with 0 kg ha\(^{-1}\) N not differing significantly from 60 and 90 kg ha\(^{-1}\) N. The higher levels of leaf Zn and Mg mineral content agreed with findings by other researchers. Safaa and Abd El Fattah (2007), and Musa and Ogbadoyi (2012), reported that N fertilizer increased mineral content in lettuce and fluted pumpkin (*Telfairia occidentalis*).

**Mineral content in roots.** The results revealed that N fertilizer had no significant effect on K, P, Mg, Fe, and Zn content of beetroot roots (data not shown) with the exception of total root N content (Fig. 1). Total root N content was highest at 150 kg ha\(^{-1}\) N, although it did not differ significantly with 120 and 60 kg ha\(^{-1}\) N. Khogali et al. (2011) found that an increase in N application increased the N content of fodder beet. Leaf harvest percentage did not have a significant effect on root mineral content (data not shown).

**Total phenolic compounds, betalains, antioxidant activity, and total protein content in roots.** There was no interaction effect between N application and percentage leaf harvest on total phenols, betalains, proteins, and antioxidants in this study. The results of this study failed to show any significant impact of N application on antioxidant-scavenging activity, total phenols, and the betalain concentration (Table 5). This could be related to the maturity of the leaves. Pandjaitan et al. (2005)...

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**Table 5. Effect of nitrogen on various bioactive compounds in roots of beetroot in Pretoria, South Africa, from Nov. 2014 to Jan. 2015.**

| Nitrogen levels (kg ha\(^{-1}\))\(^{a}\) | Antioxidants: DPPH [GAE (mg/100 g DW)]\(^{a}\) | Betalain (mg g\(^{-1}\) DW)\(^{a}\) | Phenols [GAE (mg/100 g DW)]\(^{b}\) | Conc. of total proteins [BSA (mg/100 g DW)]\(^{b}\) |
|----------------------------------------|---------------------------------------------|-----------------|-------------------------------|-----------------------------|
| 0                                      | 55.9                                        | 23.25           | 2.223                         | 318.3 \(^{a}\)             |
| 60                                     | 54.7                                        | 21.80           | 2.234                         | 292.3 \(^{b}\)            |
| 90                                     | 66.3                                        | 25.22           | 2.184                         | 281.2 \(^{b}\)            |
| 120                                    | 58.3                                        | 21.76           | 2.328                         | 244.3 \(^{c}\)            |
| 150                                    | 59.1                                        | 23.52           | 2.321                         | 247.2 \(^{c}\)            |
| LSD 0.05                               | NS                                          | NS              | NS                            | 15.97                      |

| Percentage of leaf harvest\(^{a}\) | Antioxidants: DPPH [GAE (mg/100 g DW)]\(^{a}\) | Betalain (mg g\(^{-1}\) DW)\(^{a}\) | Phenols [GAE (mg/100 g DW)]\(^{b}\) | Conc. of total proteins [BSA (mg/100 g DW)]\(^{b}\) |
|-------------------------------------|---------------------------------------------|-----------------|-------------------------------|-----------------------------|
| 0%                                  | 61.3                                        | 21.63           | 2.181                         | 253.9 \(^{b}\)            |
| 30%                                 | 57.6                                        | 23.71           | 2.276                         | 294.8 \(^{a}\)            |
| 50%                                 | 57.7                                        | 23.98           | 2.317                         | 281.3 \(^{b}\)            |
| LSD 0.05                            | NS                                          | NS              | NS                            | 35.84                      |

\(^{a}\)1 kg ha\(^{-1}\) = 0.8922 lb/acre; 1 mg/100 g = 10 ppm; 1 mg g\(^{-1}\) = 1000 ppm; DPPH = 2,2-diphenyl-1-picrylhydrazyl; DW = dry weight; GAE = gallic acid equivalents; BSA = bovine serum albumin.

\(^{b}\)Values in a column followed by the same letter are not significantly different \( (P \leq 0.05) \), using Fisher’s protected \( t \) test; NS = not significant; LSD = least significant difference.

\(^{a}\)The first leaf harvest was initiated 35 d after transplanting (DAT) by removing 0%, 30%, and 50% of the outer matured leaves and the second harvest occurred at 80 DAT by removing all the leaves.
reported that spinach (*Spinacia oleracea*) leaves harvested at the midmature stage had much higher levels of total phenolics, total flavonoids, and antioxidant capacity than immature and mature leaves. However, when the leaves were harvested at 30% or 50%, there was a tendency of an increase in betalains and phenol content, but the significant difference was found only with respect to the total protein (Table 5). The total protein content tended to decrease with an increase in N application. In terms of the percentage leaf harvest, the results showed that 30% or 50% leaf harvest resulted in a higher total protein content in the roots compared with the 0% (control) leaf harvest, although further investigation is required.

**Conclusions**

The results revealed that N application of 120 kg ha⁻¹ resulted in increased yield and quality of beetroot leaves and roots. The yield was correspondingly lower with the application of lower N levels. Application of N was shown to enhance the accumulation of Mg, Fe, and Zn in beetroot leaves. Beetroot growers can harvest 30% of beetroot leaves at 35 DAT to improve nutritional intake without adversely impacting root yield. Further studies need to be conducted to assess the effects of harvesting leaf frequencies for different beetroot cultivars on leaf and root yield and quality.

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