GROWTH, DEVELOPMENT AND CHEMICAL COMPOSITION OF BUSH TEA (*ATHRIXIA PHYLICOIDES* L.) AS AFFECTED BY SEASONAL NITROGEN, PHOSPHORUS AND POTASSIUM NUTRITION

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Growth, development and chemical composition of bush tea (*Athrixia phylicoides* L.) as affected by seasonal nitrogen, phosphorus and potassium nutrition

By

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(*Athrixia phyliciodes* L.)

DECLARATION

I declare that this dissertation, submitted for the degree of Doctor of Philosophy in Horticultural Science at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at another University.

Signature…….

Fhatuwani N. Mudau

Date……..

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DEDICATION

I would like to dedicate this dissertation to my daughter (Mutondwa F. Mudau) who was born during the crucial moment of this study.

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DEPARTMENT: Plant Production and Soil Science

DEGREE: Ph.D. (Horticultural Sciences)

ABSTRACT

Bush tea (*Athrixia phylicoides* L.*)* is an herbaceous plant that belongs to the Asteraceae family. It has predominantly been used throughout history as a medicinal herbal tea by people of South Africa. Many studies have revealed that the plant has the commercial potential to be used as a medicinal herbal beverage. The chemical profile of wild bush tea such as flavonols and total polyphenols are not yet established. Therefore, an experiment to identify the major compound in bush tea was initiated. Matured leaves were harvested in Muhuyu village (Limpopo Province) for extraction. The green leaves were cold extracted with acetone for seven days. The extract was filtered and evaporated at 50 °C under reduced pressure to yield 312 g of a green viscous liquid. Thin layer chromatography plates were visualized under UV light (240 nm) or by spraying with visualizing reagent (anisaldehyde reagent) which was made up by mixing 250 mL ethanol, 2,4 mL concentrated sulphuric acid and 6 ml

anisaldehyde. NMR spectroscopic measurements were done using a 300 MHz Bruker spectrometer, with $CDCl_3$ as solvent and TMS as an internal standard. The processed leaves of bush tea contained 5-hydroxy-6,7,8,3',4',5'-hexamethoxy flavon-3-ol as major new flavonoid.

A trial to investigate the seasonal variation of total polyphenols in bush tea leaves harvested from the wild was conducted. Leaf samples were collected from a field at Muhuyu Village (Limpopo Province) from January to December 2003, and then air dried. Total polyphenols were extracted using Folin-Ciaocalteau reagents and analyzed in a spectrophotometer. Total polyphenols showed definite seasonal variations with the lowest concentrations in March (11.8 $mg \cdot g^{-1}$), April (10.8 $mg \cdot g^{-1}$) and September (10.8 29. $mg \cdot g^{-1}$), while the highest concentrations were in June (35.5 $mg \cdot g^{-1}$) and July (35.9 $mg \cdot g^{-1}$). Thus suggesting that the ideal time for harvesting bush tea would, therefore, be during winter followed by summer season.

Seasonal nutritional requirements of bush tea were investigated. Trials for N, P or K, one at each season (autumn, winter, spring and summer), were laid out in a randomized complete block design (RCBD) with six treatments replicated eight times. Treatments consisted of 0, 100, 200, 300, 400, or 500 $kg \cdot ha^{-1}$ N, P or K. Parameters recorded were plant height, number of branches and leaves, fresh and dry stem mass, fresh and dry root mass, stem girth, fresh and dry shoot mass, leaf area and concentrations of leaf and root tissue N, P, K and total polyphenols. Results of this study demonstrated that in all trials, regardless of season, N, P or K nutrition increased bush tea fresh and dry shoot mass, plant height, number of leaves, number of branches and leaf area. Regardless of season, the optimum growth of bush tea was at 300 $kg \cdot ha^{-}$

^{1} N or P and 200 kg·ha^{-1} of K. Results for the N trial indicated that concentration of total polyphenols quadratically increased in response to N nutrition during autumn, winter, spring and summer. The optimum N level was 300 kg·ha^{-1}. The highest concentration of total polyphenols in the plant was 51.1 mg·g^{-1} in winter. For the P trial, total polyphenols quadratically increased in response to P nutrition regardless of season. Again winter had the highest concentration of total polyphenols (46.8 mg·g^{-1}). The optimum P level was 300 kg·ha^{-1}. In the K trial, regardless of season, total concentration of polyphenols reached maximum at 400 kg·ha^{-1} with most of the total polyphenol responses occurring between 0 and 200 kg·ha^{-1}. Therefore, for improved concentration of total polyphenols, 300 kg·ha^{-1}N and P and 200 kg·ha^{-1}K are recommended. Regardless of season, no significant differences in number of flowers and buds (autumn and winter), stem girth, fresh and dry root mass as well as fresh and dry stem mass were obtained.

The trial to investigate the treatment combinations of N, P and K nutrition on growth and chemical composition of bush tea were conducted in a 3^3 factorial treatment combinations arranged in a randomized block design replicated 4 times. The parameters recorded were plant height, number of branches and leaves, fresh and dry stem mass, fresh and dry root mass, stem girth, fresh and dry shoot mass, leaf area and concentrations of leaf and root tissue N, P, K and total polyphenols. The results of this study demonstrated that regardless of season, treatment combinations of N300, P300 and K200 (kg·ha^{-1}) increased fresh and dry shoot mass, number of leaves, leaf area as well as the concentrations of total polyphenols in bush tea. In all seasons, no significant differences in plant height, number of branches, number of flower buds

(autumn and winter), stem girth, fresh and dry root mass as well as fresh and dry stem mass were obtained.

In conclusion, the processed leaves of bush tea contained 5-hydroxy-6,7,8,3',4',5'-hexamethoxy flavon-3-ol as major new flavonoid. The ideal time for harvesting wild bush tea to maximize total polyphenols was during winter followed by summer season. The optimum level of nutrition for maximum growth was 300 $kg \cdot ha^{-1}$ N or P and 200 $kg \cdot ha^{-1}$ K of cultivated bush under 50% shade nets. Regardless of season, no significant differences in number of flowers and buds (autumn and winter), stem girth, fresh and dry root mass as well as fresh and dry stem mass were obtained. The total polyphenols were improved with 300 $kg \cdot ha^{-1}$ N or P and 200 $kg \cdot ha^{-1}$ K. Highest total polyphenols (51.1 $mg \cdot g^{-1}$) were obtained with nitrogen treatments during winter. Treatment combination of N300, P300 and K200 ($kg \cdot ha^{-1}$) increased fresh and dry shoot mass, number of leaves, leaf area as well as the concentrations of total polyphenols in bush tea.

Additional index words. *Athrixia phylicodes* (L.), nitrogen, phosphorus, potassium, nutrition and total polyphenol concentrations

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GENERAL INTRODUCTION

The genus *Athrixia* belongs to the Asteraceae family, tribe Inuleae and subtribe Athrixiinae. There are 14 species, which are predominantly found in southern Africa, tropical Africa and Madagascar of which 9 of these are endemic to southern Africa (Herman, Retief, Koekemoer & Welman, 2000). Bush tea (*Athrixia phylicoides* (L.) is an indigenous plant in South Africa and is commonly known as bushman tea, Zulu tea or bush tea. Botanically, it is an attractive shrub, about 50 cm to 1 m in height, branched, with thin woolly stems. Leaves are simple, alternate linear to broadly lanceoalate, tapering to a sharp point, shortly stalked, auriculate at the base, light grey-green, smooth on upper surface and white-woolly below, with margins entirely or slightly revolute. The inflorescence head is sessile or subsessile and terminal axillary in large subcorymbose panicles (Herman *et al*., 2000).

Flowering period in the coastal areas occurs during May to June and in land flowers appear during mid-summer (Roberts, 1990). Flowers vary from pink to all shades of pink and attractive purple colour depending on edaphic factors and geographical area (Van Wyk & Gericke, 2000).

The fruits consist of narrow, cylindrical and thin achenes that are approximately 0.01 to 0.06 mm wide, with an average of 2 pappus per seed of about 4 mm, which helps in the dissemination of the seed as a parachute.

Bush tea adapts well in open grassland and in thick forest margins of South Africa, especially in Limpopo Province, Free State Province, Kwazulu Natal and other parts of the Eastern Cape Province and in neighbouring Swaziland. Bush tea can successfully be by seeds and cuttings

(Hintsa, 2004). For good establishment, plants need enough space for spreading their branches and well-drained soils with full sunlight (Roberts, 1990).

The indigenous people of South Africa have used bush tea for many years as medicinal tea for cleansing or purifying the blood, treating boils, headaches, infested wounds, cuts and the solutions may also be used as foam bath. The foam bath brew can also be used as a lotion dabbed on to the boil, skin eruption or cut (Roberts, 1990). The tea is also excellent for coughs and colds and as a gargle for throat infections and loss of voice. It is also believed to have aphrodisiac properties by Vhavenda people (Mabogo, 1990). The Sotho's use strong brew preparations as a calming wash for sore feet and then bandage the washed feet with caster oil leaves (Roberts, 1990; Marnewick, Gelderblom & Joubert, 2000). The stems of bush tea are well tied in bundles for brooms and traded on a small-scale market in Limpopo, Mpumalanga and Kwazulu-Natal Provinces.

Data on chemical composition and effects of cultural practices such as mineral nutrition on growth of bush tea are not well established. Therefore, the objectives of this study were to:

(i) identify the major compound in bush tea

(ii) investigate the seasonal variation of concentrations of total polyphenols in bush tea leaves harvested from the wild

(iii) study the effects of nitrogen, phosphorus and potassium application on growth and development of bush tea under cultivation as influenced by season

(iv) determine the effect of nitrogen, phosphorus and potassium application on chemical composition of bush tea as influenced by season

(v) determine the effects of treatment combinations of N, P and K application on growth and chemical composition of bush tea