Medicinal and Aromatic Plant Science and Biotechnology ©2007 Global Science Books

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

Mogotlane I. Daniel[1] • Fhatuwani N. Mudau[2*] • Phatu W. Mashela[1] • Puffy Soundy[3]

[1] Department of Plant Production, University of Limpopo, Private Bag X 1106, Sovenga, 0726, Republic of South Africa
[2] Centre for Agrofood Processing, University of Limpopo, Private Bag X 1106, Sovenga, 0726, Republic of South Africa
[3] Department of Plant Production and Soil Science, University of Pretoria, Pretoria, 0002, Republic of South Africa

Corresponding author: * **mudaufn@ul.ac.za**

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 flavornoid (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.

Fig. 1 Bush tea (*Athrixia phylicoides* L.) in flower.

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

Received: 30 January, 2007. Accepted: 14 February, 2007.

Short Communication

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/m^2/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 bead mix (1:2:1, v/v), with AquaGro wetting agent (Aquatrols, Cherry Hill, N.J) at 0.2 kg/m^3. 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 NO_3 (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 in the form of granules.

All plants received 1% $MgSO_4$ (Mg = 20%, S = 26%), Micrel ZnO (Zn = 78.6), Micrel Fe 130 (Fe = 13%), Micrel soluble sodium borate (B = 20.5%) mono ammonium phosphate (MAP, N = 12%, P = 27%) (except for P trial), and urea (N = 46%) (except for N trial), and potassium chloride (except for K trial) (Ocean Agriculture (Pty) Ltd., Muldersdrift, South Africa) twice a week as foliar sprays to supplement the rest of the elements necessary for the production of good quality tea. At the end of each season 90 days after transplanting, all plants were harvested and leaves were washed with distilled water and freeze dried for percentage N, P, K analysis (Mudau *et al*. 2005) and assayed for total antioxidant content.

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; Endocotts 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. 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 Trolox 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 ($P \leq 0.01$) 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^1 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 ($P \leq 0.01$) 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

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

Applied fertilizer rates (kg/ha)	Autumn			Winter			Spring			Summer		
	N^x	P^y	K^z	N	P	K	N	P	K	N	P	K
0	56	15	64	75	40	69	72	20	48	68	39	54
100	94	95	88	93	94	100	95	95	100	82	82	63
200	96	97	100	92	93	100	99	99	100	85	86	62
300	100	100	100	100	100	100	100	100	100	93	94	60
400	87	88	100	99	99	100	79	79	100	85	88	61
500	76	76	100	87	88	100	75	75	100	77	78	61
Significance	Q^*	Q^*	Q^*	Q^*	Q^*	Q^*	Q^*	Q^*	Q^*	Q^*	Q^*	Q^*

(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 Odihiambo 1994).

Potassium experiments

In all seasons, total antioxidants were increased ($P \leq 0.01$) 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, 100% (winter; spring) and 63% during summer. Therefore, for improved total antioxidant contents in bush tea, 300 kg/ha^{1} 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.

REFERENCES

Andrian WJ (1973) Method of analyzing P and K content. *Analyst* **98**, 212-213

Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L (2003) Screening methods to measure antioxidant activitity of sorghum (*Sorghum bicolor*) and Sorghum products. *Journal of Science and Chemistry* **51**, 6657-6657

Ivanova D, Gerova D, Chervenkov T, Yankova T (2004) Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology* **96**, 145-150

Mashimbye MJ, Mudau FN, Soundy P, van Ree T (2006) A new flavonol from *Athrixia phylicoides* (Bush tea). *South African Journal of Chemistry* **59**, 1-2

Morel I, Lescoat G, Cillard P, Cillard J (1994) Role of flavonoids and iron chelation in antioxidant action. *Methods of Enzymology* **234**, 437-443

Mudau FN, Soundy P, du Toit ES (2007a) Nitrogen, phosphorus and potassium nutrition increases growth and total polyphenol concentrations of bush tea in a shaded nursery environment. *HortTechnology* **17**, 107-110

Mudau FN, Soundy P, du Toit ES (2007b) Effects of nitrogen, phosphorus and potassium nutrition on total polyphenol content of bush tea (*Athrixia phylicoides* L.) leaves in shaded nursery environment. *HortScience* **42**, 334-338

Mudau FN, Ngele A, Mashela PW, Soundy P (2007c) Seasonal variation of tannin content in wild bush tea. *Journal of Medicinal and Aromatic Plant Science and Biotechnology* **1**, 74-76

Mudau FN, Soundy P, du Toit ES, Olivier J (2006) Variation in polyphenolic content of *Athrixia phyliocoides* (L.) (bush tea) leaves with season and nitrogen application. *South African Journal of Botany* **72**, 398-402

Mudau FN, Soundy P, du Toit ES (2005) Plant growth and development of bush tea as affected by nitrogen, phosphorus and potassium nutrition. *HortScience* **40**, 1898-1901

Owour PO, Ng'etich KW, Obanda M (2000) Quality response of clonal black tea to nitrogen fertilizer, plucking interval and plucking standard. *Journal of the Science of Food and Agriculture* **70**, 47-52

Owour PO (1989) Black tea quality: Effects of some agronomic practices on tea quality. *Tea* **10**, 134-136

Owour PO, Odhiambo HO, Robinson JM, Tylor SJ (1990) Variations in the leaf standard, chemical composition and quality of black tea (*Camellia sinensis*) due to plucking standards. *Agricultural Journal of Biological Chemistry* **51**, 3383-3384

Owuor PO, Odhiambo HO (1994) Response of some black tea quality parameters to nitrogen fertilizer rates and plucking frequencies. *Journal of the Science of Food and Agriculture* **66**, 555-561

Owour PO, Othieno CO, Robinson JM, Baker K (1991) Responses of tea quality parameters to time of the year and nitrogen fertilizer. *Journal of the Science of Food and Agriculture* **55**, 111-446

Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends in Plant Sciences* **2**, 152-159

Roberts M (1990) *Indigenous Healing Plants* (1st Edn) Southern Book Publishers, Halfway House, South Africa, pp 56-57

SAS Institute Inc. (1999) User's guide, version 8.0 (5th Edn, Vol 2). Cary, NC, USA