Seasonal Responses of Total Antioxidant Contents in Cultivated Bush Tea (*Athrixia phylicoides* L.) Leaves to Fertilizer Rates

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**ABSTRACT**

The objective of this study was to determine the seasonal effects of nitrogen (N), phosphorus (P) and potassium (K) fertilizer on total antioxidant content of cultivated bush tea (*Athrixia phylicoides* L.) leaves. Three independent trials of N, P and K were conducted per season i.e. autumn, winter, spring and summer. Treatments consisted of 0, 100, 200, 300, 400 or 500 kg/ha N, P or K replicated four times in a randomized complete block design. At harvest, leaves were freeze dried and ground for total antioxidant using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Results of this study demonstrate that regardless of season, the application of N, P and K fertilizers quadratically increased total antioxidant content, with most of the increase occurring at 0-300 N, 300 P and 100 K kg/ha. Therefore, for improved total antioxidant contents in cultivated bush tea leaves, 300 N, 300 P and 100 K kg/ha N is recommended.

**Keywords:** bush tea, harvesting, season, total antioxidant contents

**INTRODUCTION**

Bush tea (*Athrixia phylicoides* L.) (Fig 1) contains 5-hydroxy-6,7,8,3',4,5'-hexamethoxyflavon-3-ol as a major flavonoid (Mashimbye et al. 2006). Herbal teas have antioxidant properties of a wide range of amphipathic molecules termed phenolic compounds (Ivanova et al. 2004). The antioxidant activity of phenolics are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal chelators (Morel et al. 1994; Rice-Evans et al. 1997). Antioxidant content is widely used as a parameter to characterize different plant materials for health benefits. This activity is related with compounds capable of protecting a biological system against the harmful effect of reactions that can cause excessive oxidation, involving reaction of oxygen and nitrogen species.

Agronomic practices such as plucking of leaves (Owour et al. 2000) and mineral nutrition (Owour 1989; Owour et al. 1990; Owour and Odhiambo 1994) increased the concentration of total polyphenols and total antioxidant contents in green tea. Traditionally, bush tea plant materials are only harvested from the wild for medical and herbal tea purposes and the concept of domesticating medicinal plants is critical in order to avoid wild populations becoming extinct. Total polyphenols in leaves of wild bush tea plants were lowest in March and April (autumn), September (spring) and highest in June and July (winter) (Mudau et al. 2006). A single application of 300 kg/ha N or P and 200 kg/ha K maximized shoot growth (Mudau et al. 2005) and total polyphenols (Mudau et al. 2007a), whereas the combined applications of 300 kg/ha N or P and 200 kg/ha K doubled growth and total polyphenol content of cultivated bush tea (Mudau et al. 2007b). Mudau et al. (2007c) also reported that condensed tannin contents were highest in autumn (4.82%) compared to other seasons, whereas hydrolysable tannins were lowest during summer (0.01%). Seasonal effects of fertilizer rates on antioxidant contents are not documented. Therefore, the objectives of the study was to investigate the seasonal effects of N, P and K fertilizer rates on total antioxidant content of cultivated bush tea.

**MATERIALS AND METHODS**

**Experimental site and plant materials**

The study was carried out in Morgenzon, a commercial nursery in...
Louis Trichardt (Polokwane, South Africa) (23°N 50°E, 30°S 17°E; alt. 610 m); a relatively cool subtropical climate with summer rainfall and cold, dry winter. On 13 Nov 2005, plant materials were collected from Venda at Muhuyu village (South Africa, Limpopo Province) and 1500 apical cuttings were dipped in Seradix® No. 2 hormone (0.3% IBA) (Bayer, Pretoria, South Africa) to encourage root formation and established in seed trays on a mist bed. The 5 m × 1.5 m × 1 m mist bed was supplemented with automatic misting and fogging nozzles, which are humidity based. The greenhouse temperature was recorded by a Series 3020T Datalogger (Electronic Control Design, Mulino, Oregon, USA). The measured mean minimum/maximum temperatures in the mist bed were 12.6°C/29.6°C (autumn), 9°C/27.8°C (winter), 13°C/34.2°C (spring) as well as 17°C/34.7°C (summer). The sprouted cuttings were grown with the photoperiod extended to 16 h by 1000-W, high-pressure sodium lamps (250 μmol/m²/s photosynthetic photon flux (PPF)) for 1 month.

Rooted cuttings on sand culture were transplanted into 1 L bags and placed in a hardening chamber maintained at 20°C. The transplants were grown under natural photoperiod extended to 16 h by the same lamps described above for 3 months. After 3 months, plants were transplanted into 20 L bags. The medium was pine bark:sand:styrofoam bed mix (1:2:1, v/v), with AquaGro wetting agent (Aquatrols, Cherry Hill, N.J) at 0.2 kg/m³. The chemical properties of initial media were determined using a procedure described by Hanlon et al. (1994). The EC was 0.9 dS/m and pH was 4.7. The composted pine bark contained 1.2 mg/kg NO₃ (N), 0.1 mg/kg P and 1.3 mg/kg K.

Experimental design and treatment details
Three independent trials for N, P and K were conducted, one per season (i.e. autumn, winter, spring and summer) under 50% shade nets. Treatments consisted of 0, 100, 200, 300, 400 or 500 kg/ha N, P or K, equivalent to 0, 2, 4, 6, 8 or 10 g per 20 L bag, respectively, in a randomized complete block design with six treatments replicated four times.

Fertilizers applied were limestone ammonium nitrate (LAN, N = 28%) (for N trial), single super phosphate (P = 10.5%) (for P trial) and potassium chloride (K = 50%) (for K trial) (Kynoch (Pty) Ltd., Pretoria, South Africa) applied one week after planting. The sprouted cuttings were grown with the photoperiod extended to 16 h by the same lamps described above for 1 month. After 3 months, plants were transplanted into 20 L bags. The medium was pine bark:sand:styrofoam bed mix (1:2:1, v/v), with AquaGro wetting agent (Aquatrols, Cherry Hill, N.J) at 0.2 kg/m³. The chemical properties of initial media were determined using a procedure described by Hanlon et al. (1994). The EC was 0.9 dS/m and pH was 4.7. The composted pine bark contained 1.2 mg/kg NO₃ (N), 0.1 mg/kg P and 1.3 mg/kg K.

Leaf tissue N, P and K concentrations
Total nitrogen was determined on a rapid-flow analyzer (series 300; Alpechem, Wilsonville, Oregon, USA). Phosphorus and K were analyzed using the method described by Adrian (1973).

Preparation of leaf extracts for total antioxidant content
Fifteen g of finely ground leaf material were sieved (≤1.0 mm; Endocots test sieves) for 5 min. From the sieved material, 0.5 g was mixed in 5 ml of 75% acetone for 2 h in a shaker (Nanotech 5553/630, Johannesburg, South Africa), and then centrifuged for 5 min at 40,000 × g. The supernatant was carefully decanted and the extraction procedure was repeated three times on residues. Three supernatants were combined and made-up to a volume of 15 ml of the filtrate extracts.

Determination of total antioxidant content
Total antioxidant contents were analysed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) antiradical assay protocol described by Awika et al. (2003). In this method, 24 mg DPPH was dissolved in 100 ml methanol, and mechanically shaken for 20 minutes to produce a mother solution. Ten (10) ml of the mother solution was added to 50 ml methanol, and the absorbance of the solution was adjusted to 1.1 at 515 nm by 20 ml mother solution. The extract of 2850 μl DPPH solution was added to 150 μl sample extract for 6 hours until the completion of the reaction. The absorbance was measured at 515 nm in a spectrophotometer (Cecil Instruments, Cambridge, UK) and expressed in percentages Treloso per dry basis. The assays were standardized with Trolox solution from 0 to 800 μM.

Statistical analyses
Data were subjected to analysis of variance (ANOVA) using GLM (General linear model) procedure of SAS version 8.0. (SAS Institute Inc. 1999). In all trials, treatment sums of squares were partitioned into linear and quadratic polynomial contrasts for total antioxidant contents.

RESULTS AND DISCUSSION
Nitrogen experiments
Regardless of season, there was a significant quadratic (P<0.01) increase of total antioxidants content in response to N application (Table 1). The maximum level of N was 300 kg/ha. Most of the total antioxidant contents occurred between 0-300 kg/ha, regardless of season. Similar results were also reported by Mudau et al. (2005, 2006, 2007b) on growth parameters, leaf tissue N, total polyphenols and tannin contents. Total antioxidant content decreased significantly at higher rates of N application (300 to 500) kg/ha N, presumably due to a trade-off between the synthesis of other secondary compounds such as phenolic acids and protein contents. Generally, bush tea has vigorous shoot growth (Roberts 1990), with maximum shoot growth being attained when N is applied at 300 kg/ha N (Mudau et al. 2005).

Phosphorus experiments
TAA increased (P≤0.01) quadratically with P application at 300 kg/ha, irrespective of the season (Table 1). Most of the total antioxidant contents occurred between 0-300 P kg/ha. Mudau et al. (2006) reported that the highest total polyphenol and tannin concentrations in bush tea occurred when P

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Table 1 Response of percentage total antioxidant contents to nitrogen, phosphorus and potassium nutrition as affected by season.

<table>
<thead>
<tr>
<th>Applied fertilizer rates</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>(kg/ha)</td>
<td>N&lt;sub&gt;x&lt;/sub&gt;</td>
<td>P&lt;sub&gt;y&lt;/sub&gt;</td>
<td>K&lt;sub&gt;z&lt;/sub&gt;</td>
<td>N&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>0</td>
<td>56</td>
<td>15</td>
<td>64</td>
<td>75</td>
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<tr>
<td>100</td>
<td>94</td>
<td>95</td>
<td>88</td>
<td>93</td>
</tr>
<tr>
<td>200</td>
<td>96</td>
<td>97</td>
<td>100</td>
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<tr>
<td>300</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>400</td>
<td>87</td>
<td>88</td>
<td>100</td>
<td>99</td>
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<tr>
<td>500</td>
<td>76</td>
<td>76</td>
<td>100</td>
<td>87</td>
</tr>
</tbody>
</table>

(Q) quadratic effect at P=0.01 (*)

x, y and z = value in the respective column are responses of total antioxidant contents due to nitrogen, phosphorus and potassium nutrition, respectively
was applied at 300 kg/ha. Similar finding were also evident in leaf tissue P and total polyphenols reaching a maximum at 300 kg/ha (Mudau et al. 2005). In contrast, carbon-based secondary compounds such as total polyphenols derivatives (theaflavins and thearubigins) in black tea vary with the time of year reaching a maximum at 150 kg/ha P in black tea (Owour et al. 1991; Owour and Odhiambo 1994).

**Potassium experiments**

In all seasons, total antioxidants were increased ($P\leq0.01$) quadratically with K application reaching a maximum at 100 kg/ha (Table 1). Most of the total antioxidant contents occurred between 0-100 K kg/ha. Mudau et al. (2006) reported that the highest total polyphenols and leaf tissue K concentrations occurred when K was applied at 200 kg/ha in bush tea.

In conclusion, the results of this study demonstrated that regardless of season N, P and K nutrition increased total antioxidant content in bush tea leaves. The maximum concentrations of total antioxidant contents were obtained when N or P were applied at 300 kg/ha and 100 kg/ha for K, regardless of season. For the N trial, maximum total antioxidant content was 100% during autumn, winter and spring and 93% during summer. For P trials the highest total antioxidants contents were 100% for autumn, winter and spring and 94% during summer. The highest TAA contents as affected by K nutrition were 83% during autumn, winter and spring and 63% during summer. Therefore, for improved total antioxidant contents in bush tea, 300 kg/ha for both N and P and 100 kg/ha is recommended.

**ACKNOWLEDGEMENTS**

The authors would like to acknowledge Morgenzon, a commercial nursery and the National Research Foundation (NRF) for financial assistance.

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