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

FHATUWANI N. MUDAU

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

By

Fhatuwani N. Mudau

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Supervisor: Dr P. Soundy

Co-supervisor: Professor E.S. du Toit

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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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FHATUWANI N. MUDAU

SUPERVISOR: Dr P. Soundy

CO-SUPERVISOR: Prof. E.S. du Toit

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

CHAPTER 1

LITERATURE REVIEW

1.1 BOTANY OF TEA

The tea shrub is a perennial evergreen plant (Bokuchava & Skobeleva, 1969; Purseglove, 1987). It is classified in the Theaceae family. The most predominant *Camellia* varieties are *Camellia sinensis* variety *sinensis* and *Camellia sinensis* variety *assamica* (Hara, Luo, Wickremasinghe & Yamanishi, 1995). In nature, tea tree can attain a height of 20-30 m (Purseglove, 1987). Some trees more than 1500 years old are still thriving in their original forests of Yunnan Province in south-western China (Hara *et al*., 1995). Tea plants are grown in a wide range of latitudes in the world ranging from 45°N (Russia) to 30°S (South Africa) and longitude from 150°E (New Guinea) to 60°W (Argentina). In general, the plant is kept as an evergreen shrub by pruning. The first two leaves and a bud are plucked for tea processing. In tropical countries, tea leaves are harvested all year round whereas, in temperate countries, harvesting is seasonal (Purseglove, 1987). The tea product differs in quality arising from different cultivation practices, growing conditions and processing methods (Bhatia & Ullah, 1962; Millin, 1987; Hara *et al*., 1995), despite the fact that the products are from *Camellia* species.

1.2 TYPES OF TEAS

There are currently six main types of teas depending on processing techniques including black, green, white, yellow, oolong and reprocessed tea (Hara *et al*., 1995). These types of tea are converted into a large range of tea products and there are over 300 kinds of reprocessed tea alone, of which some are well-known scented teas, such as jasmine, and brick teas (Hara *et al*., 1995). White and yellow teas have been regarded as two subclass of green tea (Harbowy & Balentine, 1997). These two types of tea are different from green tea due to differences in variety, processing, geographical and traditional distributions (Lu, 1987). There are hundreds of tea cultivars and individual cultivars may only be suited for processing into one of the six types of tea depending only on the chemical constituents of the tea, but also on its biological characteristics (Lu, 1987).

1. 3 TEA QUALITY

Quality is one of the critical factors determining the price of tea for export. Although yield is important to producers, quality also has a significant role to play. It is currently measured or valued in terms of price realisation or tea taster's scores from sensory evaluation, which is prompted to be subjective, depending upon the sensory tasting skills of the taster (Fernando & Roberts, 1984; Taylor, Baker, Owour, Orchard, Othieno & Gay, 1992).

Quality has also been reported to be influenced by active chemical compounds, mainly total polyphenols, which to date have attracted more researchers to be interested in this field (Roberts & Smith, 1961; Hilton & Ellis, 1972; Mcwell, Feakes & Gay, 1990; Owour, Ng'etich & Obanda, 2000; Venkatesan, Murugesan, Ganapathy & Verma, 2004).

The quality of tea is formed during the growth and development of the tea, when the compounds responsible for quality are synthesized (Bokuchava & Skobeleva, 1969; Obanda, Owour & Taylor, 1997). The chemical constituents synthesized in tea shoots may exert positive and or negative effects on the quality of tea the made. The quality index [(EGCG + ECG)/EGC] has been found to be directly related to the sensory properties of green tea (Yuan, 1962). Thus, the index has been used as an objective parameter for assisting the evaluation of quality green teas (Shao, Powell & Clifford, 1995).

The climatic conditions and agronomic practices of green tea and black tea are the same (Chiu, 1990; Sud & Baru, 2000). The main principal difference between black teas and other forms of teas like green tea and oolong tea is the presence of condensed catechins, i.e. polyphenols of higher molecular weight. This is formed through enzymatic oxidation with the help of enzyme polyphenol oxidase (PPO) and peroxidase (PO) and total polyphenol contents which are the main tea quality indicators (Sanderson & Grahamn, 1973).

The other chemical components of tea quality parameters are amino acids (Wang, 1996), carbohydrates (Sanderson, Co & Gonzales, 1976), organic acids (Sanderson & Selvendran, 1965), vitamins (Hu, 2001a), volatile flavour compounds and plant pigments (Taylor *et al*., 1992). Hence, the sensory quality attributes are astringent taste, bitterness, sweatiness and aroma (Hu, 2001b).

Factors that affect the above mentioned tea quality parameters can be classified into four major categories viz., cultivars (Owour *et al*., 2000), environmental conditions (Chiu, 1990), cultural practices (Taylor *et al*., 1992) and seasonal variation (Sud & Baru, 2000).

However, for the purpose of this review quality attributes of green tea, with respect to mineral nutrition, will be covered.

1.4 POTENTIAL HEALTH BENEFITS OF GREEN TEA

Cancer prevention: Polyphenols act as antioxidants and may actually inhibit the growth of existing cancer cells (Ho, Osawa, Huang & Rosen, 1994). The mechanisms to reduce cancer were by modifying enzymes and binding carcinogens to DNA, thus exerting an anti carcinogenic effect (Skibola & Smith, 2000). Quecetin is one of the most extensively studied flavonoids that possess the anticancer activities (Skibola & Smith, 2000). Proanthocyanins may participate in the prevention of cancers by acting as reducing agents (Clydesdale, 1997; Santaos-Buelga & Scalbert, 2000).

Protective activities against heart disease: The antioxidants in green tea may also be helpful in lowering cholesterol and preventing hardening of the arteries and ischemic heart disease (Muldoon & Kritchevsky, 1996; Manteiga, Park & Ali, 1997; Trevisanato & In Kim, 2000; Dufresne & Farnworth, 2001; Chantre & Lairon, 2002). The possible protective effects of polyphenols against heart diseases (Yoshikawa, Naito & Kondo, 1993; Marnewick, Gelderblom & Joubert, 2000) may be attributed to their ability to prevent the oxidation of low density lipotroteins to an atherogenic form, although anti-platelets aggregation activity and vasodilatory properties were also reported (Chen, Tang, Sutcliffe & Belton, 2000; Duthie, Duthie & Kyle, 2000; Santos-Buelga & Scalbert, 2000).

Boost energy: Other studies reported that green tea extract may boost energy levels and promote fat oxidation, and consequently be a useful tool in weight control (Benzie, Szeto, Strain & Thomlinson, 1999).

Anti-bacterial agent: Green tea is a known anti-bacterial agent in the mouth, to prevent gingivitis and periodontal disease by killing *Escherichia coli* and *Streptococcus* bacteria (Yoshoimoto, Furrkawa, Yamamoto, 1983). This anti-bacterial action can also be effective in treating bad breath (halitosis) by killing odor-causing bacteria (Bast, Haenem & Doelman, 1991; Hirasawa, Takada, Mikumura & Otake, 2002). The anti-bacterial properties made green tea a promising herbal remedy, for stomach discomfort, vomiting and to stop diarrhea (Bast *et al*., 1991). The anti-bacterial action of tea is also useful in treating infections and wounds (Bast *et al*., 1991; Ho, Chen & Shi, 1992; Stoner & Mukhutar, 1995).

Anti-oxidant agent: The chelating properties of total polyphenols may be attributed to the antioxidant activities (Cao, Guohua, Emin & Ronald, 1996; Auroma & Cuppett, 1997; Pieta, 2000; Liao, 2001). Most of the total polyphenols chelate iron (Fe^{2+}), but there were large differences in the chelating capacity (Van Acker, Van den Berg, Tromp, Grieffioen, Van Bennekom, Van der Vilgh & Bast, 1996; Zhu, Sang, Huang, Bai & Yang, 2000). Therefore, for good scavenging activity, a catechol moiety on ring B was required for the completion of oxidation process. The chelating could thus raise the scavenging activity to the level of the most active scavengers, possibly by site-specific scavenging. Therefore anti-oxidative capacity of flavonoids increased as their Fe^{2+} chelating activities increased (Santos-Buelga & Scalbert, 2000).

1.5 POLYPHENOLS IN GREEN TEA

The production of polyphenolic constituents in the tea plant was assumed to be a means of chemical defence against birds, insects and animals, which would consume the plant as food (Beart, Lilley & Haslam, 1985). Green tea is made without enzymatic oxidation of polyphenols, as polyphenol oxidase is inactivated by heat during the early stages of green tea processing (Hara *et al*., 1995). Thus, the polyphenols present in green tea should be the same as those found in fresh tea leaves (Van der Hagen, Yolton, Kaminski & Yolton, 1993). In a broader sense, green tea polyphenols consists of simple and complex compounds, the large majority of which are the flavonoid monomers catechins (C), catechin gallates (CG) and the flavan-3-ols or flavonols. The *epi*-isomers of the catechins and catechin gallates were the principal components found in tea (Van der Hagen *et al*., 1993). Tea leaves are rich sources of a group of compounds known as polyphenolic substances (Benzie *et al*., 1999), which account for almost 1/3 of dry mass of dried leaves (Roberts, 1958; Hilton, 1973; Liang, Lu & Shang, 1996). The colour of beverage and much of its taste, especially astringency was attributed to these polyphenolic compounds (Benzie *et al*., 1999). The tea catechins (C) and catechin gallates (CG) constituted 30 % (w/w) of the dry mass of tea (Baruah, Hazakira, Mahata, Korita & Murai, 1986; Harbowy & Balentine, 1997).

In a more specific sense, catechins include epicatechin (EC) and epicatechin gallate (EGC), while catechin gallate (CG) included epigallocatechin (EGC), gallochatechin (GC), epigallocatechin gallate (EGCG) and gallocatechingallate (GCG) (Forrest & Bendall, 1969; Hilton & Ellis, 1972; Hara *et al*., 1995). Two minor catechin digallates (CD), epicatechin digallate (ECDG) and epigallocatechin digallate (EGCDG) (Coxon, Holmes, Ollis, Vora, Grant & Tee, 1972; Nonaka, Kawahara & Nishioka, 1983; Hashimoto, Nonaka & Nishioka,

1987) have also been considered as catechin gallates (CG) (Opie, Clifford & Robertson, 1993). The four most common catechins and catechin gallates are EGCG, EGC, EGC and EC (Hara *et al*., 1995). Other catechins such GC (Figure 1.1) were present in smaller quantities in tea, whereas the gallates (GCG and CG) found in tea may be products of racemination and not "native" to the tea plant (Roberts, 1962).

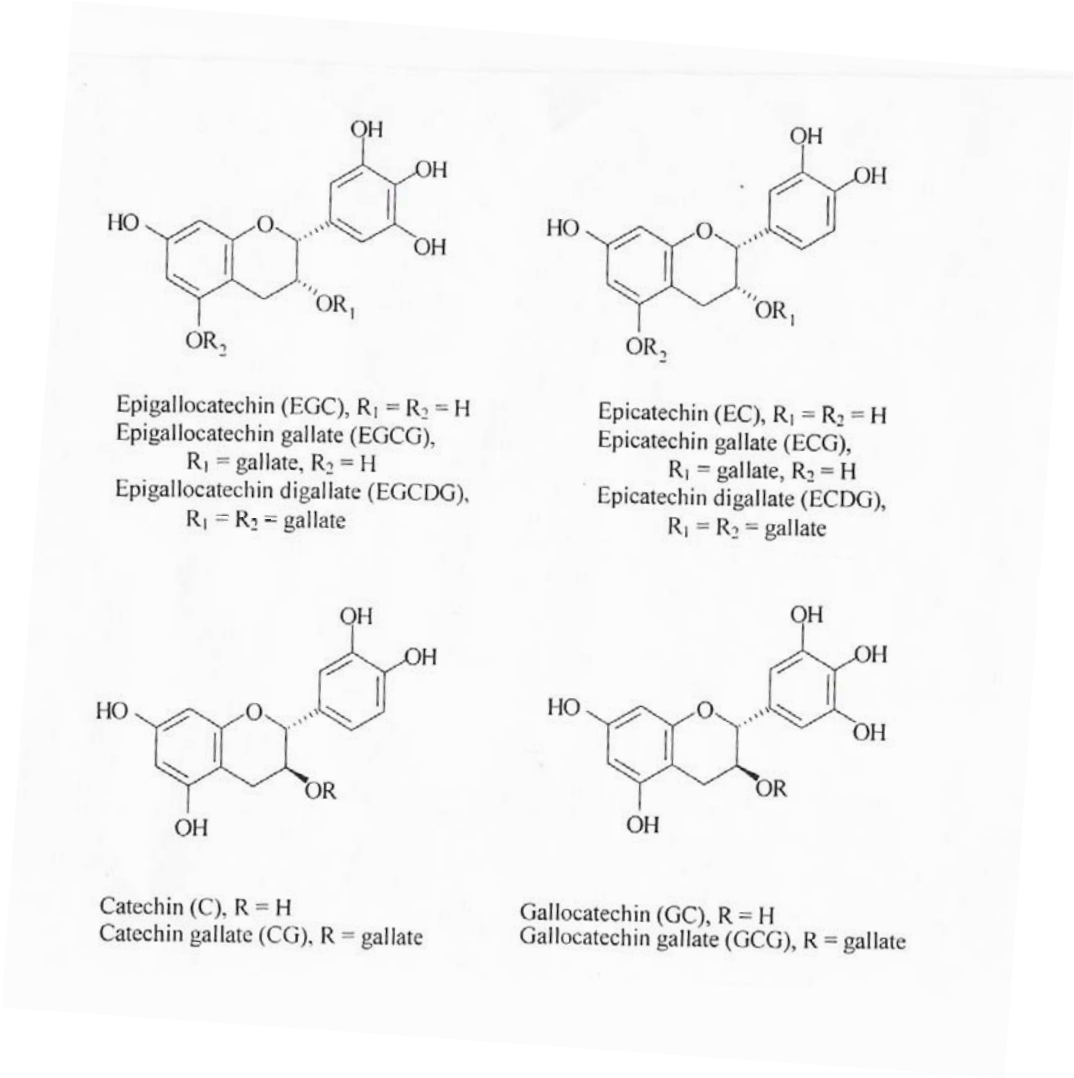

Figure1.1 Structures of tea catechins (Harbowy & Balentine, 1997)

The chemical constituents of polyphenols in tea shoots were shown to be (% total polyphenols): C 0.4; EC 1.3; GC; 2.0; EGC, 12.0; ECG; 18.1; EGCG; 58.1, while the other polyphenols were 6.67 % (Bokuchava & Skobeleva, 1969; Wu, 1974). EGCG is the major

constituent in all parts of tea shoots (Graham, 1992; Hilton, 1973). The C, GC, EC, ECG, EGC and EGCG predominantly constituted 80 % of the total polyphenols in green tea (Forrest & Bendell, 1969; Opie, Robertson & Davies, 1988). Salah, Miller, Paganga, Tijiburg, Bolwell & Rice-Evans (1995) reported that the total antioxidant activity (TAA) and the order of effectiveness of green tea polyphenols as radical scavengers were: ECG > EGCG > EGC > gallic acid > EC = catechin. Sala *et al* (1995) reported that gallic acid is the only phenolic acid obtained in green tea (Figure 1.2). Other phenolic acids are chlorogenic, theogallin and kaempferol glycosides (Sala *et al*., 1995).

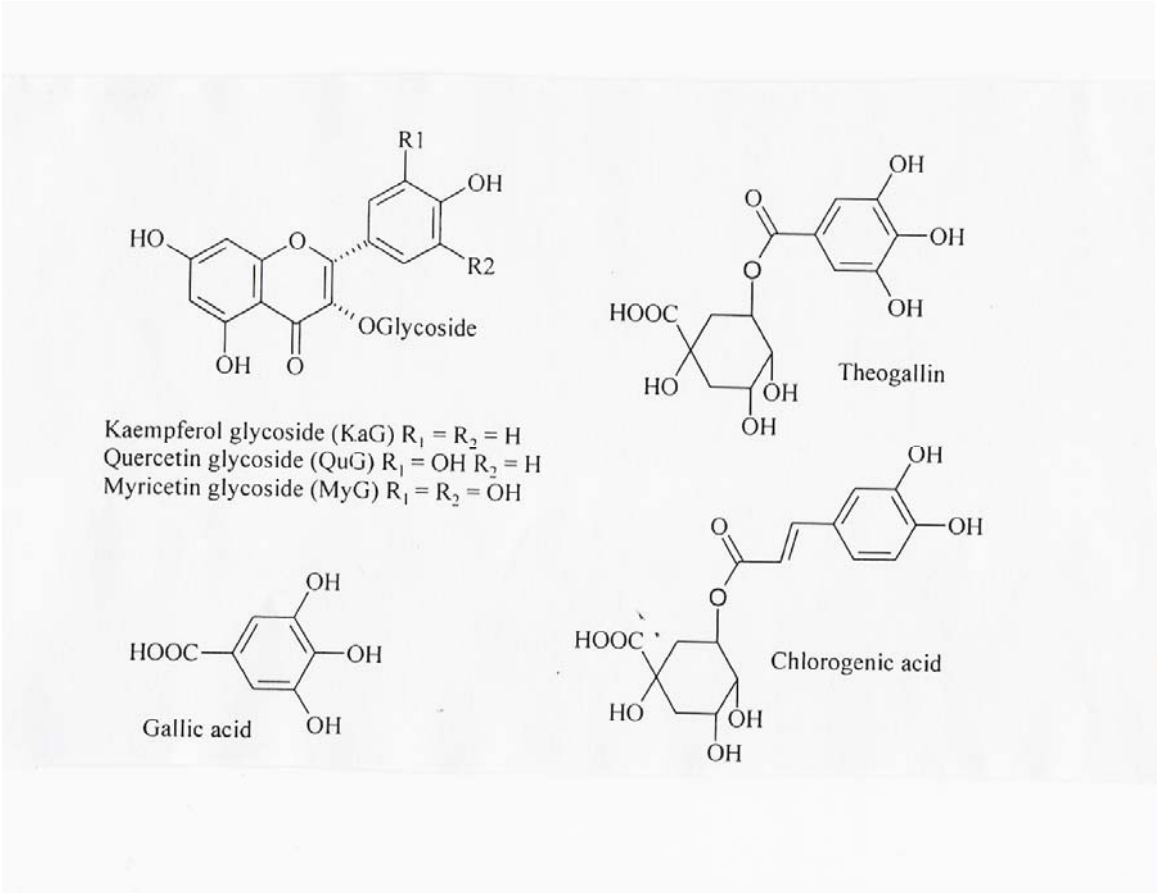

Figure 1.2 Structures of phenolic acids (Sala *et al*., 1995)

1.6 MACRO ELEMENTS

1.6.1 Effects of nitrogen nutrition on quality of green tea

Fatty acids: Saturated fatty acids do not contribute to the volatile flavour compounds (VFC) as they do not break down to more volatile compounds during black tea manufacture (Kamau, Owour & Wanyoko, 1999; Ng'etich, 1999). It is evident that unsaturated fatty acids (FA) in the young tender shoots of a tea breakdown through lipoxygenase-initiated oxidation during manufacture of black tea to form volatile flavour compounds (VFC) (Hatanaka, Kajiwara & Sekiiya, 1987). In the process, linoleic acids further break down to form hexanal which reduces to 1-hexanal (Hatanaka *et al*., 1987; Odhiambo, 1989). The authors further reported that unsaturated fatty acids, that had been associated with nitrogen and the fatty acids that influenced quality of tea were linolenic, linoleic, palmitic, steric acids, oleic and palmitoleic acids. However, the unsaturated fatty acids (FA) namely linolenic and linoleic acid were responsible for the production of undesirable volatile flavour compounds in green tea, thus concurring with the results of Fernado & Roberts (1984), who reported that the VFC produced by FA breakdown normally impart inferior green flavour. The optimum level of nitrogen level of 275 $kg \cdot ha^{-1}$ caused production of high amounts of VFC, thus resulting in reduction in flavour and quality of green tea (Owour & Obanda, 1998).

Amino acids: Liang, Liu, Xu & Hu (1990) reported that theanine was the major amino acid and its content reached 37.7 to 57 % of the total amino acids in green tea. The second predominant amino acids were glutamic acids followed by arginine and histidine (Cartwright, Roberts & Woods, 1954; Owour, Munavu & Muritu, 1990). The levels of

many of these flavour components were altered immediately after detachment of the leaf. Therefore, it was imperative that processing commenced as soon as possible after harvest, and preferably within one hour improved tea quality (Nge'tich, 1999). Although amino acid content of tea might influence quality, further studies needs to be expanded on how amino acids can be increased with time of the year with nitrogen application.

Plant pigments: The plant pigments viz., chlorophyll and carotenoid composition of fresh green leaf tea for selection of clones have been evaluated, to predict quality of black tea (Taylor *et al*., 1992). The chlorophyll content was reported to improve the development of the blackness of the processed tea (Wicknemasinghe & Perera, 1966; Van Lelyveld, Fraser, Smith & Visser, 1990). Increasing levels of nitrogen increased the chlorophyll levels of fresh green tea leaves (Wicknemasinghe & Perera, 1966) and the higher the chlorophyll content in the flush, the higher the concentration of phaeophytin, which in turn increased tea blackness. In contrast, chlorophyll was connected to the undesirable 'grassy' taste of black tea (Van Lelyveld, Rooster & Smith, 1989). However, it was not known whether it was the VFC compounds or their degradation products, which contributed to the quality of green tea. It was interesting to note that caretonoid rather than chlorophyll appeared to be the more significant compound in determining the quality of green tea. Further studies need to be carried out to investigate seasonal trends in response to nitrogen application of tea in order to predict quality from caretonoid and chlorophyll composition of fresh tea leaves together with other agronomic practices, environmental conditions and various manufacturing techniques.

Total polyphenols: The specific total polyphenols derivatives, such as theaflavins (TF) and thearubigins (TR) of green tea, have been established as important non-volatile green tea

constituents, with TF contributing to the brightness and briskness and TR (Langat, Otieno & Musau, 1998; Owour & Obanda, 1998) contributing to the depth of colour, mouthfeel and body of green tea (Hilton & Ellis, 1972; Cloughley, 1983; Kato & Shibamoto, 2001). Owour, Othieno, Robinson & Baker (1991) reported that in green tea quality parameters, i.e. theaflavins (TF), thearubigins (TR) and caffeine vary with time of the year and nitrogen fertiliser. The levels of TR and flavour index (FI) were generally high when there was no application of nitrogen and TF levels varied with the time of the year, but there was no clear trend to nitrogen fertiliser application (Roberts & Smith, 1963; Marwaha, 1999; Nge'tich, 1999; Kamau, Owour & Wanyoko, 1998). The plots receiving 300 $kg{\cdot}ha^{-1}N$ had generally higher caffeine levels than plots without fertiliser. It is therefore interesting that where nitrogen was not applied, less caffeine was produced throughout the whole season (Mwakha & Anyuka, 1984; Owour, Othieno, Horita, Tsushida & Murai, 1987; Macwell *et al*., 1990; Owour & Odhiambo, 1994; Kamau *et al*., 1999; Obanda, Owour & Rutto, 1999; Wachira & Nge'tich, 1999), thus indicating the benefit of caffeine formation drawn from nitrogen fertiliser application (Cloughley, 1982; Owour *et al*., 1987, Obanda *et al*., 1999). Thus, suggesting that nitrogen increased caffeine content of tea.

Volatile flavour compounds: The evaluation of green tea quality with respect to volatile flavour compounds (VFC) has been the subject of a number of investigations (Wickermarisnghe, 1974; Fernando & Roberts, 1984; Owino-Gerro, Keter & Mbuvi, 1999). The VFC were divided into two groups: group I VFC (those compounds known to impart inferior, green, grassy flavour) and group II VFC (compounds known to impart a sweet, flowery aroma) (Wickermarisnghe, 1974; Owour *et al*., 1987; Owour, Tsushida, Horita & Murai, 1988; Owour, Othieno, Odhiambo, Ng'etich, 1997). Low group I and group II VFC were noted where nitrogen was not applied, although the fluctuations in VFC levels were

not monitored continuously throughout the year. The major volatile flavour compounds variations were noted at 300 kg·ha^{-1} N for both NPKS 25:5:5:5 and NPK 20:10:10. The ratio of group II VFC to group 1 (flavour index, FI) was demonstrated as a quality indicator of the tea (Owour, Wanyako, Obanda, Othieno, 1998).

The response of tea quality parameters to time of the year and nitrogen with respect to VFC (Owour *et al*., 1991), showed that the VFC as groups I and II and FI increased with monthly variation. Thus, suggesting that VFC were more sensitive to monthly environmental conditions, irrespective of whether nitrogen fertiliser was applied or not.

1.6.2 Effects of phosphorus nutrition on quality of green tea

To date, no direct response from phosphate applications to mature tea on quality have been reported.

1.6.3 Effects of potassium nutrition on quality of green tea

Amino acids: Free amino acids in tea leaves were an important chemical constituent that considerably influenced the quality of tea, especially green tea (Ruan, Wu, Ye & Härdter, 1998). In field and pot experiments, biomass production and free amino acid content in tea leaves considerably increased following the application of potassium at a rate of 600 mg·kg^{-1} K (Ruan *et al*., 1998). Amino acids viz. theanine and arginine increased by application of sulphur containing products, indicating that potassium containing sulphur compound increased nitrogen metabolism, leading to an increased synthesis of amino acids (Ruan, Wu

& Härdter, 1999). This could also suggest that the effect of potassium containing sulphur products may result in an increase of amino acids.

Polyphenol and aromatic substances: In field experiments conducted in two seasons, i.e. spring and autumn, the content of polyphenol derivatives i.e. theaflavins and thearubigins, in tea leaves considerably increased following the application of potassium at a rate of 300 $kg \cdot ha^{-1}$ (Ruan *et al*., 1999). The three aromatic substances i.e. nerolidol, α-farnesene and (Z) 3-hexenyl hexanoate, increased by 12 and 23 % with potassium application (Owour *et al*., 1998; Ruan *et al*., 1999). The study was conducted with different types of tea including green tea and further studies may be required to strategically develop production protocol for high yielding tea and high quality tea in different seasons.

1.6.4 Effects of magnesium nutrition on quality of green tea

Amino acids: The application of soil-applied magnesium improved amino acid content in the leaves (Ruan *et al*., 1998). The maximum amino acid accumulations occurred with the application of 600 $mg \cdot kg^{-1}$ MgO. The larger amount of 800 $mg \cdot kg^{-1}$ MgO caused a decline in amino acid content. This may be explained by antagonistic effects between Mg^{2+} and K^{+} at this high rate, depressing the uptake of K^{+}. Theanine and arginine increased with an application containing sulphur-based Mg^{2+} (Ruan *et al*., 1998). However, an interesting observation was noted when comparing the two fertiliser sources of $MgSO_4$ and MgO. Cysteine and methionine were markedly increased with sulphur containing products thus suggesting that the responses were largely caused by sulphur (Mengel & Kirby, 1987).

Polyphenol and aromatic substances: The polyphenol derivatives theaflavin and thearubigin increased significantly with an increase of magnesium concentration of 300 $kg \cdot ha^{-1}$ MgO indicating that magnesium played a role in total polyphenols (Ruan *et al.*, 1999).

1.6.5 Effects of calcium nutrition on quality of green tea

Calcium (Ca) is one of the constituents of plant cell wall and plays an important role in cell division and activation of shoot growing points (Kler, 1995). However, it should be noted that calcium wis highly adsorbed on exchange sites of the free space, which is probably a limiting factor in Ca delivery to other plant organs. Similar findings were obtained by Xie (1998), who reported that Ca-oxalate and Ca-carbonate precipitated in the vacuoles. Calcium deficiency may cause shoot malformation and terminal die back (Wachira & Ng'etich, 1999), presumably due to the lack of phloem transport and immobility in the plant.

1.7 MICRO ELEMENTS

1.7.1 Effects of sulphur on quality of green tea

Sulphur (Su) application improved amino acid content in the leaves (Ruan *et al*., 1998). The maximum biomass production and amino acid accumulation occurred with the application of 600 $mg \cdot kg^{-1}$ $MgSO_4$. Magnesium containing sulphur compounds increased theanine and arginine, suggesting that the effect of $MgSO_4$ may be attributed to the S containing products (Ruan *et al*., 1998). Cysteine and methionine were also increased

(Mengel & Kirkby, 1987), probably due to the response to sulphur. This was not surprising because S plays a role in protein synthesis and it is a constituent of amino acids such as cysteine and methionine.

1.7.2 Effects of selenium on quality of green tea

Selenium (Se) has been identified as a constituent of glutathione perioxidase, which shows anti oxidative activity in higher, plants and reduced active oxygen free radical oxidation (Hu, 2001a). A trial was conducted to evaluate the response of green tea to selenium selinite and orga nic selenium at 50 $g \cdot ha^{-1}$ in older leaves of green tea with newly a growing bud and two young leaves collected two days after spraying (Hu, 2001a). The sensory quality evaluated parameters of unsprayed control tea extracts had a astringent taste, bitterness and the least sweetness and aroma (Hu, 2001b). However, when comparing sodium selenite and organically bound Se treatments, the treated extracts of green tea sprayed with sodium selenite tested was more astringent, bitter and less sweat than those sprayed with organically bound Se (Hu, 2001b). Furthermore, the extracts of green tea sprayed with Se had a better aroma than the control extracts.

Vitamim C content of green tea was significantly increased with Se spray (Hu, 2001a) and a significant difference in Vit C content was also found between sodium selenite and organically bound Se treatments. Both sodium selenite and organically bound Se treatments increased polyphenol content and chemical qualities of green tea which were harvested in the summer tea (Hu, 2001b). Although this trial was conducted in only one season, it could be interesting to determine if the chemical analyses would vary with respect to seasonal variation and time of the year after Se sprays.

1.7.3 Effects of zinc, iron, copper and boron on quality of green tea

To date, there has been no response reported of zinc (Zn), iron (Fe), copper (Cu) and boron (B) on tea quality parameters, suggesting that this is a subject that still needs to be investigated.

1.8 SUMMARY AND CONCLUSIONS

This review has generally examined the important information about phenolic compounds in green tea, beginning with total polyphenolics and their health benefits as the most potential quality indicators in green tea. It was suggested from this review that some of the individual phenolics, aromatics and amino acids occurring in tea shoots were the potential indicators for tea quality.

Nitrogen influenced the production of fatty acids, such as linolenic, palmitic steric acids, oleic, and palmitoleic acids, in the young shoots, catalysed by lipoxygenase enzyme to form volatile flavour compounds, which determine the quality of tea. It also improved amino acids such theanine, glutanic, argine and histadine, which generally improve the flavour of tea. Polyphenols derivatives such as thearubigins (TR) and theaflavins (TF) have been established as important polyphenol derivatives in green tea constituent with TF contributing to brightness and briskness (Owour & Odhiambo, 1994). The TR contributing to the depth of colour, mouthfeel and body of tea (Owour & Odhiambo, 1994). The application of nitrogen at an optimum level of 300 $kg \cdot ha^{-1}$ N increased the caffeine, but the volatile flavour compounds were lower where nitrogen was not applied. No direct responses on phosphorus have been reported on tea quality.

Free amino acids such as theanine and arginine content in green tea leaves considerably increased following the application of potassium at an optimum level of 600 $mg \cdot kg^{-1}$. The polyphenols derivatives ,viz. thearubigins and theaflavin, were considerably increased at an optimum level of 300 $kg \cdot ha^{-1}$ K. Soil-applied $MgSO_4$ at an optimum level of 600 $mg \cdot kg^{-1}$ K improved amino acid content such as theanine and arginine. Cysteine and methionine were also markedly increased with magnesium containing sulphur compounds.

The application of selenium improved vitamin C content, polyphenols of green tea and sensory quality parameters such as sweetness and aroma. No responses of zinc, iron, copper, and boron on tea quality parameters have been reported and therefore needs further investigations.

CHAPTER 2

A NEW FLAVONOL FROM *ATHRIXIA PHYLICOIDES* (L.) (BUSH TEA)

2.1 INTRODUCTION

Bush tea (*Athrixia phylicoides* L.) belongs to the Asteraceae family (Bremer, 1973). It has small, dark green pointed leaves with white woolly backs and small pink or mavvy pink daisy flowers with a bright yellow center (Roberts, 1990). Bush tea is a popular beverage used as a herbal tea and medicinal plant for cleansing or purifying the blood, treating boils, headaches, infested wounds, cuts and the solutions may also be used as a foam bath (Van Wyk & Gericke, 2000). It is also used as an aphrodisiac by Vhavenda people (Mabogo, 1990).

Flavonoids, or bioflavonoids, are an ubiquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers (Schewe & Sies, 2005). A great number of plant medicines contain flavonoids, which have been reported by many authors as having anti-bacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti-neoplastic, anti-thrombotic and vasodilatory activities (Hirasawa, *et al*., 2002). Multiple combinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures create the various classes of flavonoids: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins and isoflavones (Schewe & Sies, 2005). Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals

(Schewe & Sies, 2005). The structural components common to these molecules include two benzene rings on either side of a 3-carbon ring (Schewe & Sies, 2005) (Figure 2.1).

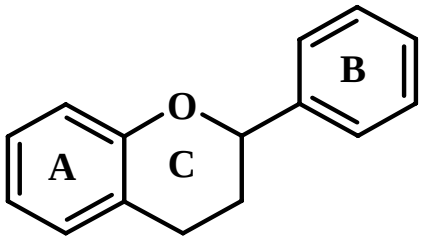

Figure 2.1 Structure of flavonol (Schewe & Sies, 2005)

Free radicals, including the superoxide radical (O2ú-), hydroxyl radical (.OH), hydrogen peroxide (H_2O_2) and lipid peroxide radicals have been implicated in a number of disease processes, including asthma cancer (Bast *et al*., 1991; Green, 1995), cardiovascular disease (Steinberg, Parthsarathy & Carew, 1989; Hertog, Feskens & Hollman, 1993) cataracts (Varma & Kinoshita, 1976; Gerster, 1989), diabetes (Kahler, Kuklinski, Ruhlmann & Lpotz, 1993), gastrointestinal inflammatory diseases (Yoshikawa *et al*., 1993; Smirnov, 1994), liver disease (Miguez, Anundi, Sainz-Pardo & Lindros, 1994), mascular degeneration (Lebuisson, Leroy & Rigel, 1986; Van der Hagen *et al*., 1993), periodontal disease (Bobyrev, Rozkulupa & Skripnikova, 1994) and other inflammatory processes. These radical oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body (Arabbi, Genovese & Lajolo, 2004) and as a result of increased exposure to adverse environmental and or dietary xenobiotics. In herbal teas, flavonols are

known to be the quality potential indicators since they are antioxidants in nature. Therefore, the objective of this study was to identify the major compound of bush tea.

2.2 MATERIALS AND METHODS

2.2.1 Experimental Procedures

Silica gel (0.063 – 0.2 mm) was used as a stationary phase and a mixture of hexane and ethyl acetate was used as mobile phase in the chromatographic separations (Figure 2.2).

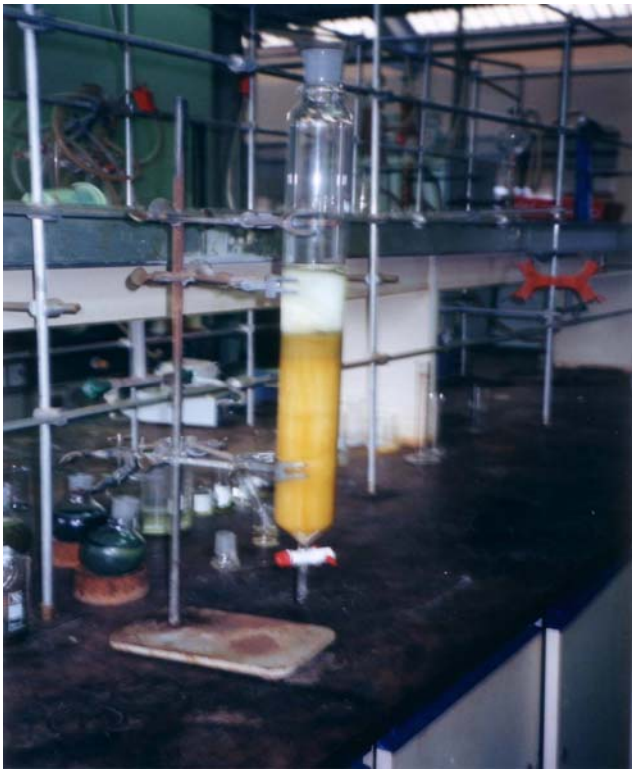

Figure 2.2 Column chromatograph with extracts of bush tea leaves

Thin layer chromatography plates, which were packed with silica gel, were used to isolate major components of the fractions from the minor ones. 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 (Labotech, Johannesburg), with $CDCl_3$ as solvent and TMS as an internal standard.

2.2.2 Extraction and Fractionation

The plant material (Figure 2.3) was harvested in Muhuyu village, Limpopo Province (South Africa).

Figure 2.3 Plant materials of wild bush tea

The green leaves (567 g) 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. Thirty (30) g silica was added and the mixture evaporated to dryness. The dry mixture was added on top of a chromatographic column containing 250 g of silica gel with

hexane as mobile phase. The mixture was chromatographed with a hexane : ethyl acetate gradient of increasing polarity to yield several low polarity phytosterol mixtures and a more polar, yellowish, crystalline product identified as 5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavon-3-ol.

2.2.3 Identification of the isolates

Thirty (30) mg crystallized as yellowish crystals from hexane/ethyl acetate (m.p. 127-129.5°C). ^{1}H NMR ($CDCl_3$) : δ 3.88 (3H, s, OMe), 3.92 (6H, s, 2xOMe), 3.93 (3H, s, OMe), 3.97 (3H, s, OMe), 4.02 (3H, s, OMe), 6.49 (1H, s, 5-OH), 7.47 (2H, s, 2'-H, 6'-H), 12.46 (1H, s, 3-OH). ^{13}C NMR ($CDCl_3$): δ 179.30 (S, C-4), 155.14 (S, C-2), 153.20 (S, C-3', C-5'), 148.82 (S, C-7), 147.95 (S, C-5), 144.83 (S, C-8a), 140.70 (C-4'), 139.06 (C-2', C-6'), 130.49 (C-3), 127.08 (C-6), 125.53 (C-1'), 122.23 (C-8), 106.01 (C-4a), 61.60 (Q, 8-OMe), 61.03 (Q, 7(4')-OMe), 61.00 (Q, 4'(7)-OMe), 60.27 (Q, 6-OMe), 56.24 (Q, 3'-OMe, 5'-OMe).

2.3 RESULTS AND DISCUSSION

The compound identified as ($C_{21}H_{22}O_{10}$) is a flavonol characterized for the first time in this study (Figure 2.4). Its structure was deduced from the data obtained from ^{1}H and ^{13}C NMR experiments (Table 2.1). The proton connectivity pattern was determined by analysis of the proton-proton coupling constants and the correlations observed in the ^{1}H-^{1}H COSY spectrum. The signals of the proton-bearing carbon atoms were correlated with specific proton resonances in a ^{1}H-^{13}C COSY experiment, which allowed the assignment of the structure (Figure 2.4) to the flavonol.

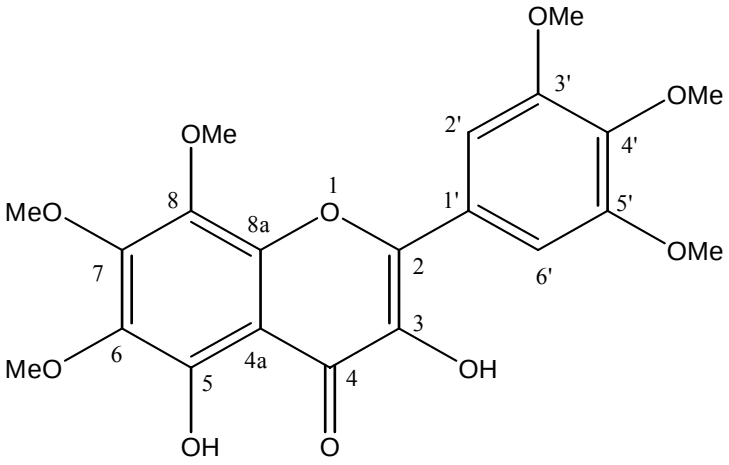

Figure 2.4 Five (5)-hydroxy-6,7,8,3',4',5'-hexamethoxy flavon-3-ol of bush tea

The ^{1}H NMR spectrum is dominated by five singlets (δ_H 4.019, 3.973, 3.928, 3.920 (6H), 3.876 ppm) due to six methoxy groups. Two aromatic protons resonate as a singlet at δ_H 7.467 ppm, leading to the conclusion of a 3',4',5'-trimethoxyphenyl substituent. A one-proton singlet at δ_H 12.46 ppm is typical of a strongly hydrogen-bonded hydroxyl group, and a very weak singlet at δ_H 6.49 indicated the presence of a phenolic hydroxyl group.

Table 2.1 NMR data for flavonol

Atom	**δ_H, ppm**	**δ_C, ppm**
2	-	155.1 S
3	12.46 (OH)	130.49 S
4	-	179.30 S
4a	-	106.01 S
5	6.49 (OH)	147.95 S
6	-	127.08 S
7	-	148.82 S
8	-	122.23 S
8a	-	144.83 S
1’	-	125.53 S
2’, 6’	7.467 (2H)	139.06 D
3’,5’	-	153.20 S
4’	-	140.7 S
6-MeO 3’,5’-diMeO 4’(7)-OMe 8-MeO 7(4’)-MeO	3.876 (3H) 3.920 (6H) 3.928 (3H) 3.973 (3H) 4.019 (3H)	60.27 Q 56.24 Q 61.00 Q 61.60 Q 61.03 Q

In the ^{13}C NMR spectrum the six methoxy groups were again clearly discernable at δ_C 56.24 (2 Me groups), 60.27, 61.00, 61.03, and 61.60 ppm. Apart from six aromatic carbon atoms, an enone carbonyl resonating at δ_C 179.30 ppm, two alkene carbon atoms at δ_C 155.1 and 130.49 ppm complete the structure.

2.4 SUMMARY

The objective of this experiment was to identify the major compound in bush tea. The plant materials were harvested in Muhuyu village (Limpopo Province, South Africa). 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. Silica gel (0.063 – 0.2 mm) was used as stationary phase and a mixture of hexane and ethyl acetate was used as mobile phase in the chromatographic separations. Thin layer chromatography plates, which were packed with silica gel, were used to isolate major components of the fractions from the minor ones. 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'-hexamethoxyflavon-3-ol as a major new flavonoid.

CHAPTER 3

RESPONSE OF TOTAL POLYPHENOL CONTENT IN BUSH TEA (*ATHRIXIA PHYLICOIDES* L.) TO SEASONAL VARIATION

3.1 INTRODUCTION

Total polyphenols were substances that have anti-oxidant, anti-bacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti-neoplastic, anti-thrombotic anti-cancer, anti-hypertensive, anti-cholesterolemic and anti-microbial activities as well as vasodilatory properties (Hirasawa *et al*., 2002). The major polyphenol anti-oxidant reported in green tea is epigallocatechin-3-gallate (EGCG), which reduces the amount of free radicals and inflammatory prostaglandins (Katiyar & Mukhtar, 1996). Herbal tea helps to protect against cancers of the lungs, skin, liver, pancreas, and stomach (Anon. 1992), reduces heart problems by lowering cholesterol levels and lowering the tendency of blood platelets to stick together (Stensveld, Tversdal & Solvoll, 1992). Herbal teas have been profoundly affected by seasons (Chiu, 1990). For example, Paochung tea produced best quality tea during winter followed by autumn tea. The summer tea was the worst in terms of quality possibly due to high temperatures and strong sunshine, which apparently produced higher tannin and low concentrations of polyphenols (Chiu, 1990). Polyphenols are potential quality indicators and bush tea leaves (Figure 3.1) are a rich source of polyphenols, which are potent antioxidants in nature. Therefore, the objective of the trial was to investigate the seasonal variation of total polyphenols and leaf nitrogen in bush tea leaves harvested from the wild.

3.2 MATERIALS AND METHODS

3.2.1 Sample collection

Approximately 200 g of leaf (matured leaves) samples of bush tea were randomly collected from end of January to December 2003 at Muhuyu Village (24°N 50'E, 31°S17'E; alt 610m; subtropical-type climate, i.e. summer rainfall, cold and dry winter) and air-dried. The annual rainfall is 650 mm per annum with temperatures ranging from 13-18 °C in winter and 27 to 39 °C in summer (Limpopo Province); autumn (March to May, winter (June to August), spring (September to November) and summer (December to February) and air-dried in the shade. The soil type is sandy loam with pH ranging from 5.6 – 7.4.

Figure. 3.1 Bush tea leaves

3.2.2 Preparation of the extracts

Approximately, 15 g of finely ground material was sieved (≤1.0 mm; Endocotts test sieves) for 5 minutes. From the sieved material, 0.5 g sample with 5 mL acetone were mixed for 2 hours in a shaker, and then centrifuged for 5 minutes at 4000 rpm. The supernatant was carefully decanted and the extraction procedure was repeated three times on residues. Three supernatants were combined and a volume of 15 mL with 75 % acetone was prepared. The residues were discarded.

3.2.3 Concentration of total polyphenols

Concentration of total polyphenol was determined using the Folin-Ciocalteu method (Waterman & Mole, 1994). In this method, 0.5 mL of the filtrate extracts was added to a 50 mL volumetric flask containing deionized water. The contents were swirled to mix and 0.5 mL of the solution was pipetted and mixed into a test tube containing 2.5 mL of Folin-Ciocalteu phenol reagent (Fluka Ltd, Johannesburg). Sodium carbonate solution (7.5 mL) was added to the mixture and the volume made up to exactly 50 mL with deionized water. The mixture was shaken thoroughly, by inventing it several times, and allowed to stand for 2 hrs for completion of the reaction when a blue colour formed. Measurements were done at 760 nm using a spectrophotometer (Cecil Instruments, Cambridge, UK). The standards (preparations of 0.05 g tannic acid) were dissolved in the extracting solvent (75 % acetone) up to 50 mL. The standard serial dilutions of 1, 0.8, 0.6, 0.4, 0.2, 0, 0.08, 0.06 and 0.02 $mg \cdot mL^{-1}$ were prepared, respectively. The optical densities were converted into concentrations from a standard curve using 1 to 0.02 $mg \cdot mL^{-1}$ of tannic acid with phenol reagent and sodium

carbonate in a similar manner. The standard curve obtained had a minimum r^2 value of 0.9869, passing through the origin.

3.2.4 Leaf and root N content

Total N was determined using the Auto-analyser method (Anon. 1972) on a Sanplus Segmented Flow Analysis System (Skalar Instruments, Netherlands), and expressed as % N per dry mass.

3.3 RESULTS AND DISCUSSION

3.3.1 Concentration of total polyphenols in leaves of wild bush tea

Concentration of total polyphenols showed seasonal variations in wild *A. phylicoides* (Figure 3.2). The lowest total polyphenol in the leaves concentrations were in March (11.8 $mg.g^{-1}$), April (10.8 $mg{\cdot}g^{-1}$) and September (10.8 $mg{\cdot}g^{-1}$), while the highest concentrations were in June (35·5 $mg{\cdot}g^{-1}$) and July (35.9 $mg{\cdot}g^{-1}$). The difference between the lowest and the highest concentration of total polyphenols in bush tea leaves was 25.1 $mg{\cdot}g^{-1}$ dry mass.

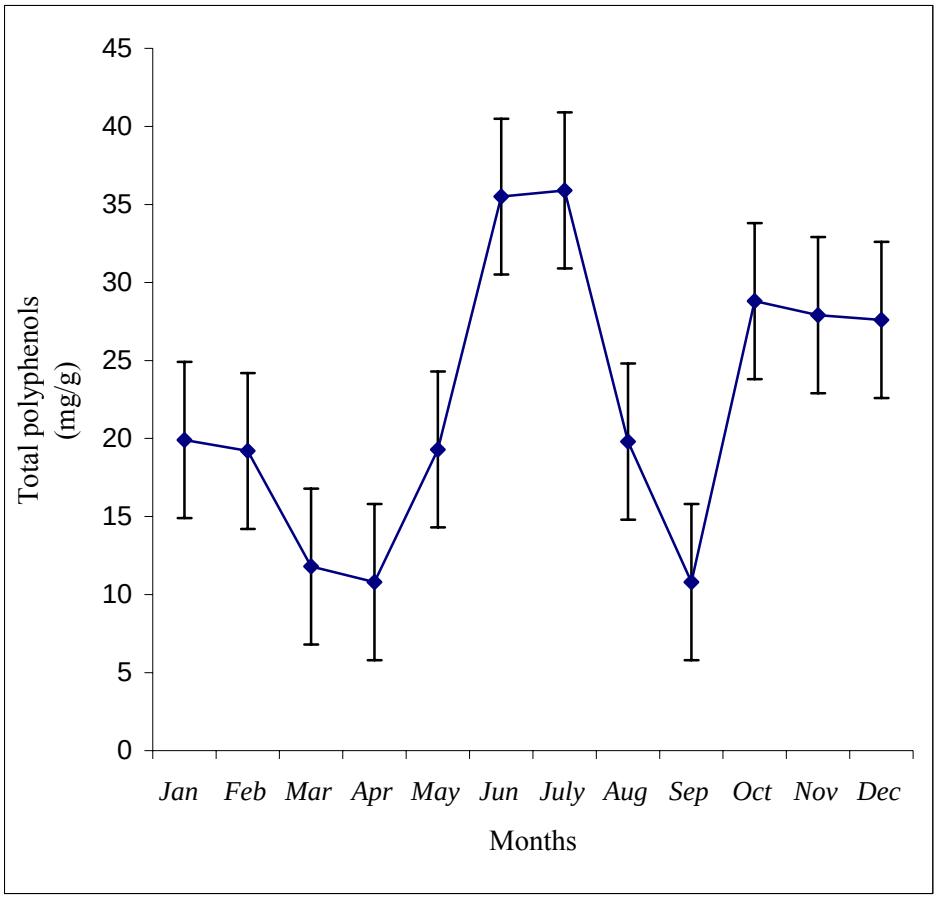

Figure 3.2 Measured monthly total polyphenols ($P<0.05$) in wild bush tea for the year 2002

Similar responses were reported by Owour (1992), where the concentrations of total polyphenols in black tea seedlings were highest during June (22.9 $mg \cdot g^{-1}$) and July (24.1 $mg \cdot g^{-1}$) in the eastern highlands of Kenya. In a field trial conducted by Ruan *et al*. (1999) during spring and autumn, there were differential responses on concentration of total polyphenols due to season. However, besides season, other studies reported that agronomic practices such as processing techniques (Fernando & Roberts 1984); plucking (Owour *et al*., 2000) and mineral nutrition (Owour *et al*., 1991) increased concentration of total polyphenols in green tea. Results in Figure 3.2 showed that the best time of harvesting bush tea from the wild was during winter or summer.

3.3.2 Concentration of total nitrogen in leaves of wild bush tea.

Concentration of total nitrogen in the leaves of wild harvested *A. phylicoides* showed seasonal variations (Figure 3.3). The lowest concentrations were in September (0.7 %), June and August (0.9 %), while the highest was in May (1.6 %) and showing inconsistent trend with the seasonal variation of total polyphenols of wild bush tea in Figure 3.2. The difference between the lowest and the highest concentration of total leaf tissue N content in bush tea leaves was about 1 %. Under field conditions, Owour & Odhiambo (1994) reported that zero application of nitrogen tended to reduce the concentration of total leaf nitrogen by 0.8 %. Other studies have demonstrated that concentration of leaf nitrogen increased with increasing nitrogen until an optimum level of 300 kg·ha^{-1} N, with percentage leaf tissue nitrogen ranging from 3 to 3.4 % in CTC black tea throughout the whole year (Wanyoko, 1983).

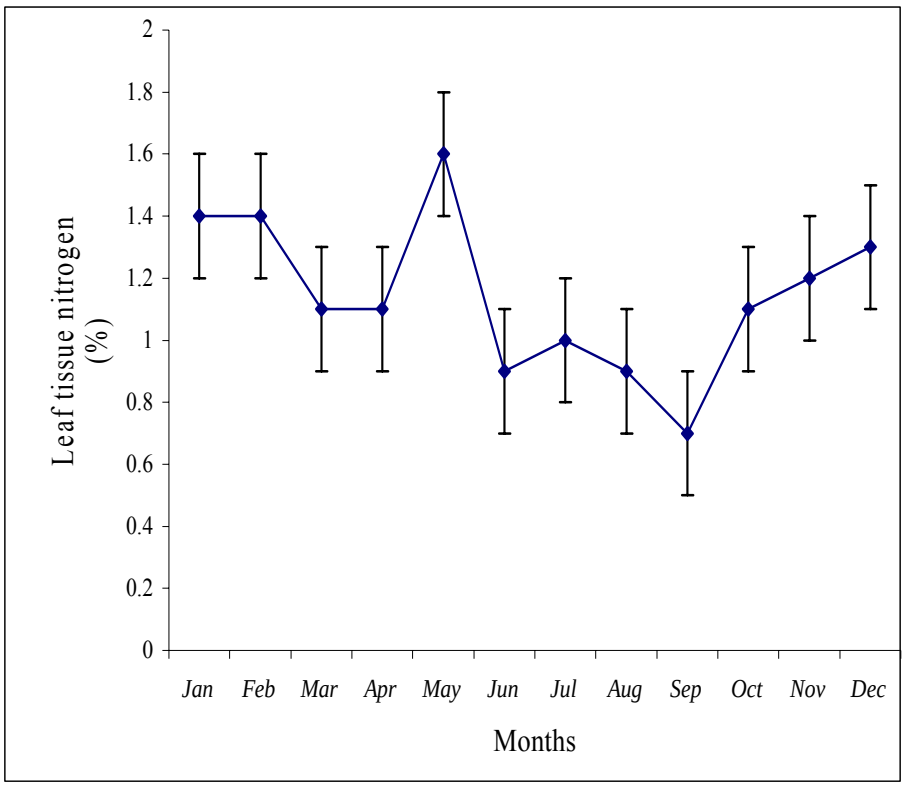

Figure 3.3 Measured monthly total leaf nitrogen ($P<0.05$) in bush tea harvested from the wild for the year 2002

In conclusion, the best time to harvest wild bush tea would therefore be in winter or summer in order to maximize the possible potential antioxidants.

3.4 SUMMARY

In herbal tea polyphenols are quality indicators since they are antioxidants in nature. Therefore, the objective of the trial was to investigate the seasonal variation of total polyphenols in bush tea leaves harvested from the wild. Leaf samples were collected from the field at Muhuyu Village (Limpopo Province) from January to December in 2003, and then dried. Total polyphenols were extracted using Folin-Ciaocalteau reagents and analyzed in a spectrophotometer. Total polyphenols showed definite seasonal variations. The lowest concentrations were in March (11.8 $mg \cdot g^{-1}$), April (10.8 $mg \cdot g^{-1}$) and September (10.8 $mg \cdot g^{-1}$), while the highest were in June (35.5 $mg \cdot g^{-1}$) and July (35.9 $mg \cdot g^{-1}$). There was no consistent trend on leaf tissue N. Thus, suggesting that the total polyphenols were largely not attributed to total leaf nitrogen. Therefore, the ideal time for harvesting bush tea would, therefore, be during winter followed by summer.

CHAPTER 4

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

4.1 INTRODUCTION

Cultural practices have a significant influence on tea growth and productivity of herbal teas (Owour, 1989; Ruan *et al*., 1999; Venkatesan *et al*., 2004). Among such cultural practices, mineral nutrition (Hilton & Palmer-Jones, 1973; Barauh *et al*., 1986; Owour, Odhiambo, Robinson & Taylor, 1990; Owour & Odhiambo, 1994), plucking (Owour *et al*., 2000), and irrigation (Stephens & Carr, 1991; Nge'tich, 1999) have been widely reported to improve tea growth and maximize productivity.

In mineral nutrition, the application of nitrogen, potassium and phosphorus fertilizers are the main normal agronomic practices and several studies have shown improvement on growth and yield of tea (Owour & Odhiambo, 1994; Marschener, Kirby & Cakmak., 1996; Keen & Zidenberg-Cherr, 2000; Owour *et al*., 2000).

The plant materials of bush tea are only harvested from the wild and the concepts of domesticating wild plants is very important in order to avoid the natural population from becoming extinct from its native environment. Presently, the mineral nutrition on bush tea is not well established. Data are lacking on the response of N, P and K on growth and productivity of bush tea. Therefore, the objective of this study was to determine the effects of

nitrogen, phosphorus and potassium application on growth and development of bush tea under cultivation as influenced by season.

4.2 MATERIALS AND METHODS

4.2.1 Experimental site and plant material

The study was carried out in Morgenzon, a commercial nursery in Louis Trichardt (23°N 50'E, 30°S 17'E; alt 610 m; subtropical-type climate i.e. summer rainfall and cold, dry winter). On 13 November 2002, plant material was collected from Venda (Limpopo Province) and 1500 cuttings were dipped in Seradix® No. 2 hormone (0.3 % IBA) (Bayer, Pretoria, South Africa) and established in seed trays on a mist bed. Rooted cuttings were transplanted into 1 L bags and placed in a hardening-off chamber for 3 months (Figure 4.1). After 3 months, seedlings were transplanted into 20 L bags.

Figure 4.1 Rooted cuttings of bush tea transplanted into 1 L bags

The medium was a 1 pine bark: 2 sand : 1 stryofoam bead mix (v/v), with AquaGro wetting agent (Aquatrols, Cherry Hill, N.J) at 0.2 $kg \cdot m^{-3}$. The initial media test chemical analyses was determined using procedure of (Hanlon, Gonzalez & Bartos, 1994). The EC was 0.9 $dS \cdot m^{-1}$ and pH was 4.7 of the growth media. The pine bark contained 1.2 $mg \cdot kg^{-1}$ NO_3-N, 0.1 $mg \cdot kg^{-1}$ P and 1.3 $mg \cdot kg^{-1}$ K.

4.2.2. Experimental design and treatments

Three (N, P and K) parallel trials were conducted under 50 % shade nets with one at each season (summer, autumn, winter and spring) in a randomized complete block designed with six treatments replicated eight times. Meterological data on temperature (°C), rainfall (mm),

relative humidity (%) and evaporation (mm) were supplied by Agrometerorological Division at Morgenzon, a commercial nursery (Louis Trichardt, Limpopo Province, South Africa) (Table 4.1).

Table 4.1 Average seasonal variations in temperature, rainfall, relative humidity, and evaporation on growth of bush tea under 50 % shade nets in 2003/4

Season	Temperature (°C)	Rainfall (mm)	Relative humidity (mm)	Evaporation (mm)
Autumn	28	300	51	68
Winter	24	100	44	45
Spring	34	400	86	65
Summer	38	500	77	75

Fertilizer sources used were limestone ammonium nitrate (for N trial), single super phosphate (for P trial) and potassium chloride (for K trial) applied as post treatments in the form of granules. The treatments consisted of 0, 100, 200, 300, 400 or 500 $kg \cdot ha^{-1}$N, P or K. All plant received 1 % $MgSO_4$, ZnO, Microfel® Fe (Fe = 29 %), mono ammonium phosphate (Climax®) [52 % P_2O_3 (P = 22%), 34 % K_2O (K = 28.2 %)] and urea (N = 46 %) (except for N and P trial), sodium borate [$Na_2B_4O_7 \cdot 10H_2O$ (27 % boron and 18 % Na)] and KCℓ (except for K trial) were applied twice per week as foliar sprays to supplement the rest of the elements necessary for the production of good quality tea.

4.2.3 Data collection

For all the treatments, at harvest (autumn, 30 May 2003; winter, 30 August 2003; spring, 30 November and summer, 28 February 2004), plant height (cm), number of branches, number of leaves, flowers and flower buds (autumn and winter), stem girth (mm), fresh and dry root mass (g), fresh and dry stem mass (g), leaf area (measured by a LI-3100 area meter; LI-COR, Lincoln, Neb), fresh and dry shoot mass (g), and percentage leaf and root N, P or K content were recorded.

4.2.4 Leaf and root N content

Total N was determined using the Auto-analyser method (Anon., 1972) on a Sanplus Segmented Flow Analysis System (Skalar Instruments, Netherlands), and expressed as % N per dry mass.

4.2.5 Leaf, root P and K tissue content

Phosphorus and potassium were analyzed using the method of Adrian (1973), and expressed as % P and % K per dry mass.

4.2.6 Statistical analysis

Analyses of variance were performed on data using the GLM (General linear model) procedure of SAS version 8.0 (SAS Institute Inc., 1999). Treatment sums of squares were partitioned into linear and quadratic polynomial contrasts.

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of nitrogen on growth and development of bush tea

Regardless of season, plant height, number of branches and leaves, leaf area, and fresh and dry shoot mass increased quadratically in response to nitrogen nutrition (Tables 4.2, 4.3, 4.4 and 4.5). Regardless of season, the optimum level of N was 300 kg·ha^{-1}. Most of the growth responses occurred between 0 and 300 kg·ha^{-1} N. Wanyoko (1983) reported that biomass production of green tea increased to an optimum at 250 kg·ha^{-1} N. Krishnapillai & Pethiyagoda (1979) reported that when different forms of nitrogenous fertilizer such as ammonium sulphate, ammonium nitrate, urea and calcium nitrate were applied at 300 kg·ha^{-1} N, biomass production of young tea (*Camellia sinensis* L.) was increased.

Regardless of season, leaf tissue N and root tissue content increased quadratically to reach an optimum at 300 kg·ha^{-1} N (Tables 4.2, 4.3, 4.4 and 4.5). Wanyoko (1983) reported that the normal harvestable tea leaves had leaf tissue N content of 3 to 3.4 %. Regardless of season, root tissue nitrogen content increased quadratically to reach an optimum at 300 kg·ha^{-1} N (Tables 4.2, 4.3, 4.4 and 4.5). Anandacoomaraswamy, De Costa, Tennakoon & Van Der Werf (2002) reported that 375 kg·ha^{-1} N increased assimilates of N partitioned towards the

shoots at the expense of roots in young clonal tea, possibly due to a high photosynthetic rates in the leaves than in the roots. No significant differences in stem girth, fresh and dry root mass, number of flowers and flower buds as well as fresh and dry stem mass were recorded in the present study.

4.3.2 Effect of phosphorus on growth and development of bush tea

Results in Tables 4.6, 4.7, 4.8 and 4.9 showed that regardless of season, P increased in a quadratic fashion the plant height, number of leaves, branches, fresh and dry shoot mass, leaf area and root tissue. The optimum level was 300 kg·ha^{-1} P. However, most of the growth responses occurred between 0 and 300 kg·ha^{-1} P throughout the seasons (Tables 4.6, 4.7, 4.8 and 4.9). Ruan *et al*. (1999) reported that P nutrition applied as a single super phosphate at 225 kg·ha^{-1} P, increased biomass production of green tea. Leaf tissue P was increased quadratically to reach an optimum at 300 kg·ha^{-1} P (Tables 4.6, 4.7, 4.8 and 4.9). Wanyoko (1983) also reported that the normal harvestable tea leaves had a leaf tissue P content of 0.5 to 0.8 %. Percentage root tissue P was also increased quadratically to reach an optimum at 300 kg·ha^{-1} P, regardless of season (Tables 4.6, 4.7, 4.8 and 4.9).

Table 4.2 Response of growth characteristics of bush tea to N nutrition during autumn

Applied nitrogen ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of flower buds	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
0	77	36	32	411	32	17	818	1.7	0.5
100	86	113	38	598	39	22	935	2.1	0.6
200	88	102	39	688	50	29	1084	2.6	1.4
300	96	104	37	926	56	32	1479	3.2	2.0
400	95	121	35	702	55	33	1197	3.1	2.3
500	88	117	32	762	53	32	935	2.8	2.2
Response	Q^{**}	Q^{**}	NS	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.3 Response of growth characteristics of bush tea to N nutrition during winter

Applied nitrogen ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of flower	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf Tissue N (%)	Root tissue N (%)
0	25	3	23	124	20	12	260	1.4	1.0
100	49	7	29	192	26	18	431	2.4	1.9
200	55	6	25	235	35	20	465	3.1	2.6
300	61	6	20	346	48	23	1107	3.8	3.4
400	73	7	17	310	49	22	575	3.7	3.4
500	70	7	19	317	49	22	550	3.6	3.5
Response	Q^{**}	Q^{**}	NS	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.4 Response of growth characteristics of bush tea to N nutrition during spring

Applied nitrogen ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
0	63	17	841	37	16	425	1.6	1.2
100	109	36	947	69	23	896	3.2	1.8
200	114	40	1115	76	30	927	3.5	2.2
300	118	54	1479	78	37	965	3.8	2.6
400	114	28	1088	76	37	813	3.7	2.7
500	103	18	1076	74	37	553	3.6	2.7
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.5 Response of growth characteristics of bush tea to N nutrition during summer

Applied nitrogen ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf Tissue N (%)	Root tissue N (%)
0	73	23	844	47	21	658	1.6	1.3
100	122	37	949	79	28	1431	1.9	1.6
200	119	40	1115	86	35	1389	2.5	1.8
300	110	54	1089	88	42	1772	2.6	1.9
400	114	28	1479	86	42	1651	1.6	2.1
500	103	18	1137	84	42	1443	1.6	2
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.6 Response of growth characteristics of bush tea to P nutrition during autumn

Applied phosphorus ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Number of flower buds	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf Area (cm^2)	Leaf tissue P (%)	Root tissue P (%)
0	50	22	219	20	27	18	349	0.1	0.1
100	89	40	353	22	34	26	751	0.2	0.2
200	79	45	357	23	45	34	789	0.3	0.3
300	89	46	439	19	51	41	1014	0.5	0.4
400	83	32	345	20	50	41	733	0.4	0.4
500	81	29	318	17	51	38	705	0.3	0.3
Response	Q^{**}	Q^{**}	Q^{**}	NS	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.7 Response of growth characteristics of bush tea to P nutrition during winter

Applied phosphorus ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Number of flower	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue P (%)	Root tissue P (%)
0	27	5	61	15	11	8	142	0.4	0.4
100	36	11	177	16	14	11	312	0.6	0.6
200	50	10	259	16	19	12	342	0.7	0.7
300	40	15	260	18	26	16	463	0.7	0.8
400	38	13	257	18	25	16	409	0.7	0.7
500	37	13	216	17	25	16	371	0.7	0.7
Response	Q^{**}	Q^{**}	Q^{**}	NS	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.8 Response of growth characteristics of bush tea to P nutrition during spring

Applied phosphorus ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue P (%)	Root tissue P (%)
0	73	7	294	29	17	355	0.2	0.2
100	96	15	311	41	21	521	0.3	0.3
200	97	12	413	44	22	631	0.4	0.4
300	104	20	444	48	25	729	0.6	0.5
400	95	17	355	48	22	577	0.5	0.5
500	82	16	301	47	22	400	0.5	0.5
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.9 Response of growth characteristics of bush tea to P nutrition during summer

Applied phosphorus ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue P (%)	Root tissue P (%)
0	85	9	423	44	25	356	0.2	0.2
100	127	14	1186	56	29	548	0.3	0.5
200	143	16	1304	59	30	577	0.4	0.5
300	142	22	1372	63	33	717	0.4	0.6
400	128	19	1362	63	31	717	0.4	0.6
500	154	19	1302	62	30	401	0.4	0.5
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P=0.05 (*), 0.01 (**) or non significant (NS)

No significant differences in number of flower and buds (winter and autumn), stem girth, fresh and dry root mass as well as fresh and dry stem mass were recorded.

4.3.3 Effect of potassium on growth and development of bush tea

Results in Tables 4.10, 4.11, 4.12 and 4.13 showed that the plant height (cm), number of branches, number of leaves (spring), leaf area, as well as fresh and dry shoot mass increased quadratically in response to potassium. The optimum level was 200 kg·ha^{-1}K throughout the seasons (Table 4.10, 4.11, 4.12 and 4.13). Most of the growth responses occurred between 0 and 200 kg·ha^{-1} K. Ruan *et al.* (1999) reported that biomass production considerably increased following the potassium application, reaching the maximum at 800 mg·kg^{-1} K_2O. Percentage leaf and root tissue K quadratically increased at an optimum level of 200 kg·ha^{-1} K (Tables 4.10, 4.11, 4.12 and 4.13). Similar findings were also confirmed by Ruan *et al.* (1998), whereby K application increased leaf and root tissue K content by 3.5 to 4.1 % K in green tea. Wanyoko (1983) reported that the normal harvestable tea leaves had leaf tissue K content of 1.5 to 1.8 %. No significant differences in number of flowers buds (autumn and winter), number of leaves (autumn and summer), stem girth, fresh and dry root mass as well as fresh and dry stem mass were recorded. In conclusion, the results of this study demonstrated that regardless of season, N, P and K nutrition increased bush tea fresh and dry shoot mass, plant height and number of branches.

The optimum level of bush tea for improved growth was 300 kg·ha^{-1} for N or P and 200 kg·ha^{-1} for K regardless of season. In all trials, regardless of season, no significant differences in stem girth, fresh stem and root mass and dry and stem mass were obtained.

Table 4.10 Response of growth characteristics of bush tea to K nutrition tea during autumn

Applied potassium ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of flower buds	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue K (%)	Root tissue K (%)
0	39	17	17	229	17	13	464	2.3	0.1
100	60	46	17	226	21	17	1138	3.6	0.2
200	61	48	19	229	24	18	1589	4.4	0.3
300	53	38	18	215	24	17	1479	4.7	0.4
400	53	35	20	252	23	17	1550	4.8	0.4
500	49	28	19	218	22	16	1398	4.8	0.3
Response	Q^{**}	Q^{**}	NS	NS	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.11 Response of growth characteristics of bush tea to K nutrition during winter

Applied potassium ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Num of flowers	umber of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue K (%)	Root tissue K (%)
0	44	7	13	87	12	9	121	2.3	0.4
100	60	15	14	95	15	11	212	2.8	0.6
200	117	17	14	210	16	13	366	3.3	0.7
300	73	16	14	187	17	14	328	3.7	0.7
400	57	14	14	122	18	13	255	3.8	0.7
500	40	13	14	116	17	13	215	3.7	0.7
Response	Q^{**}	Q^{**}	NS	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.12. Response of growth characteristics of bush tea to K nutrition during spring

Applied potassium ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue K (%)	Root tissue K (%)
0	70	8	363	27	12	233	1.7	0.2
100	100	18	613	50	24	287	2.3	0.3
200	102	22	712	52	30	348	2.4	0.4
300	99	13	427	50	20	310	2.5	0.5
400	79	13	609	49	19	256	2.3	0.5
500	73	13	651	46	17	233	2.2	0.5
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P = 0.05 (*), 0.01 (**) or non significant (NS)

Table 4.13 Response of growth characteristics of bush tea to K nutrition during summer

Applied potassium ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of branches	Number of leaves	Fresh shoot mass (g)	Dry shoot mass (g)	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
0	77	9	233	41	16	369	0.8	0.2
100	101	19	289	64	29	673	1.6	0.3
200	106	23	294	66	35	712	2.2	0.4
300	104	15	291	64	34	500	1.3	0.4
400	100	12	257	63	24	645	1.2	0.4
500	95	13	256	60	21	651	0.7	0.4
Response	Q^{**}	Q^{**}	NS	Q^{**}	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P=0.05 (*), 0.01 (**) or non significant (NS)

4.4 SUMMARY

Bush tea was grown under varying nitrogen, phosphorus and potassium levels in all four seasons (autumn, winter, spring and summer) to determine the seasonal nutrient requirements for improved plant growth. Three parallel 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·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 percentage leaf and root tissue N, P and K.

Results of this study demonstrated that, in all trials regardless of season, N, P or K nutrition increased fresh and dry shoot mass, plant height, number of leaves, number of branches and leaf area of bush tea. Regardless of season, the optimum level of bush tea was 300 kg·ha^{-1} N or P and 200 kg·ha^{-1} K. 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.

CHAPTER 5

RESPONSE OF LEAF TOTAL POLYPHENOL CONCENTRATIONS TO NITROGEN, PHOSPHORUS AND POTASSIUM NUTRITION OF BUSH TEA *(ATHRIXIA PHYLICOIDES* L.) IN A SHADED NURSERY ENVIRONMENT

5.1 INTRODUCTION

Herbal teas have high concentrations of total polyphenols (Owour *et al*., 2000, Venkatesan *et al*., 2004). Polyphenols are substances that have anti-oxidant, anti-bacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti-neoplastic, anti-thrombotic, anti-cancer, anti-hypertensive, anti-cholesterolemic and anti-microbial activities as well as vasodilatory action (Hirasawa *et al*., 2002). The major polyphenol antioxidant reported in green tea is epigallocatechin-3-gallate (EGCG), which reduced the amount of free radicals and inflammatory prostaglandins (Katiyar & Mukhtar, 1996).

Agronomic practices such as plucking (Owour *et al*., 2000) and mineral nutrition (Owour, 1989; Owour *et al*., 1990; Owour & Odhiambo, 1994) improved the concentration of total polyphenols in green tea. Among such agronomic practices, the application of mineral nutrition is the main normal agronomic practice and several studies have reported total polyphenol improvement due to the addition of N, P and K (Owuor *et al*., 1991; Owour *et al*., 2000). Nitrogen, P and K application improved the accumulation of plant carbohydrates and thus plant growth (Wanyoko, 1983) and also increased photosynthetic rates (Haukioja,

Ossipove, Koricheva, Honkanen, Larsson & Lempa, 1998). This resulted in the biosynthesis of carbon based secondary metabolites, such as flavonoids, phenolic acids and tannins, known as total polyphenols which are anti-oxidant in nature (Haukioja *et al*., 1998).

Presently, agronomic practices such as mineral nutrition on total polyphenols of bush tea are not well established. The plant materials are only harvested from the wild for medicinal and herbal tea purposes. The concentrations of total polyphenols in tea leaves are the main potential indicators for medicinal potential due to their anti-oxidant activities (Hirasawa *et al*., 2002). Therefore, the objective of the study was to determine the effect of nitrogen (N), phosphorus (P) and potassium (K) application on total polyphenols of bush tea, as influenced by season.

5.2 MATERIALS AND METHODS

5.2.1 Experimental site and plant material [Refer to Chapter 4]

5.2.2 Experimental design [Refer to Chapter 4]

5.2.3 Leaf tissue N, P and K content [Refer to Chapter 4]

5.2.4 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using PROC 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 polyphenols, and total leaf tissue nitrogen, phosphorus and potassium.

5.3 RESULTS AND DISCUSSION

5.3.1 Response of leaf total polyphenols of bush tea to nitrogen nutrition

Results in Table 5.1 show that total polyphenols increased quadratically in response to nitrogen, regardless of season. Total polyphenol level peaked at 300 kg·ha^{-1} N during autumn, winter, spring and summer. The application of 300 kg·ha^{-1} N improved the total polyphenol content during autumn (38.0 mg·g^{-1}), winter (51 mg·g^{-1}), spring (43.5 mg·g^{-1}) and summer (48.9 mg·g^{-1}) (Table 5.1). Most of the total polyphenol response to N occurred between 0 to 300 kg·ha^{-1} N with the highest total polyphenols obtained during winter (51 mg·g^{-1}) (Table 5.1). Owour (1989) showed that total polyphenols of black tea deteriorated with increasing nitrogenous fertilizer rates from 450 to 600 kg·ha^{-1} N.

Table 5.1 Response of leaf total polyphenols of bush tea to nitrogen nutrition

	Concentration of total polyphenols ($mg \cdot g^{-1}$)			
Applied N ($kg \cdot ha^{-1}$)	Autumn	Winter	Spring	Summer
0	26.0	28.0	28.4	26.6
100	37.2	46.2	39.6	38.9
200	37.9	47.8	41.7	42.1
300	38.0	51.1	43.5	48.9
400	37.8	51.0	42.9	47.7
500	37.7	50.1	42.8	47.3
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P=0.05 (*), 0.01 (**) or non significant (NS)

There were positive correlation and significant linear relationship between leaf tissue N and total polyphenol content of bush tea leaves, regardless of season (Figure 5.1).

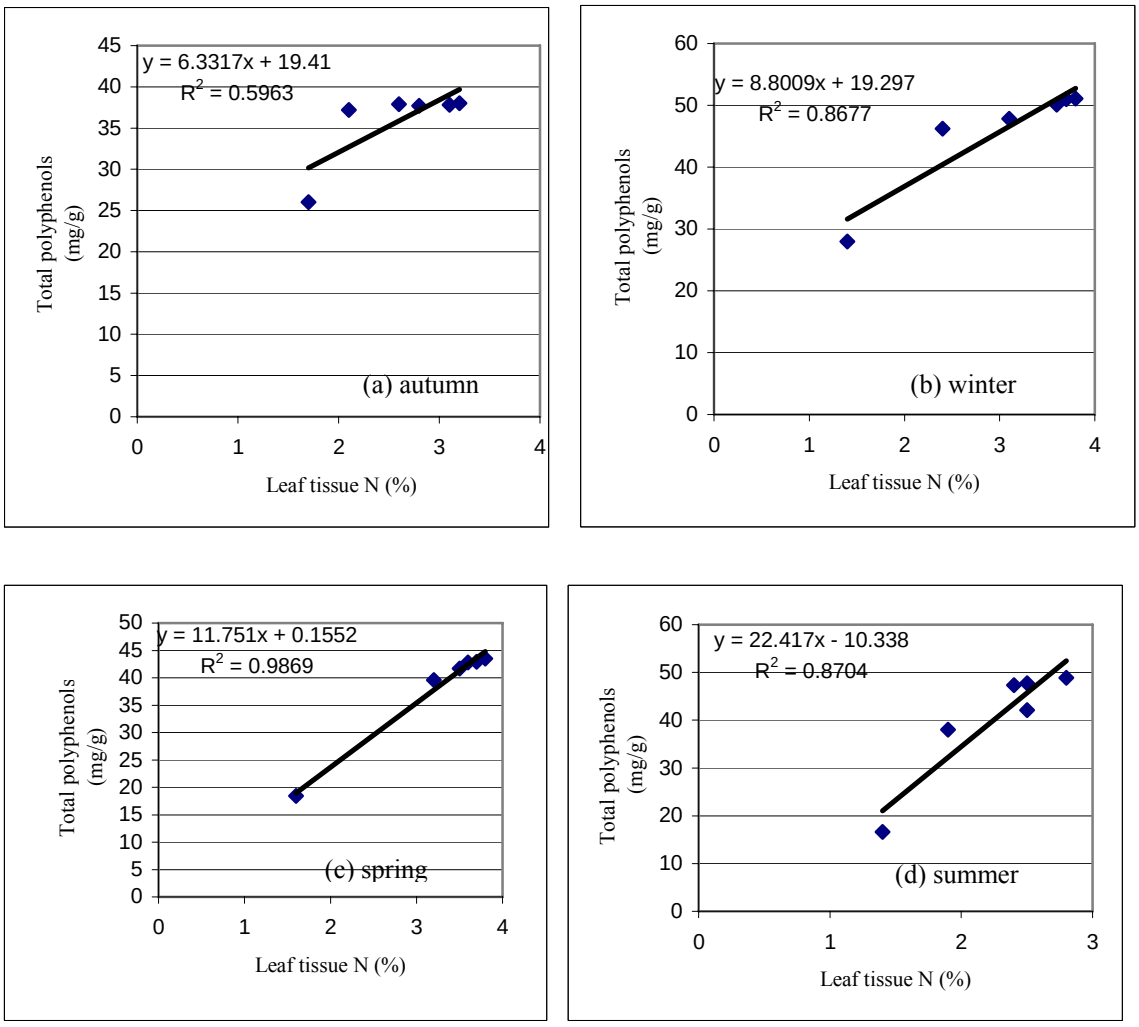

Figure 5.1 Correlation and regression between total polyphenols and leaf tissue nitrogen of bush tea with respect to season

Bryant, Clausen & Werner (1987) and Tuomi, Niemelä, Haukioja, Siré & Neuvonen (1984) reported a negative correlation between concentrations of carbon based secondary compounds (CBSCs) such as total phenolics and low nutrient availability in plant tissues of aspen tortix (*Populus tremuloides*) and aska paper (*Choritoneura conflitana*) seedlings. Similar results were also reported by Muzika & Pregitzer (1993) and Kainulanaine, Holopainen, Palomäki & Holopainen (1996).

The carbon-nutrient balance (CNB) hypothesis emphasized that only when a plant is restricted to mineral nutrient availability, mainly nitrogen does the CBSCs accumulates in plant tissues (Haukioja *et al*., 1998; Hamilton, Zangerl, Delucia & Berenbaum, 2001) which in practice could result in significant reduction in yield and productivity. Other factors such as moisture stress, shading and elevated CO_2 have been reported to induce the accumulation of total phenolics in plants, whereas no consistent changes were observed in terpenoids (Peñuelas, Estiarte & Llsiá, 1997; Peñuelas & Estiarte, 1998). Haukioja *et al*. (1998) reported that the inconsistency of the results of total phenolics were largely due to lower leaf nitrogen content, presumably due to increase in carbohydrate concentration when plants were stressed. Therefore, in contrast with the results in Table 5.1, nitrogen treatments applied under 50 % shade net considerably improved the concentrations of total polyphenols of bush tea, regardless of season. Roberts (1990) reported that bush tea had vigorous shoots, thus accumulation of carbohydrates reserves could have been channeled towards the production of total polyphenols. This resulted in higher accumulations of concentration of polyphenols in the leaves. Similar results in green tea were also reported by Venkatesan *et al*. (2004) and Owour *et al*. (2000), whereby the application of 450 $kg{\cdot}ha^{-1}$ nitrogen improved yield, polyphenols and amino acid content. In South African tea industry, N applications from 200 to 270 $kg{\cdot}ha^{-1}$ increased yield and concentration of total polyphenols in black tea (Rooster, Synman, Smith, Fourie, de Villiers, Willers & Schwarts., 1985). Therefore, CNB hypothesis in practice has limitations in that there is a negative implication when nutrients are not restricted for plant growth and productivity (Hamilton *et al*., 2001). Therefore, our results also suggested that the carbon-nutrient balance hypothesis in bush tea is not plausible as it is generally reported. Thus, suggesting that the carbon nitrogen balance (CNB) hypothesis still needs further investigation on agronomic practices such as mineral nutrition.

Percentage leaf tissue nitrogen quadratically increased with increasing N ranging from 2.1 to 3.1 % (autumn), 2.4 to 3.8 % (winter), 3.2 to 3.8 % (spring) and 1.9 to 2.6 % (summer) (Figure 5.2). Wanyoko (1983) reported that the current leaf norm in a normal harvestable (*Camellia sinensis* L.) tea crop was 3 to 3.4 % N.

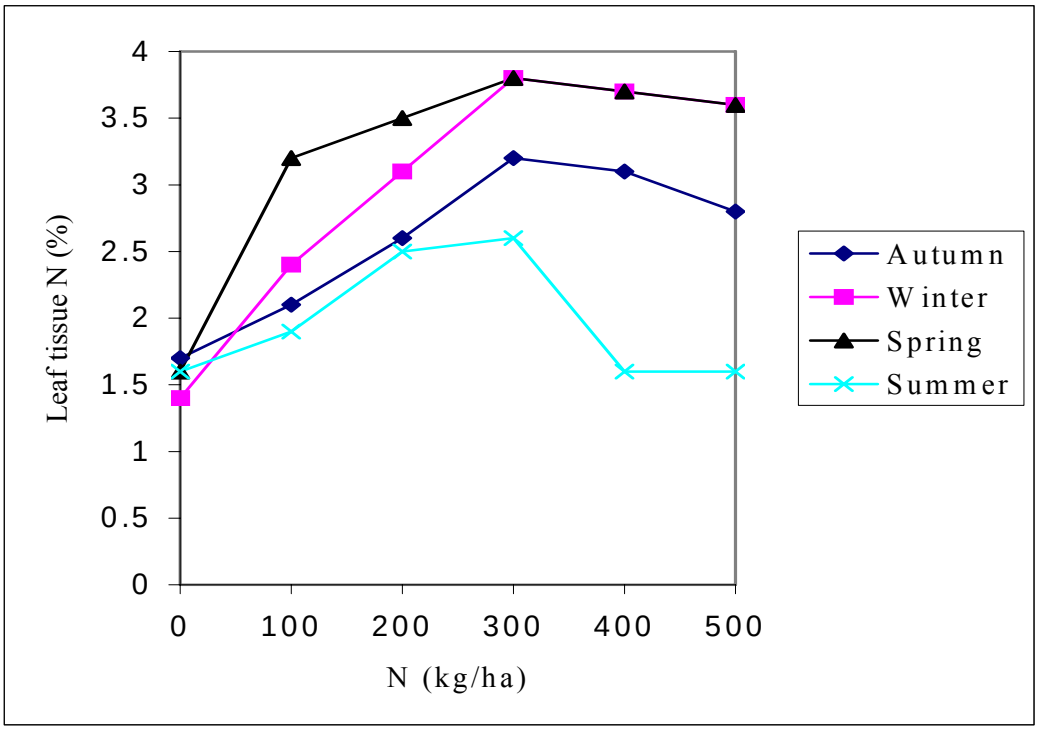

Figure 5.2 Leaf tissue nitrogen content in bush tea

5.3.2 Response of leaf total polyphenols of bush tea to phosphorus nutrition

Results in Table 5.2 showed that regardless of season, total polyphenols were quadratically increased by phosphorus nutrition. Highest concentrations were at 300 kg·ha^{-1} P. Most of the total polyphenol response to P occurred between 0 to 300 kg·ha^{-1} P. The highest total polyphenols were at 300 kg·ha^{-1} P, and were 46.8 mg·g^{-1} in winter, 44.9 mg·g^{-1} in summer, 38.7 mg·g^{-1} in spring and 38.4 mg·g^{-1} in autumn.

Table 5.2 Response of leaf total polyphenols of bush to P nutrition

	Concentration of total polyphenols ($mg \cdot g^{-1}$)			
Applied P ($kg \cdot ha^{-1}$)	Autumn	Winter	Spring	Summer
0	8.5	14.1	13.1	22.2
100	13.2	34.7	20.9	31.5
200	30.9	36.9	34.5	33.8
300	38.4	46.8	38.7	44.9
400	37.3	43.6	36.8	42.2
500	35.9	43.3	36.3	41.7
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P=0.05 (*), 0.01 (**) or non significant (NS)

Percentage leaf tissue phosphorus was quadratically increased and ranged from 0.2 to 0.3 % (autumn), 0.6 to 0.7 % (winter), 0.2 to 0.5 % (spring) and 0.1 to 0.5 % (summer) (Figure 5.3). Wanyoko (1983) reported that the current leaf norm in a normal harvestable tea (*Camellia sinsensis* L.) crop is 0.5 to 0.8 % P.

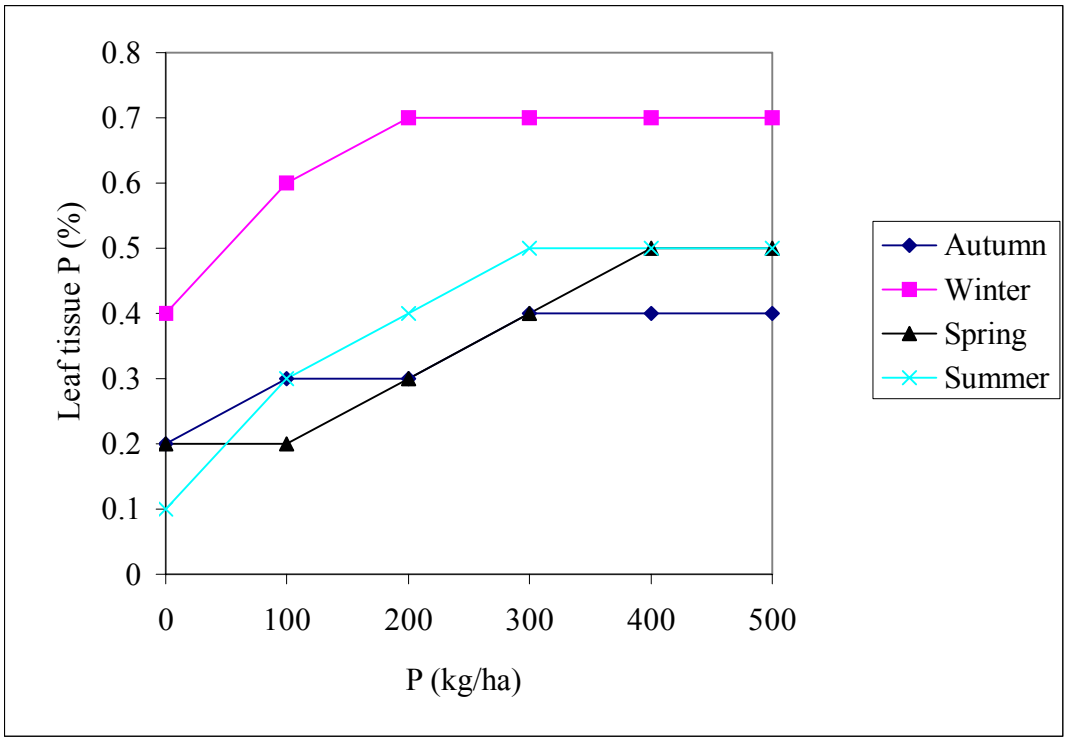

Figure 5.3 Leaf tissue phosphorus content of bush tea

There were positive correlation and significant linear relationship between leaf tissue N and total polyphenols content, regardless of season (Figure 5.4).

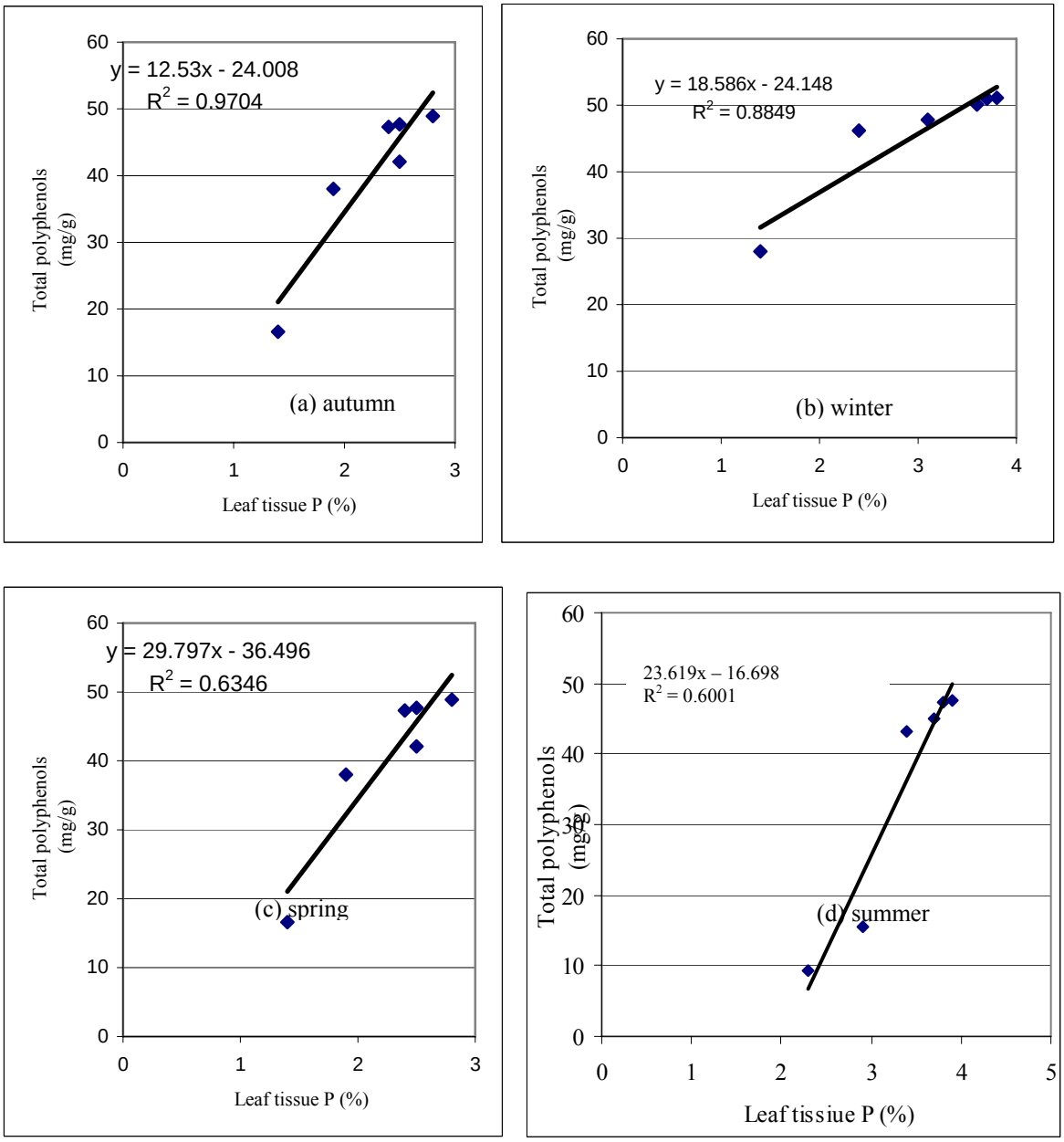

Figure 5.4 Correlation and regression between total polyphenol and leaf tissue phosphorus of bush tea with respect to season

The specific total polyphenol derivatives such as theaflavins (TF) and thearubigins (TR) of green tea have been established as important non-volatile green tea constituents with TF contributing to the brightness and briskness (Owour & Obanda, 1998; Liang, Liu, Xu & Hu, 2003), and TR contributing to the depth of colour, mouthfeel and body of green tea (Kato & Shibamoto, 2001). However, Owour *et al.* (1991) reported that in green tea, quality

parameters, i.e. theaflavins (TF), thearubigins (TR), which have been derived from polyphenols derivatives and caffeine vary with time of the year with application of 150 kg·ha^{-1} P, which concur with the results in Table 5.3. Kamau *et al*. (1999) reported that the levels of TR and flavour index (FI) were generally high when phosphorus at 250 kg·ha^{-1} P were applied.

5.3.3 Response of leaf total polyphenols of bush tea to potassium nutrition

Results in Table 5.3 show that there was a quadratic increase of total polyphenols with application of potassium. In the K trial, regardless of season, total polyphenols reached their maximum at 400 kg·ha^{-1} with total polyphenols concentrations of 47.7 mg·g^{-1} during winter. Most of total polyphenol response to K occurred between 0 to 200 kg·ha^{-1}K. In growth and production studies of bush tea, the application of 200 kg·ha^{-1}K for maximum biomass production occurred between 0 and 200 kg·ha^{-1}K (Mudau, Soundy & du Toit., 2005). In contrast with the results of bush tea, Ruan *et al*. (1999) reported that total polyphenols significantly increased with K applications at maximum levels of 150 kg·ha^{-1} K during spring and autumn in black tea. In other herbal teas, such as oolong tea and green tea total polyphenols and other aromatic compounds such as (Z)-3-hexenyl hexanoate, farnesene and nerolidol were considerably increased with 300 kg·ha^{-1} K applied as potassium sulphate (Ruan *et al*., 1998).

Table 5.3 Response of leaf total polyphenols of bush to K nutrition

	Concentration of total polyphenols ($mg \cdot g^{-1}$)			
($kg \cdot ha^{-1}$)	Autumn	Winter	Spring	Summer
K applied				
0	8.4	9.2	14.2	8.7
100	14.0	15.6	29.8	26.1
200	35.9	43.3	38.7	37.5
300	38.4	45.1	43.8	43.9
400	39.2	47.7	43.2	44.4
500	39.1	47.2	42.8	44.1
Response	Q^{**}	Q^{**}	Q^{**}	Q^{**}

Linear (L) or quadratic (Q) effects significant at P=0.05 (*), 0.01 (**) or non significant (NS)

Percentage leaf tissue potassium was quadratically increased and ranged from 3.6 to 4.8% (autumn), 2.3 to 3.8 % (winter), 2.2 to 2.5 % (spring) and 0.7 to 2.2 % (summer) (Figure 5.5). Wanyoko (1983) reported that the current leaf norm in a normal harvestable tea (*Camellia sinsensis* L.) was 1.5 to 1.8 % K.

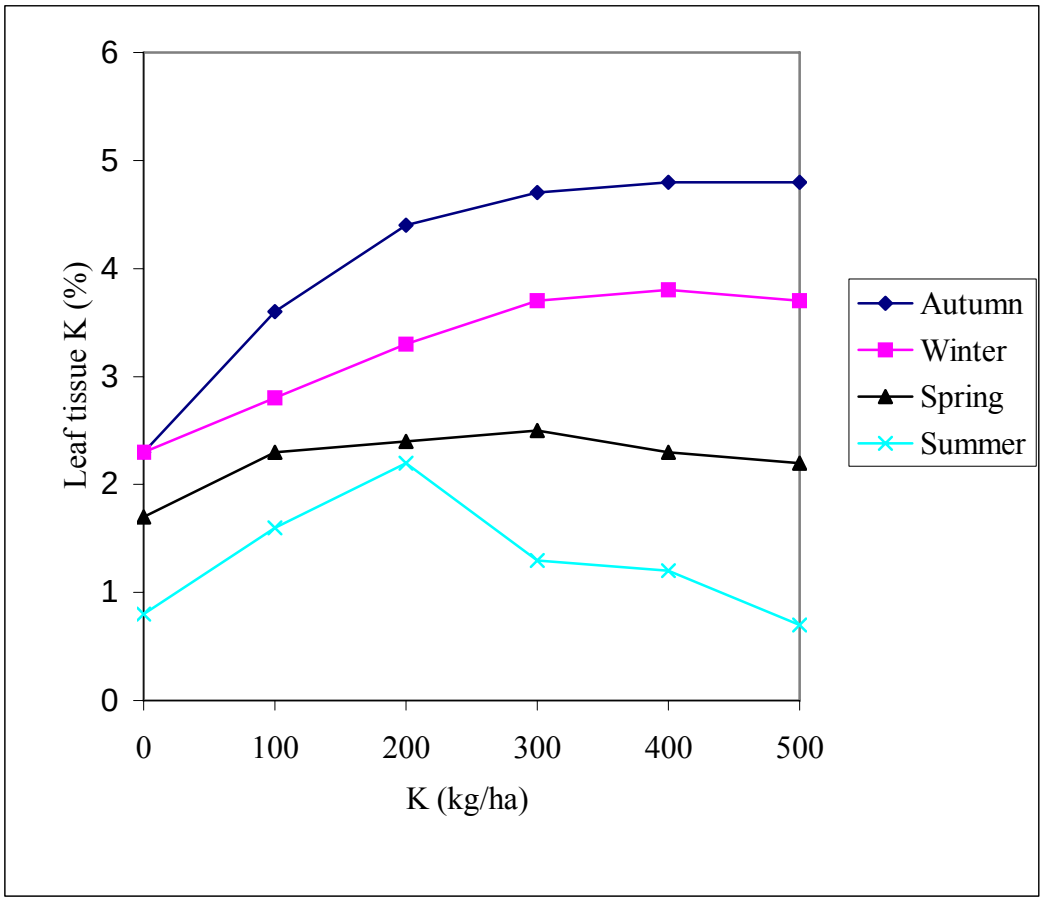

Figure 5.5 Leaf tissue potassium content of bush tea

There was positive correlation and significant linear relationship between leaf tissue K and total polyphenols content of bush tea leaves, regardless of season (Figure 5.6).

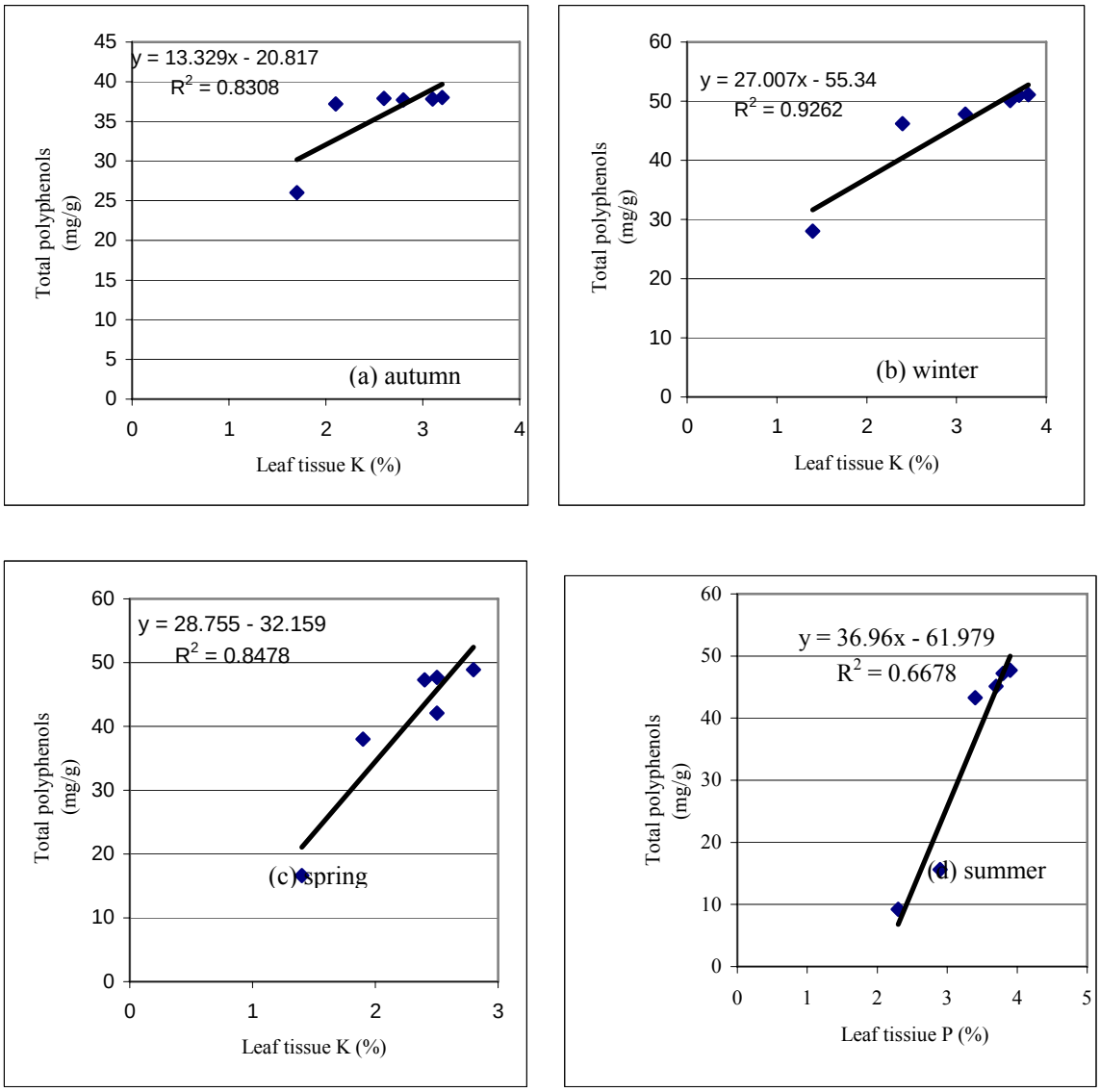

Figure 5.6 Correlation and regression between total polyphenols and leaf tissue K of bush tea with respect to season

In conclusion, the results of this study demonstrated that regardless of season, N, P and K nutrition significantly improved the total polyphenol content in bush tea with the highest total polyphenol produced during winter. Nitrogen treatments had highest total polyphenols (51.1 $mg \cdot g^{-1}$) during winter. Therefore, for improved total polyphenols of bush tea grown under 50 % shade nets on a composted pinebark, 300 $kg \cdot ha^{-1}$ N or P and 200 $kg \cdot ha^{-1}$ K is recommended.

5.4 SUMMARY

Bush tea was grown in 20 L bags containing a composted pine bark medium under varying nitrogen (N), phosphorus (P) and potassium (K) levels for four seasons to determine the seasonal nutrient requirements for high total polyphenol content of leaves. Treatments consisted of 0, 100, 200, 300, 400 or 500 kg·ha^{-1} N, P or K in a randomized complete block design under 50 % shade nets. Three (N, P and K) parallel trials were conducted per season (autumn, winter, spring and summer). Total polyphenols were determined, using Folin-Ciaocalteau reagents, and analyzed in a spectrophotometer. Regardless of season, results for the N trial indicated that total polyphenols increased quadratically in response to N nutrition. High total polyphenols were at 300 kg·ha^{-1} N with a concentration of 51.1 mg·g^{-1} in winter. For the P trial, total polyphenols also increased quadratically in response to P nutrition, regardless of season. Again winter had the highest total polyphenols concentration (46.8 mg·g^{-1}) at 300 kg·ha^{-1} P. In the K trial, regardless of season, total polyphenols reached their maximum at 200 kg·ha^{-1} with a seasonal total polyphenols concentrations of 47.7 mg·g^{-1}. Most of total polyphenol response to K occurred between 0 to 200 kg·ha^{-1}K. In all trials, there was a correlation and lniar relationship between leaf tissues N, P K and total polyphenols content of bush tea leaves, regardless of seasons. Therefore, for highest total polyphenols content, 300 kg·ha^{-1} N and P and 200 kg·ha^{-1} K is recommended regardless of the season, for bush tea plants grown in mixed composted pine bark growing medium under 50 % shade nets.

CHAPTER 6

EFFECTS OF N, P AND K NUTRITION ON GROWTH AND CHEMICAL COMPOSITION OF BUSH TEA (*ATHRIXIA PHYLICOIDES* L.) AS INFLUENCED BY SEASON

6.1. INTRODUCTION

Agronomic practices such as mineral nutrition have been reported to improve growth and quality of black tea (Owour & Odhiambo, 1994). The applications of nitrogen, phosphorus and potassium have been a normal agronomic practice (Owour, 1989; Owour *et al*., 1990; Owour & Odhiambo, 1994; Keen & Zidenberg-Cherr, 2000) and several studies have shown yield and quality improvement (Owour *et al*., 2000; Venkatasen *et al*., 2004). Wanyoko (1983) reported that the normal harvestable tea crop contains 3 to 3.4 % nitrogen (N), 0.2 to 0.3 % phosphorus (P) and 1.5 to 1.8 % potassium (K) when a plucking standard of two leaves and a bud was practiced. Regardless of season, the optimum nutrient levels for bush tea growth and concentration of polyphenols were 300 kg·ha^{-1} N, 300 kg·ha^{-1} P and 200 kg·ha^{-1} K (Mudau *et al*., 2005). It also contains 5-hydroxy 6,7,8 3',4'5,'-hexamethyoxyflavon-3-ol which has been identified as a major flavonol compound (Mashimbye, Mudau, Soundy & van Ree, 2006). Data is lacking on the response of growth and chemical composition with respect to treatment combinations of N, P and K in bush tea. Therefore, the objective of this investigation was to determine the effects of treatment combinations of N, P and K nutrition on growth and concentration of polyphenols in bush tea.

6.2 MATERIALS AND METHODS

6.2.1 Experimental site and plant materials

The study was carried out in Morgenzon, a commercial nursery in Louis Trichardt (23°N 50'E, 30°S 17'E; alt 610 m; subtropical-type climate, i.e. summer rainfall and cold, dry winter). The annual rainfall is 650 mm per annum with temperatures ranging from 13 to 18 °C in winter and 27 to 39 °C in summer. On 13 November 2003, plant materials were collected from Venda (Limpopo Province) and 1200 cuttings were dipped in Seradix® No. 2 hormone (0.3 % IBA concentration) (Bayer, Pretoria, South Africa) and established in seed trays on a mist bed. Rooted cuttings (Figure 6.1) were transplanted into 1 L bags and placed into a hardening chamber at 20 °C for 3 months.

Figure 6.1 Rooted cutting of bush tea

Irrigation was supplied with sprinklers at an amount of 550 mm per day for 3 months. After 3 months, plants with approximately 25 leaves were transplanted into 20 L bags. The medium was a 1 pinebark : 2 sand : 1 stryofoam bead mix (v/v), with AquaGro wetting agent (Aquatrols, Cherry Hill, N.J) at 0.2 $kg \cdot m^{-3}$. The initial media chemical analyses were determined using procedure described by Hanlon *et al*. (1994). The EC was 0.9 $dS \cdot m^{-1}$ and pH was 4.7. The composted pine bark contained 1.2 $mg \cdot kg^{-1}$ NO_3-N, 0.1 $mg \cdot kg^{-1}$ P and 1.3 $mg \cdot kg^{-1}$ K.

All plants received 1% $MgSO_4$, ZnO, microfel Fe and sodium borate as foliar spray to supplement Mg, S, Fe, and Zn necessary for the production of quality tea. The initial plant height was about 40 cm before the application of treatments.

6.2.2 Experimental design and treatments

Four trials one at each season (autumn, winter, spring and summer), were conducted under 50 % shade net in a 3^3 factorial experiment arranged in randomized block design for N, P and K with 4 replications. During autumn, three levels of N were 300, 350 and 400 $kg \cdot ha^{-1}$, P levels were 200, 250 and 300 $kg \cdot ha^{-1}$ and K levels were 150, 200 and 250 $kg \cdot ha^{-1}$. During winter, spring and summer, the N levels were 250, 300 and 350 $kg \cdot ha^{-1}$, P levels were 250, 300 and 350 $kg \cdot ha^{-1}$, and K levels were 150, 200 and 250 $kg \cdot ha^{-1}$.

6.2.3 Data collection

From each replicate at harvest (autumn, 30 May 2004; winter, 30 August 2004; spring, 30 November and summer, 28 February 2005) records were taken of plant height, number of branches, number of leaves, number of flowers and flower buds (autumn and winter), stem

girth, fresh and dry root mass, fresh and dry stem mass, leaf area (measured by a LI-3100 area meter; LI-COR, Lincoln, Neb) and fresh and dry shoot mass. The percentage leaf and root concentrations of N, P, K and leaf concentrations of total polyphenols were also determined.

6.2.4 Leaf and root N, P, K and total polyphenol content

Refer to (Chapter 3 & 4) where this was firstly described.

6.2.5 Statistical analysis

In all seasons, data were analyzed using the GLM (General linear model) procedure of SAS version 8.0 (SAS Institute Inc., 1999). Mean comparisons were performed using Duncan Multiple Range Test (DMRT) procedure.

6.3 RESULTS AND DISCUSSION

6.3.1 Effects of N, P and K application on growth of bush tea

Regardless of season, treatments of N300, P300 and K200 ($kg \cdot ha^{-1}$) increased fresh and dry shoot mass, number of leaves as well as leaf area (Tables 6.1, 6.2, 6.3 and 6.4). Other treatments did not consistently affect concentration of leaf N, P and K during the study period, although again the plants that received N 300, P 300 and K 200 ($kg \cdot ha^{-1}$) always had the highest concentration of leaf N, P and K and lowest concentration of root N, P and K (Tables 6.5, 6.6, 6.7 and 6.8). Mudau *et al*. (2005) reported that the percentage leaf tissue nitrogen quadratically increased, with increasing N, ranging from 2.1 to 3.1 % (autumn), 2.4 to 3.8 % (winter), 3.2 to 3.8 % (spring) and 1.9 to 2.6 % (summer) in bush tea. Mudau *et al*. (2005) also reported that the percentage leaf tissue phosphorus in bush tea was quadratically

increased ranging from 0.2 to 0.3 % (autumn), 0.6 to 0.7 % (winter), 0.2 to 0.5 % (spring) and 0.1 to 0.5 % (summer). Percentage leaf tissue potassium of bush tea was quadratically increased ranging from 3.6 to 4.8% (autumn), 2.3 to 3.8 % (winter), 2.2 to 2.5 % (spring) and 0.7 to 2.2 % (summer). There are only a few reports on application of treatment combinations for N, P and K application on tea. The standard commercial application in South Africa for black tea is 200 to 270 kg·ha^{-1} N, 50 kg·ha^{-1} K and 50 kg·ha^{-1} P, depending on the soil type and the results of leaf analysis (Rooster *et al.,* 1985). Results reported by Wanyoko (1983) when N was applied indicated that biomass production of green tea increased to an optimum at 250 kg·ha^{-1} N. Ruan *et al.* (1999) reported that P nutrition applied as a single superphosphate at 225 kg·ha^{-1} P, increased biomass production of green tea. Ruan *et al.* (1998) reported that biomass production considerably increased, following potassium application, reaching a maximum at 800 mg·kg^{-1} K_2O. In the present study, the applications of N and P at a rate of 300 kg·ha^{-1} and K at a rate of 200 kg·ha^{-1} increased the concentrations of N, P and K in leaf tissues of bush tea, whereas the concentrations of N, P and K in root tissues tended to be inconsistently affected and significantly lower, regardless of season (Tables 6.5, 6.6, 6.7 and 6.8). Wanyoko (1983) reported that the normal harvestable tea crop contains 3 to 3.4 % nitrogen (N), 0.2 to 0.3 % phosphorus (P) and 1.5 to 1.8 % potassium (K), when a plucking standard of two leaves and a bud was practiced. 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, regardless of season.

6.3.2 Effects of N, P and K application on total polyphenols in bush tea

Regardless of season, the treatment combinations of N300, P300 and K200 ($kg \cdot ha^{-1}$) improved the concentrations of total polyphenols (Tables 6.5, 6.6, 6.7 and 6.8). There is a distinct lack of published reports on multiple combinations of N, P and K studies in green tea. Ruan *et al.* (1999) reported that caffeine and the concentration of total polyphenols significantly increased with K applications with an optimum level at 150 $kg \cdot ha^{-1}$ K during spring and autumn in black tea. Similar responses were also evident in oolong tea and green tea, where concentrations of total polyphenols and other aromatic compounds such as (Z)-3-hexenyl hexanoate, farnesene and nerolidol were considerably improved with 300 $kg \cdot ha^{-1}$ K applied as potassium sulphate (Ruan *et al*., 1999).

From this study it can be concluded that regardless of season, treatment combinations of N300, P300 and K200 ($kg \cdot ha^{-1}$) increased fresh and dry shoot mass, number of leaves, leaf area and the concentration of total polyphenols in bush tea when N, P and K were simultaneously applied. Treatments applied did not consistently affect concentration of leaf N, P and K during the study period, although the treatment that received N300, P300 and K200 ($kg \cdot ha^{-1}$) always had the highest concentration of leaf N, P and K and the lowest concentration of root N, P and K. There were 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, regardless of season.

Table 6.1 Growth characteristics of bush tea as affected by N, P and K nutrition during autumn

($kg \cdot ha^{-1}$)	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)
N300P250K150	88.5 b	46.0 bcd	716.5 b	1602.3 b
N300P250K200	88.2 b	46.0 bcd	706.2 b	1061.5 b
N300P250K250	88.5 b	46.0 bcd	700.0 b	1641.0 b
N300P300K150	87.7 b	44.0 bcde	710.0 b	1758.5 b
N300P300K200	99.0 a	56.0 a	942.2 a	3663.5 a
N300P300K250	86.2 b	47.0 bdc	749.5 b	1688.5 b
N300P350K150	89.0 b	47.0 bcde	703.0 b	1554.5 b
N300P350K200	89.0 b	51.0 b	708.5 b	1627.0 b
N300P350K250	89.0 b	44.5 b	705.2 b	1590.8 b
N350P250K150	89.0 b	51.0 b	720.2 b	1628.3 b
N350P250K200	89.0 b	51.0 b	707.7 b	1671.0 b
N350P250K200	88.2 b	50.3 b	712.2 b	1600.0 b
N350P300K150	88.0 b	50.0 b	704.5 b	1641.0 b
N350P300K200	89.0 b	51.0 b	710.0 b	1632.0 b
N350P300K250	87.5 b	48.8 bc	705.2 b	1605.0 b
N350P350K150	83.0 bcd	44.9 bcd	702.7 b	1617.5 b
N350P350K200	75.6 ef	37.0 f	700.0 b	1610.0 b
N350P350K200	79.0 de	40.5 edf	711.0 b	1632.0 b
N400P250K150	75.7 ef	38.5 ef	725.7 b	1296.0 b
N400P250K200	72.7 f	36.0 f	703.7 b	1643.0 b
N400P250K250	80.2 cde	41.7 cdef	709.5 b	1650.3 b
N400P300K250	83.5 bcd	48.0 bc	706.5 b	1640.3 b
N400P300K150	85.5 bc	47.3 bcd	705.0 b	1620.5 b
N400P300K250	77.2 ef	38.0 ef	720.7 b	1602.8 b
N400P350K200	83.0 bcd	47.0 bcd	712.0 b	1643.0 b
N4000P250K250	88.0 b	46.0 bcd	711.0 b	1619.0 b
N400P350K150	87.5 b	47.0 bdc	704.7 b	1619.0 b

Means followed by the same letter in a column are not significantly different at 5 % level

Table 6.2 Growth characteristics of bush tea as affected by N, P and K nutrition during winter

($kg \cdot ha^{-1}$)	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)
N250P250K150	53.2 b	26.2 ef	784.3 cd	1447.5 b
N250P250K200	53.0 b	33.7 cdef	592.3 d	1326.2 c
N250P250K250	53.5 b	27.7 ef	914.0 cd	1544.4 b
N250P300K150	54.0 b	31.2 def	945.5 bcd	1447.5 b
N250P300K200	52.2 b	47.2 ab	977.3 bcd	1447.5 b
N250P300K250	53.2 b	38.7 bcd	935.0 bcd	1447.5 b
N250P350K150	53.0 b	34.5 cdef	761.3 cd	1447.5 b
N250P350K250	54.2 b	40.2 bcd	752.3 cd	1447.5 b
N300P250K150	53.7 b	32.2 def	746.3 d	1447.5 b
N300P250K200	52.7 b	34.0 cdef	709.8 d	1447.5 b
N300P250K200	53.2 b	33.7 cdef	733.5 d	1447.5 b
N300P250K250	53.2 b	33.5 cdef	703.1 d	1447.5 b
N300P300K150	53.0 b	39.7 bcd	754.5 cd	1447.5 b
N300P300K200	70.7 a	56.7 a	1683.8 a	1765.6 a
N300P300K250	53.0 b	27.7 f	1409.5 ab	1447.5 b
N300P350K150	52.7 b	39.0 bcd	987.8 bcd	1447.5 b
N300P350K200	53.7 b	33.0 cdef	859.4 cd	1447.5 b
N300P350K250	53.2 b	36.2 cdef	857.8 cd	1447.5 b
N350P250K150	53.8 b	35.2 cdef	1228.5 abc	1447.5 b
N350P250K200	53.7 b	33.0 cdef	784.3 cd	1447.5 b
N350P250K250	52.7 b	38.7 bcd	745.0 d	1447.5 b
N350P300K150	53.7 b	39.2 bcd	722.0 d	1447.5 b
N350P300K200	53.7 b	36.2 cdef	747.8 d	1447.5 b
N350P300K250	52.5 b	38.5 bcd	739.5 d	1447.5 b
N350P350K150	53.7 b	38.2 bcde	748.3 d	1447.5 b
N350P350K200	53.2 b	35.5 cdef	746.3	1447.5 b
N350P350K250	53.2 b	43.0 bc	759.8 cd	1447.5 b

Means followed by the same letter in a column are not significantly different at 5 % level

Table 6.3 Growth characteristics of bush tea as affected by N, P and K nutrition during spring

($kg \cdot ha^{-1}$)	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)
N250P250K150	106.5 d	77.7 efg	703.3 c	927.5 c
N250P250K200	110.5 c	85.0 bcdef	752.5 bc	953.5 c
N250P250K250	114.7 b	92.0 abcd	982.3 bc	953.2 c
N250P300K150	115.2 b	96.7 ab	699.3 c	911.5 c
N250P300K200	116.7 b	94.0 abc	902.8 bc	883.7 c
N250P300K250	114.5 b	92.0 abcd	815.8 bc	942.2 c
N250P350K150	117.2 b	88.7 abcde	989.4 bc	889.2 c
N250P350K250	116.5 b	86.2 bcdef	933.1 bc	926.0 c
N300P250K150	115.7 b	88.7 abcde	930.3 bc	924.7 c
N300P250K200	114.7 b	89.7 abcde	936.5 bc	926.9 c
N300P250K200	114.7 b	92.2 abcd	911.5 bc	953.2 c
N300P250K250	115.7 b	96.7 ab	941.0 bc	1403.0 b
N300P300K150	117.0 b	88.7 abcd	951.3 bc	924.8 c
N300P300K200	138.7 a	100.5 a	1810.5 a	1940.9 a
N300P300K250	115.2 b	88.0 abcd	1034.5 bc	957.1 c
N300P350K150	115.5 b	88.0 abcd	912.8 bc	929.9 c
N300P350K200	114.5 b	92.2 abcd	988.0 bc	997.5 c
N300P350K250	115.7 b	88.0 abcd	991.8 bc	934.9 c
N350P250K150	116.5 b	86.2 bcdef	932.8 bc	957.1 c
N350P250K200	116.2 b	86.2 bcdef	914.5 bc	968.2 c
N350P250K250	117.0 b	88.7 abcd	1089.5 bc	949.1 c
N350P300K150	116.5 b	88.0 abcd	969.0 bc	915.3 c
N350P300K200	115.0 b	92.2 abcd	955.5 bc	940.2 c
N350P300K250	117.2 b	88.7 abcd	967.8 bc	945.5 c
N350P350K150	116.2 b	86.2 bcdef	925.5 bc	938.7 c
N350P350K200	116.0 b	86.2 bcdef	892.0 bc	979.0 c
N350P350K250	114.5 b	92.2 abcd	930.0 bc	981.1 c

Means followed by the same letter in a column are not significantly different at 5 % level

Table 6.4 Growth characteristics of bush tea as affected by N, P and K nutrition during summer

($kg \cdot ha^{-1}$)	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)
N250P250K150	65.0 h	36.2 h	1191.0 c	1477.3 f
N250P250K200	78.2 cdef	50.0 bcdefg	1191.0 c	1889.3 abc
N250P250K250	73.2 gh	43.7 fgh	1191.0 c	1803.3 abcd
N250P300K150	76.0 defg	42.7 gh	1191.0 c	1890.3 abc
N250P300K200	79.0 cdefg	47.7 defg	1191.0 c	1709.0 cde
N250P300K250	82.2 abcdefg	52.0 bcdefg	1191.0 c	1831.5 abc
N250P350K150	76.5 defg	42.5 gh	1191.0 c	1539.5 ef
N250P350K250	88.5 ab	59.7 ab	1191.0 c	1876.3 abc
N300P250K150	79.5 bcdefg	48.5 defg	1261.0 b	1874.5 abc
N300P250K200	87.5 abc	59.5 abc	1281.0 a	1919.8 abc
N300P250K200	80.5 bcdefg	50.2 bcdefg	1191.0c	1778.3 abcd
N300P250K250	78.2 cdefg	48.2 defg	1191.0 c	1863.8 abc
N300P300K150	85.2 abcd	55.0 abcde	1191.0 c	1725.0 bcde
N300P300K200	91.2 a	64.2 a	1191.0 c	1954.5 a
N300P300K250	78.2 cdefg	46.2 efgh	1191.0 c	1607.0 fde
N300P350K150	74.7 efg	43.5 fgh	1191.0 c	1860.0 abc
N300P350K200	79.2 bcdefg	49.5 cdefg	1191.0 c	1877.5 abc
N300P350K250	75.0 efg	43.2 fgh	1191.0 c	1751.0 abcd
N350P250K150	73.5 fgh	43.0 gh	1191.0 c	1801.0 abcd
N350P250K200	75.0 efg	45.0 efgh	1191.0 c	1801.0 abcd
N350P250K250	73.2 gh	42.0 gh	1191.0 c	1801.0 abcd
N350P300K150	76.5 defg	44.2 fgh	1191.0 c	1801.0 abcd
N350P300K200	86.5 abc	56.7 abcd	1191.0 c	1801.0 abcd
N350P300K250	86.5 abc	57.0 abcd	1191.0 c	1801.0 abcd
N350P350K150	86.2 abcdef	50.0 bcdefg	1191.0 c	1801.0 abcd
N350P350K200	83.2 abcde	53.2 bcdef	1191.0 c	1801.0 abcd
N350P350K250	80.5 bcdef	49.5 cdefg	1191.0 c	1801.0 abcd

Means followed by the same letter in a column are not significantly different at 5 % level

Table 6.5 Leaf and root tissue N, P, K and total polyphenols of bush tea during autumn

($kg \cdot ha^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)	Total polyphenols ($mg \cdot g^{-1}$)
N300P250K150	2.9 b	2.5 ab	0.3 b	0.4 ab	2.8 ab	2.5 ab	52.4 bcde
N300P250K200	2.8 b	2.8 ab	0.4 b	0.5 a	2.8 ab	2.5 ab	53.7 bcde
N300P250K250	2.8 b	2.5 ab	0.3 b	0.2 ab	3.9 ab	2.5 ab	43.5 bcde
N300P300K150	2.8 b	2.5 ab	0.3 b	0.3 ab	2.8 ab	2.0 b	47.2 bcde
N300P300K200	3.9 a	2.2 b	0.7 a	0.1 b	4.4 a	1.9 b	94.9 a
N300P300K250	2.8 b	2.7 ab	0.3 b	0.2 ab	2.8 ab	2.5 ab	48.0 bcde
N300P350K150	2.9 b	2.6 ab	0.3 b	0.2 ab	2.9 ab	2.5 ab	62.1 bc
N300P350K200	2.9 b	2.5 ab	0.3 b	0.2 ab	2.9 ab	2.5 ab	56.7 bcd
N300P350K250	2.8 b	2.6 ab	0.3 b	0.1 b	2.9 ab	2.5 ab	55.8 bcd
N350P250K150	2.8 b	2.7 ab	0.3 b	0.2 ab	2.9 ab	2.2 ab	36.6cde
N350P250K200	2.8 b	2.9 ab	0.3 b	0.1 b	2.8 ab	2.5 ab	41.4 bcde
N350P250K200	2.8 b	2.9 ab	0.3 b	0.2 ab	2.9 ab	2.6 ab	51.7 bcde
N350P300K150	2.8 b	2.9 ab	0.3 b	0.2 ab	3.9 ab	2.4 ab	65.4 b
N350P300K200	2.8 b	2.7 ab	0.3 b	0.3 ab	2.8 ab	2.5 ab	60.4 bcd
N350P300K250	2.8 b	3.1 a	0.3 b	0.2 ab	2.8 ab	2.3 ab	42.3 bcde
N350P350K150	2.8 b	2.2 b	0.3 b	0.2 ab	2.5 b	2.3 ab	35.6 cde
N350P350K200	2.8 b	2.8 ab	0.3 b	0.3 ab	2.9 ab	2.8 a	66.4 b
N350P350K200	3.4 b	2.9 ab	0.3 b	0.1 b	2.9 ab	2.3 ab	50.4 bcde
N400P250K150	3.3 b	2.8 ab	0.3 b	0.1 b	2.9 ab	2.3 ab	33.2 cde
N400P250K200	3.2 b	2.9 ab	0.3 b	0.2 ab	2.8 b	2.8 a	58.3 bcd
N400P250K250	3.3 b	3.1 a	0.3 b	0.2 ab	2.9 ab	2.6 ab	48.6 bcde
N400P300K250	3.3 b	3.1 a	0.3 b	0.1 b	2.8 b	2.8 a	41.6 bcde
N400P300K150	2.7 b	3.1 a	0.3 b	0.2 ab	2.4 b	2.0 b	33.9 cde
N400P300K250	3.1 b	3.1 a	0.3 b	0.2 ab	3.9 ab	2.8 a	31.8 de
N400P350K200	3.4 b	3.1 a	0.4 b	0.3 ab	2.8 b	2.5 ab	37.8 bcde
N4000P250K250	2.6 b	3.1 a	0.3 b	0.1 b	3.9 ab	2.3 ab	25.2 e
N400P350K150	3.3 b	3.1 a	0.3 b	0.1 b	2.8 b	2.6 ab	55.2 bcd

Means followed by the same letter in a column are not significantly different at 5 % level

Table 6.6 Leaf and root tissue N, P, K and total polyphenols of bush tea during winter

($kg \cdot ha^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)	Total polyphenols ($mg \cdot g^{-1}$)
N250P250K150	2.6 defg	2.4efg	0.3 c	0.1 b	1.9 g	1.5 fg	57.4 cdef
N250P250K200	2.8 cdefg	2.6 bcdefg	0.4 bc	0.3 a	2.2 fg	2.0 bcdef	71.1 bc
N250P250K250	2.6 defg	2.7 bcdefg	0.3 c	0.2 ab	2.5 cdefg	2.3 abcde	68.5 bcd
N250P300K150	2.8 cdefg	2.4 defg	0.3 c	0.2 ab	2.2 fg	2.0 bcdef	57.2 cdef
N250P300K200	3.0 bcdefg	2.7 bcdefg	0.3 c	0.2 ab	2.1 fg	1.9 cdef	55.7 def
N250P300K250	2.4 fg	3.5 a	0.2 c	0.2 ab	2.9 bcd	2.7 a	64.9 bcde
N250P350K150	3.8 b	3.3 abc	0.2 c	0.1 b	2.6 bcdef	2.4 abcde	67.1 bcd
N250P350K250	2.8 cdefg	2.8 abcdef	0.3 c	0.2 ab	2.6 bcdef	2.2 abcde	64.2 bcde
N300P250K150	3.5 bc	2.8 abcdef	0.2 c	0.1 b	2.1 fg	1.8 def	65.8 bcd
N300P250K200	3.5 bc	3.1 abcd	0.3 c	0.2 ab	2.8 bcde	2.1 abcdef	64.1 bcde
N300P250K200	2.8 cdefg	3.4 a	0.2 c	0.1 b	3.1 b	2.5 abc	61.4 bcde
N300P250K250	3.1 bcdef	3.1 abcde	0.3 c	0.2 ab	2.9 bcd	2.4 abcd	61.7 bcde
N300P300K150	3.0 bcdef	3.0 abcde	0.3 c	0.2 ab	2.5 cdefg	2.1 abcdef	72.9 b
N300P300K200	4.3 a	1.1 h	0.7 a	0.1 b	3.8 a	1.1 g	90.4 a
N300P300K250	2.9 cdefg	2.2 fg	0.3 c	0.2 ab	2.0 g	1.7 efg	57.3 cdef
N300P350K150	2.8 cdefg	2.1 g	0.3 c	0.2 ab	2.4 defg	2.2 abcde	50.6 ef
N300P350K200	2.9 cdefg	2.5 cdefg	0.3 c	0.2 ab	2.5 cdefg	2.2 abcde	66.4 bcd
N300P350K250	2.9cdefg	2.5 cdefg	0.3 c	0.1 b	2.7 bcdef	2.2 abcde	62.9 cde
N350P250K150	2.5 cdefg	2.9 abcdef	0.2 c	0.1 b	2.2 efg	1.9cdef	46.2 f
N350P250K200	3.5 bc	3.2 abc	0.2 c	0.1 b	3.0 bc	2.5 abc	63.3 bcde
N350P250K250	3.2 bcdef	3.4 ab	0.2 c	0.1 b	2.8bcde	2.6 ab	56.1 def
N350P300K150	3.3 bcd	2.5 cdefg	0.2 c	0.1 b	2.8 bcde	2.5abc	59.1 bcdef
N350P300K200	2.2 g	3.3 ab	0.2 c	0.1 b	2.2 fg	2.0 bcdef	61.4 bcde
N350P300K250	2.9 cdefg	3.4 ab	0.3 c	0.2 ab	2.8 bcde	2.4 abcd	54.3 def
N350P350K150	3.2 bcdef	3.2 abc	0.5 b	0.3 a	2.9 bcd	2.5 abc	50.3 ef
N350P350K200	2.4 efg	2.8 abcdef	0.2 c	0.1 b	2.4 defg	2.0bcdef	60.2 bcdef
N350P350K250	3.2 bcde	3.2 abc	0.2 c	0.1 b	2.8 bcde	2.4 abcde	72.7 b

Means followed by the same letter in a column are not significantly different at 5 % level

Table 6.7 Leaf and root tissue N, P, K and total polyphenols of bush tea during spring

($kg \cdot ha^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)	Total polyphenols ($mg \cdot g^{-1}$)
N250P250K150	2.8 cdef	2.7 bcdef	0.3 cd	0.1 cde	2.0 g	1.5 e	59.6 bc
N250P250K200	2.8 cdef	2.6 bcdef	0.4 cd	0.3 a	2.2 fg	2.0 bcde	58.6 bc
N250P250K250	2.6 edf	2.7 bcdef	0.3 cd	0.2 abc	2.5 cdefg	2.3 abcd	63.5 abc
N250P300K150	2.8 cdef	2.4 edf	0.3 cd	0.2 abc	2.2 fg	2.1 abcd	59.7 bc
N250P300K200	3.0 bcdef	2.7 bcdef	0.3 cd	0.2 abc	2.1 fg	2.1 abcde	60.5 bc
N250P300K250	2.4 ef	3.5 a	0.2 cd	0.2 abc	2.9 abcd	2.3 abcd	54.9 c
N250P350K150	3.3 abcd	3.3 abc	0.2 cd	0.1 cde	2.6 bdef	2.4 abcd	62.1 abc
N250P350K250	2.8 cdef	2.8 abcde	0.3 cd	0.2 abc	2.6 bcdef	2.2 abcd	54.2 c
N300P250K150	3.5 abc	2.8 abcdef	0.2 cd	0.1 e	2.1 fg	1.8 de	63.3 abc
N300P250K200	3.5 abc	3.1 abcd	0.3 cd	0.2 abc	2.8 bcde	2.3 abcd	58.1 bc
N300P250K200	3.7 ab	3.4 a	0.2 cd	0.1 e	3.1 b	2.5 ab	53.9 c
N300P250K250	3.5 abc	3.1 abcde	0.3 cd	0.2 cde	2.9 abcd	2.4 abc	56.7 bc
N300P300K150	3.0 bcde	3.0 abcde	0.3cd	0.2 cde	2.5 cdefg	2.1 abcde	62.9 abc
N300P300K200	3.9 a	1.4 g	0.5 a	0.1 e	3.4 a	0.9 a	75.4 a
N300P300K250	2.9 cdef	2.3 ef	0.3 cd	0.2 abc	2.0 g	2.2 abcd	67.3 abc
N300P350K150	2.8 cdef	2.1 fg	0.3 cd	0.2 abc	2.4 edfg	2.2 abcd	70.6 ab
N300P350K200	2.9 bcdef	2.5 cdef	0.3 cd	0.2 abc	2.6 bcdef	2.5 ab	66.4 abc
N300P350K250	2.9 cdef	2.5 cdef	0.3 cd	0.1 e	2.7 bcdef	2.2 abcd	60.4 bc
N350P250K150	2.5 ef	2.9 abcde	0.2 cd	0.1 e	2.2 efg	1.9 cde	58.2 bc
N350P250K200	3.5 abc	3.2 abc	0.2 cd	0.1 e	3.0 abc	2.1 abcde	65.8 abc
N350P250K250	3.2 abcde	3.4 ab	0.2 cd	0.1 e	2.8 bcde	2.6 a	58.6 bc
N350P300K150	3.3 abcd	2.5 cdef	0.2 cd	0.1 e	2.8 bcde	2.5 abc	61.6 abc
N350P300K200	2.2 f	3.3 ab	0.2 cd	0.1 e	2.2 fg	2.0 bcde	63.9 abc
N350P300K250	2.9 bcdef	3.4 ab	0.3 cd	0.2 abc	2.8 bcde	2.4 abc	64.3 abc
N350P350K150	3.2abcde	3.2 abc	0.5 cd	0.3 ab	2.9 abcd	2.5 abc	60.3 bc
N350P350K200	2.4 ef	2.8 abcdef	0.2cd	0.1 e	2.4 edfg	2.2 abcd	60.2 bc
N350P350K250	3.3 abcd	3.3 abc	0.2 cd	0.1 e	3.0 abc	2.4 abc	62.7 abc

Means followed by the same letter in a column are not significantly different at 5 % level

Table 6.8 Leaf and root tissue N, P, K and total polyphenols of bush tea during summer

($kg \cdot ha^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)	Total polyphenols ($mg \cdot g^{-1}$)
N250P250K150	3.5 bc	3.3 ab	0.6 ab	0.1 c	2.6 cdef	1.2 c	57.4 bcd
N250P250K200	2.8 cde	2.6 abcdef	0.4 d	0.3 a	2.2 cdef	2.0 b	63.6 bcd
N250P250K250	2.6 ed	2.7 abcdef	0.3 d	0.2 b	2.5 cdef	2.3 b	43.5 d
N250P300K150	2.8 cde	2.4 abcdef	0.3 d	0.2 b	2.4 cdef	2.0 b	57.2 bcd
N250P300K200	3.0 bcde	2.7 abcdef	0.3 d	0.2 b	2.1 cdefg	1.9 c	48.0 cd
N250P300K250	2.4 e	3.5 a	0.3 d	0.2 b	2.9 cdef	2.7 a	57.4 bcd
N250P350K150	3.8 ab	3.3 ab	0.3 d	0.1 c	2.6 cdef	2.4 b	62.1 bcd
N250P350K250	2.8 cde	2.8 abcdef	0.3 cd	0.1 c	2.8 cde	2.2 b	56.7 bcd
N300P250K150	3.5 bc	2.8 abcdef	0.3 d	0.1 c	2.5 cdef	1.8 c	55.8 bcd
N300P250K200	3.5 bc	3.1 abcd	0.3 d	0.2 b	2.8 cde	2.1 b	59.1 bcd
N300P250K200	2.8 cde	3.4 ab	0.3 d	0.2 b	3.1 cb	2.5 b	53.9 bcd
N300P250K250	3.1 bcde	3.1 abcd	0.3 d	0.2 b	2.9 cde	2.4 b	61.7 bcd
N300P300K150	3.0 bcde	3.0 abcde	0.3 cd	0.2b	2.5 cdef	2.1 b	65.4 bcd
N300P300K200	4.5 a	1.9 g	0.8 a	0.1 c	4.4 a	0.9 d	85.9 a
N300P300K250	2.9 cde	2.2 afg	0.3 d	0.2 b	2.3 cdef	1.7 c	67.3 abc
N300P350K150	2.8 cde	2.1 fg	0.3 d	0.2 b	2.4 cdef	2.2 b	63.1 bcd
N300P350K200	2.9 cde	2.5 cdefg	0.3 d	0.2 a	2.4 cdef	2.2 b	73.9 ab
N300P350K250	2.9 cde	2.5 cdefg	0.3 d	0.1 c	2.5 cdef	1.9 c	67.9 abc
N350P250K150	2.5 ed	2.9 abcde	0.3 d	0.1 c	2.7 cdef	2.5 b	63.2 bcd
N350P250K200	3.5 bc	3.2 abc	0.3 d	0.1 c	3.0 bcd	2.6 b	58.3 bcd
N350P250K250	3.2 bcde	3.4 ab	0.3 d	0.1 c	2.8 cde	2.5 b	56.1 bcd
N350P300K150	3.3 bcd	2.5 cdefg	0.3 d	0.1 c	2.8 cde	1.5 c	61.6 bcd
N350P300K200	2.5 ed	2.8 abcdef	0.4 dc	0.1 c	2.8 cde	2.4 b	61.4 bcd
N350P300K250	2.9 cde	3.4 ab	0.3 d	0.1 c	2.5 cdef	2.5 b	59.3 bcd
N350P350K150	3.2 bcde	3.2 abcd	0.5 bc	0.1 c	2.8 cde	2.4 b	62.8 bcd
N350P350K200	2.4 ed	2.8 abcdef	0.3 d	0.1 c	2.6cdef	2.5 b	65.2 abcd
N350P350K250	3.2 bcde	3.2 abcd	0.7 ab	0.1 c	3.5 b	2.0 b	70.2 abc

Means followed by the same letter in a column are not significantly different at 5 % level

6.4 SUMMARY

Four trials, one at each season (autumn, winter, spring and summer), were conducted under 50 % shade net in a 3^3 factorial experiment replicated 4 times arranged in a randomized block design for N, P and K combinations. The objective of this investigation was to determine the effects of simultaneous applications of for N, P and K nutrition on growth and quality of bush tea. 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 percentage concentrations of leaf and root tissue N, P, K and total polyphenols. The results of this study demonstrated that regardless of season, there was some increased tendency of treatment combinations of N300, P300 and K200 ($kg \cdot ha^{-1}$) on increase fresh and dry shoot mass, number of leaves, leaf area as well as on theconcentrations of total polyphenols of bush tea. Regardless of season, no significant differences in plant height, number of branches, number of flowers buds (autumn and winter), stem girth, fresh and dry roots mass, as well as fresh and dry stem mass were obtained.

GENERAL DISCUSSION AND CONCLUSIONS

An experiment (Chapter 2) to identify the major compound of bush tea was conducted. It was found that bush tea contained 5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavon-3-ol. This compound is also identified as ($C_{21}H_{22}O_{10}$) and is characterized for the first time in bush tea. Flavonoids are known to be potent anti-oxidants (Schewe & Sies, 2005) and are capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals. Therefore, this compound identified in this study could be used to justify the medicinal potential of bush tea as a herbal tea with medicinal value. It would, therefore be appropriate to test this compound for anti-bacterial, anti-microbial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti-neoplastic, anti-thrombotic, and vasodilatory properties if it constitutes any of this activities.

In herbal teas, the total polyphenols are known to be potential quality indicators since they are anti-oxidant in nature. As a result, a field trial (Chapter 3) to investigate the seasonal variation of total polyphenols in bush tea, leaves harvested from the wild was established. Results in this study indicated that, the best or ideal time for harvesting wild bush tea would be during winter followed by summer season. These results will be used to assist the herbalist and traditional healers to know the ideal or best time to harvest wild bush tea for medicinal purposes. It is thus interesting to note that harvesting wild bush tea showed seasonal variations, with the lowest concentrations of total polyphenols of 10.8 ($mg.g^{-1}$) during autumn and spring, while the highest total polyphenol concentrations were in winter (35·5 $mg{\cdot}g^{-1}$) followed by summer. Similar responses were also confirmed by Owour (1992), where the concentrations of total polyphenols in black tea seedlings were highest during June (22.9 $mg{\cdot}g^{-1}$) and July (24.1 $mg{\cdot}g^{-1}$) vary due to seasons in the eastern highlands of Kenya. In a

field trial conducted by Ruan *et al*. (1999) during spring and autumn, there were also differential responses on the concentration of total polyphenols due to season.

Although, bush tea is only harvested from the wild, the concepts of domesticating wild plants are very critical in order to avoid the natural population from becoming extinct from its native environment. Experiments (Chapter 4) were carried out to determine the agronomic practices of nitrogen, phosphorus and potassium application on growth and development of bush tea, as influenced by season under shaded nursery environment. Fertilizer sources used were limestone ammonium nitrate (for N trial), single superphosphate (for P trial) and potassium chloride (for K trial), applied as post treatments in the form of granules. Results of this study confirmed that in all trials, regardless of season, N, P or K nutrition increased bush tea fresh and dry shoot weight, plant height, number of leaves, number of branches and leaf area. Therefore, for optimum levels for bush tea growth, 300 $kg{\cdot}ha^{-1}$ N or P and 200 $kg{\cdot}ha^{-1}$ for K. 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. Further studies should be investigated using different fertilizer sources in order to compare which N, P or K will yield better results comparing to the fertilizer sources used in the current studies.

For chemical composition experiments (Chapter 5), the optimum N level for total polyphenols was 300 $kg{\cdot}ha^{-1}$. For the P trial, total polyphenols were quadratically increased in response to P nutrition, regardless of season. Winter had the highest concentration of total polyphenols (46.8 $mg{\cdot}g^{-1}$). The optimum P level for total polyphenols was 300 $kg{\cdot}ha^{-1}$. In the K trial, total concentration of polyphenols reached maximum at 200 $kg{\cdot}ha^{-1}$, regardless of season. Most of the total polyphenols and biomass production occurred between 0 to 200 $kg{\cdot}ha^{-1}$ K. Therefore, for improved concentration of total polyphenols and biomass production 300

kg·ha^{-1} N and P is recommended. In K trial, the optimum level of bush tea growth was 200 kg·ha^{-1} K. However, for increased total polyphenols content, 400 kg·ha^{-1} K in bush tea is recommended. In these results, it was also observed that N treatments yielded the highest total polyphenols 51.1 mg·g^{-1} in winter compared to P and K trials.

In cultivated bush tea, total polyphenols were higher as compared to the total polyphenols from bush tea harvested from the wild. The differences between total polyphenols from the wild bush tea and cultivated bush tea were also exhibited during the study period. Total leaf tissue nitrogen was 3.2 % (autumn), 3.8 % (spring and winter) and 2.6 % (summer) in cultivated bush tea, whereas total leaf tissue N of wild bush tea did not exceed 1.7 % throughout the seasons. Therefore, these results suggest that the concentration of total polyphenols of cultivated bush tea under 50 % shade nets were largely attributed due to high total leaf tissue N in cultivated bush tea. Further research should also be conducted to investigate the influence of carbohydrate contents of wild bush tea leaves and cultivated bush tea leaves, to find out if there is any relationship in bush tea. This concur with the results reported by Bryant *et al*. (1987) and Tuomi *et al*. (1984), who reported negative correlation between concentrations of carbon based secondary compounds (CBSCs) and low nutrients availability in plant tissues. Other factors such as moisture stress, shading and elevated CO_2 has been reported to induce the accumulations of total phenolics in plants (Haukioja *et al*., 1998; Hamilton *et al*. 2001), thus suggesting another field of research that needs further investigation.

An experiment (Chapter 6) was established to determine the treatment combinations of N, P and K on growth and chemical composition of bush tea. The results of this study demonstrated that regardless of season, the treatment combinations 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 of bush tea than separately applied N, P and K nutrition (Chapter 5). No significant differences in plant height, number of branches, number of flowers buds (autumn and winter), stem girth, fresh and dry roots mass as well as fresh and dry stem mass were obtained regardless of season.

Based on the results and conclusions from this study, the following future prospects are suggested: sensory data (aroma, astringent and colour) on quality of a brew or liquor should be investigated and possibly, correlated with the concentration of total polyphenols. The effects of micro elements on chemical composition of bush tea and also determination of the effects of N, P and K application on concentrations on specific phenolic acids, flavonoids, total anti-oxidant activities, amino acids and tannins if any on bush tea need further investigations.

GENERAL SUMMARY

An experiment was carried out to identify the major compound in bush tea. The plant material was harvested in Muhuyu village (Limpopo Province, South Africa). 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. Silica gel (0.063 – 0.2 mm) was used as stationary phase and a mixture of hexane and ethyl acetate was used as mobile phase in the chromatographic separations. 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 contain 5-hydroxy-6,7,8,3',4',5'-hexamethoxy flavon-3-ol as a new flavonoid.

Another trial was done to investigate the seasonal variation of total polyphenols in bush tea leaves harvested from the wild. Leaf samples were collected from the field at Muhuyu Village (Limpopo Province) from January to December in 2003, and then dried. Total polyphenols were extracted using Folin-Ciaocalteau reagents and then 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 concentration 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 time would, therefore, be during winter followed by summer season.

Seasonal nutritional requirement and chemical composition of bush tea were investigated. Trials for N, P or K with one for 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·ha^{-1} N, P or K. Parameters recorded were plant height, number of branches, number of leaves, fresh and dry stem mass, fresh and dry root mass, stem girth, fresh and dry shoot mass, leaf area and the concentration 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 300 kg·ha^{-1} N or P and 200 kg·ha^{-1} K. Results for the N trial indicated that concentration of total polyphenols increased quadratically in response to N nutrition during autumn, winter, spring and summer. The highest concentration of total polyphenols was 51.1 mg·g^{-1} in winter. For the P trial, total polyphenols also increased quadratically in response to P nutrition regardless of season. Again, winter had the highest concentration of total polyphenols (46.8 mg·g^{-1}). In the K trial, regardless of season, total concentration of polyphenols reached their maximum at 200 kg·ha^{-1} with most of the total polyphenols occurring between 0 to 200 kg·ha^{-1} K. Therefore, for improved concentration of total polyphenols, 300 kg·ha^{-1} N and P and 200 kg·ha^{-1} K is recommended. 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.

Experiments to determine the effects of treatment combinations for N, P and K nutrition on growth and chemical composition of bush tea were investigated. 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 for N300, P300 and K200 ($kg \cdot ha^{-1}$) increased fresh and dry shoot mass, number of leaves, leaf area as well as the concentration of total polyphenols in bush tea. No significant differences in plant height, number of branches, number of flowers buds (autumn and winter), stem girth, fresh and dry roots mass as well as fresh and dry stem mass were obtained, regardless of the season.

RESEARCH OUTPUTS RESULTING FROM THIS PROJECT

This study has been published or presented in part as follows:

MUDAU F.N., SOUNDY, P. & DU TOIT E.S., 2005. Plant growth and development of bush tea (*Athrixia phylicoides* L.) as affected by nitrogen, phosphorus and potassium nutrition. *HortScience* 40(6), 1898-1901.

MUDAU, F.N., MASHIMBYE, M.J., SOUNDY, P. & VAN REE, I., 2005 A new flavonol from *Athrixia phylicoides* (Bush tea). *S. Afr. J. Chem.* 59, 1-2.

MUDAU F.N., SOUNDY, P., DU TOIT, E.S. & OLIVIER, J., 2004. Response of total polyphenol content in bush tea (*Athrixia phylivoides* L.) to seasonal variation. Poster presented at the 7th Indigenous Plant Use Forum (IPUF) Conference, 49. 5-8 July 2004, Augsburg Agricultural School, Clanwilliam, Western Cape Province, South Africa.

MUDAU F.N., SOUNDY, P., DU TOIT, E.S. & OLIVIER, J., 2005. Response of total polyphenols in leaves of bush tea (*Athrixia phylicoides* L.) to seasonal variation and nitrogen application. *South African Journal of Botany* (Accepted) Ref No SAJB 04-105.

MUDAU F.N., SOUNDY, P. & DU TOIT, E.S., 2005. Response of leaf total polyphenol content to nitrogen, phosphorus and potassium nutrition of bush tea (*Athrixia phylicoides* L.). *HortScience* (Submitted). Oral paper presented at the 102th American Society for Horticultural Sciences (ASHS), July 18-21, Las Vegas, Nevada (USA).

MUDAU F.N., SOUNDY, P. & DU TOIT, E.S., 2004. Plant growth and development of bush tea (*Athrixia phylicoides* L.) as affected by year-round nitrogen, phosphorus and potassium nutrition. Oral paper presented at the 7th Indigenous Plant Use Forum (IPUF) Conference, 48. 5-8 July 2004, Augsburg Agricultural School, Clanwilliam, Western Cape Province, South Africa.

MUDAU F.N., SOUNDY, P. & DU TOIT, E.S. Effects of N, P and K nutrition on growth and chemical composition of bush tea (*Athrixia phylicoides* L.) as influenced by season. Submitted to HortTechnology.

ACADEMIC AWARD FROM THIS PROJECT

MASHIMBYE, M.J., **MUDAU, F.N.,** SOUNDY, P. & VAN REE, T., 2005. A new flavonol from *Athrixia phylicoides* L. (bush tea). Poster presented at the Combined Congress 2005, 104. 10-13 January 2005. Awarded a prize for the Best Poster presented at the 12th National Congress of the Southern African Society for Horticultural Sciences, 11 -13, January 2005, North West University, North West Province, South Africa.

IMPLICATIONS AND INTELLECTUAL PROPERTY OF THIS PROJECT

Prior to this study, no published research was conducted on flavonol and total polyphenols of bush tea. The results have shown that bush tea has a major flavonol and contains total polyphenols, thus suggesting that bush tea has medicinal potential. Total polyphenols of wild bush tea were highest during winter and summer. In cultivated bush tea, the highest polyphenols were during winter followed by summer, spring and autumn. Therefore, the results of this study suggest that bush tea has commercial significance. Thus, the marketing and development strategy of the plant need to be established worldwide.

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APPENDICES

SUMMARY OF ANALYSES OF VARIANCE (ANOVA)

APPENDIX A

Nitrogen experiments

Table A-1 Analysis of variance for N nutrition on growth characteristics of bush tea during autumn

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
Model	12	420.0	2127.4	420.1	163.8	47449.8	313628.0	1.5	2.7
Treatment	5	282.9*	2632.2*	663.3*	282.9*	58731.8*	396993.7*	2.7*	6.4*
Nitrogen (linear)	1	2.9^{NS}	2740.3^{NS}	185.1^{NS}	100.3^{NS}	60235.6^{NS}	30810.9^{NS}	1.8^{NS}	2.3^{NS}
Nitrogen (quadratic)	1	832.0**	9888.0**	2001.4**	872.3**	117117.9**	555503.7**	7.3**	11.3**
Error	35	197.7	2630.0	277.0	169.3	59103.2	17758.7	0.3	0.2

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table A-2 Analysis of variance for N nutrition on growth characteristics of bush tea during winter

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
Model	12	212.9	32.9	614.7	77.6	30722.2	265558.9	3.0	4.2
Treatment	5	126.5*	21.7*	1191.6*	127.9*	51549.6*	529776.3*	7.1*	9.4*
Nitrogen (linear)	1	20.6^{NS}	0.1^{NS}	193.0^{NS}	4.8^{NS}	10803.6^{NS}	376120.9^{NS}	1.8^{NS}	1.0^{NS}
Nitrogen (quadratic)	1	276.6**	8.6**	2795.7**	529.8**	142793.2**	751016.2**	24.2**	27.7**
Error	35	327.1	15.6	176.1	51.9	18988.8	188930.8	0.3	0.2

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table A-3 Analysis of variance for N nutrition on growth characteristics of bush tea during spring

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
Model	12	235.9	847.6	1040.5	361.1	347470.9	232766.2	0.4	0.3
Treatment	5	150.6*	1342.3*	1967.6*	649.7*	375223.7*	393748.9*	0.9*	0.9*
Nitrogen (linear)	1	13.8^{NS}	946.1^{NS}	45.9^{NS}	109.9^{NS}	623007.0^{NS}	29448.0^{NS}	3.8^{NS}	0.2^{NS}
Nitrogen (quadratic)	1	656.7**	1100.8**	9425.4**	916.8**	599600.1**	1097252.2**	26.8**	0.3**
Error	35	717.5	374.8	521.6	123.5	185206.5	105837.7	0.2	0.8

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table A-4 Analysis of variance for N nutrition on growth characteristics of bush tea during summer

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
Model	12	270.2	846.6	1040.5	361.8	358952.7	632706.4	0.9	0.7
Treatment	5	195.4*	1347.8*	1967.6*	650.7*	374220.6*	580206.2*	1.8*	1.4*
Nitrogen (linear)	1	11.3^{NS}	979.0^{NS}	45.9^{NS}	108.4^{NS}	620498.0^{NS}	12140.3^{NS}	0.3^{NS}	0.2^{NS}
Nitrogen (quadratic)	1	893.2**	1088.0**	9425.4**	1918.6**	638395.4**	2639433**	2.3**	2.7**
Error	35	713.4	376.3	521.6	123.6	157835.8	531041.1	0.3	0.3

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

APPENDIX B

Phosphorus experiments

Table B-1 Analysis of variance for P nutrition on growth characteristics of bush tea during autumn

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue P (%)	Root tissue P (%)
Model	12	510.8	410.9	443.2	368.4	37998.5	74727.9	0.7	0.5
Treatment	5	125.8*	303.0*	711.9*	591.5*	16329.9*	96160.9*	0.4*	0.7*
Phosphorus (linear)	1	28.0^{NS}	282.9^{NS}	211.8^{NS}	89.3^{NS}	3726.0^{NS}	147871.8^{NS}	0.2^{NS}	0.2^{NS}
Phosphorus (quadratic)	1	179.2**	192.4**	2126.8*	1897.8*	24235.9**	49345.1**	0.3**	0.6**
Error	35	497.2	461.7	275.3	239.3	36196.8	47850.2	0.2	0.2

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table B-2 Analysis of variance for P nutrition on growth characteristics of bush tea during winter

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
Model	12	212.9	78.9	162.6	44.7	22848.9	127676.6	0.5	0.6
Treatment	5	363.9*	86.2*	306.0*	82.2*	36920.3*	84960.0*	0.6*	0.4*
Phosphorus (linear)	1	246.0^{NS}	0.9^{NS}	26.9^{NS}	0.7^{NS}	408.9^{NS}	264226.6^{NS}	0.2^{NS}	0.3^{NS}
Phosphorus (quadratic)	1	995.7**	339.5**	781.7**	234.1**	147260.6**	327583.8**	0.4**	0.5**
Error	35	131.5	53.9	103.5	23.7	15129.1	51098.1	0.1	0.1

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table B-3 Analysis of variance for P nutrition on growth characteristics of bush tea during spring

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue N (%)	Root tissue N (%)
Model	12	673.6	176.9	422.2	46.3	25910.7	160168.6	0.4	1.5
Treatment	5	560.1*	164.4*	442.4*	32.7*	31560.1*	158459.5*	0.7*	2.9*
Phosphorus (linear)	1	101.5^{NS}	2.0^{NS}	15.7^{NS}	1.6^{NS}	48360.5^{NS}	3516.8^{NS}	0.1^{NS}	0.1^{NS}
Phosphorus (quadratic)	1	749.1**	531.0**	1932.6**	154.3**	32900.4**	312215.7**	0.3**	27.7**
Error	35	697.2	132.9	258.7	95.7	26428.3	81395.5	0.2	0.2

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table B-4 Analysis of variance for P nutrition on growth characteristics of bush tea during summer

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue P (%)	Root tissue P (%)
Model	12	445.2	138.3	422.2	46.3	49956.9	158081.9	0.3	0.3
Treatment	5	620.3*	114.9*	442.4*	32.7*	60109.8*	186100.2*	0.5*	0.8*
Phosphorus (linear)	1	0.3^{NS}	4.5^{NS}	15.7^{NS}	1.6^{NS}	66200.8^{NS}	1726.9^{NS}	0.2^{NS}	0.9^{NS}
Phosphorus (quadratic)	1	3074.5**	570.4**	1932.6**	154.3**	193286.5**	371859.9**	1.4**	0.6**
Error	35	587.7	118.1	258.7	95.7	43860.9	85137.2	0.1	0.3

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

APPENDIX C

Potassium experiments

Table C-1 Analysis of variance for K nutrition on growth characteristics of bush tea during autumn

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue K (%)	Root tissue K (%)
Model	12	384.1	263.3	41.3	24.2	14029.2	47740.3	3.6	3.6
Treatment	5	537.9*	66.4*	41.5*	21.9*	15725.6*	33565.8*	7.4*	7.7*
Potassium (linear)	1	28.0^{NS}	14.3^{NS}	8.9^{NS}	0.6^{NS}	2740.3^{NS}	26048.8^{NS}	1.5^{NS}	0.2^{NS}
Potassium (quadratic)	1	1974.9**	56.6**	166.5**	94.4**	2530.7**	12543.0**	29.3**	26.7**
Error	35	193.7	343.6	49.0	38.8	8795.7	33494.5	0.7	0.5

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table C-2 Analysis of variance for K nutrition on growth characteristics of bush tea during winter

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue K (%)	Root tissue K (%)
Model	12	1091.2	223.2	41.7	16.8	13130.4	31038.4	1.4	1.5
Treatment	5	1462.4*	191.7*	36.8*	23.7*	17860.5*	54871.5*	3.0*	3.2*
Potassium (linear)	1	751.7^{NS}	3.8^{NS}	5.2^{NS}	4.2^{NS}	24.1^{NS}	2136.1^{NS}	0.6^{NS}	0.7^{NS}
Potassium (quadratic)	1	3336.9**	370.0**	141.0**	79.8**	20305.8*	139668.8**	9.4**	10.6**
Error	35	687.1	126.9	43.2	17.8	5075.4	42581.7	0.3	0.3

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table C-3 Analysis of variance for K nutrition on growth characteristics of bush tea during spring

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue K (%)	Root tissue N (%)
Model	12	1091.2	223.16	41.3	24.2	11215.9	47740.3	0.3	0.4
Treatment	5	1462.4*	191.7*	41.5*	21.9*	16751.4*	33565.8*	0.7*	0.9*
Potassium (linear)	1	751.75^{NS}	3.8^{NS}	8.9^{NS}	0.6^{NS}	23058.8^{NS}	26048.8^{NS}	0.3^{NS}	0.1^{NS}
Potassium (quadratic)	1	3336.9**	370.0**	166.5**	94.4**	19674.7**	12543.0**	3.2**	4.2**
Error	35	687.1	126.8	49.0	38.8	6784.7	33494.5	0.2	0.2

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table C-4 Analysis of variance for K nutrition on growth characteristics of bush tea during summer

Sources of variation	Df	Mean squares[z]							
		Plant height (cm)	Number of branches	Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Leaf tissue K (%)	Root tissue K (%)
Model	12	721.2	208.5	540.3	405.6	7293.1	116943.3	1.2	1.1
Treatment	5	928.9*	212.3*	688.94*	416.6*	4971.9*	136926.6*	2.5*	2.5*
Potassium (linear)	1	2.0^{NS}	1.1^{NS}	6.1^{NS}	27.7^{NS}	8811.3^{NS}	67172.1^{NS}	0.2^{NS}	0.2^{NS}
Potassium (quadratic)	1	4092.0**	380.0**	32.81**	969.6**	13172.0**	476666.3**	2.7**	2.4**
Error	35	369.6	109.9	484.2	524.0	7615.3	59404.7	0.5	0.3

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

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APPENDIX D

Seasonal Variation

Table D-1 Analysis of variance for N nutrition on seasonal variation of leaf concentration of polyphenols of bush tea

Sources of variation	Df	Mean squares[z]			
		Concentration of total polyphenols ($mg \cdot g^{-1}$)			
		Autumn	Winter	Spring	Summer
Model	12	116.7	165.4	394.9	613.9
Treatment	5	184.6**	197.2**	767.6**	1193.7**
Nitrogen (linear)	1	0.5NS	4.9NS	4.3NS	9.1NS
Nitrogen (quadratic)	1	919.7**	835.7**	3762.6**	5378.4**
Error	35	172.4	77.3	107.4	241.8

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

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Table D-2 Analysis of variance for P nutrition on seasonal variation of leaf concentration of polyphenols of bush tea

Sources of variation	Df	Mean squares[z]			
		Concentration of total polyphenols ($mg \cdot g^{-1}$)			
		Autumn	Winter	Spring	Summer
Model	12	643.6	580.8	312.7	441.1
Treatment	5	1383.7*	1133.3*	588.9*	881.9*
Phosphorus (linear)	1	554.5NS	2.0 NS	0.5 NS	273.5 NS
Phosphorus (quadratic)	1	3442.1**	4843.8**	1860.1**	2767.2**
Error	35	211.4	278.3	300.7	236.3

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

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Table D-3 Analysis of variance for K nutrition on seasonal variation of leaf concentration of polyphenols of bush tea

Sources of variation	Df	Mean squares[z]			
		Concentration of total polyphenols ($mg \cdot g^{-1}$)			
		Autumn	Winter	Spring	Summer
Model	12	720.3	1244.9	232.5	742.1
Treatment	5	1581.5**	2437.9**	1085.7**	1631.3**
Potassium (linear)	1	1024.5^{NS}	1837.1^{NS}	139.9^{NS}	276.8^{NS}
Potassium (quadratic)	1	4130.4^{**}	6236.2^{**}	4326.2^{**}	6174.0^{**}
Error	35	171.4	238.9	232.6	370.9

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

APPENDIX E

FACTORIAL EXPERIMENT

Table E-1 Analysis of variance for N x Px K nutrition on growth and chemical composition of bush tea during autumn

Sources of variation	Df	Means squares[z]										
		Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Total polyphenols ($mg \cdot g^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)
Model	29	112.3	85.7	7622.0	636910.4	835.9	0.3	0.4	0.6	0.1	0.4	2.3
Treatment	26	122.0*	93.7*	70033.3*	883862.2*	806.3*	0.4*	0.3*	0.4*	0.7*	0.5*	2.2*
Error	78	12.5	18.9	2123.1	356871.1	275.8	0.1	0.2	0.2	0.6	0.4	2.0

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table E-2 Analysis of variance for N x Px K nutrition on growth and chemical composition of bush tea during winter

Sources of variation	Df	Means squaresz										
		Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Total polyphenols ($mg \cdot g^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)
Model	29	55.8	206.0	208461.8	15194.3	281.3	0.8	1.0	0.1	0.1	0.5	2.3
Treatment	26	46.0*	150.6*	22745.1*	16611.2*	303.3*	2.5*	1.1*	0.4*	0.5*	0.4*	3.3*
Error	78	24.3	56..5	116690.9	9810.9	75.2	0.4	0.2	0.2	0.4	0.2	2.3

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table E-3 Analysis of variance for N x P x K nutrition on growth and chemical composition of bush tea during spring

Sources of variation	Df	Means squares[z]										
		Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Total polyphenols ($mg \cdot g^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)
Model	29	91.4	127.4	140912.0	1599.4	95.4	0.6	0.8	0.2	0.4	0.5	0.4
Treatment	26	101.3*	141.4*	150747.6*	17732.4*	92.7*	0.7*	0.9*	0.4*	0.3*	0.6*	0.5*
Error	78	7.2	73.7	63306.3	27350.8	69.3	0.3	0.2	0.1	0.1	0.1	1.2

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability

Table E-4 Analysis of variance for N x P x K nutrition on growth and chemical composition of bush tea during summer

Sources of variation	Df	Means squares[z]										
		Fresh shoot mass (g)	Dry shoot mass (g)	Number of leaves	Leaf area (cm^2)	Total polyphenols ($mg \cdot g^{-1}$)	Leaf tissue N (%)	Root tissue N (%)	Leaf tissue P (%)	Root tissue P (%)	Leaf tissue K (%)	Root tissue K (%)
Model	29	119.9	155.2	1145.5	42976.5	247.8	0.8	0.7	0.4	0.1	0.8	0.7
Treatment	26	133.5*	171.9*	1266.9*	46989.8*	255.3*	0.3*	0.3*	0.1*	0.2*	0.9*	0.8*
Error	78		50.7	92.6	21748.9	166.1	0.4	0.8	0.1	0.1	0.2	0.2

[z]F-values significant (*), highly significant (**) or not significant different (NS) at 5% level of probability