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Full Length Research Paper

The effect of pruning on growth and chemical composition of cultivated bush tea (*Athrixia phylicoides* D.C)

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A trial to determine the effect of pruning at different heights on growth and quality of cultivated bush tea was conducted. Pruning of bush tea largely led to crop losses. Unpruned bush tea plants remained the tallest plants, with higher number of branches, bigger leaf area and a larger biomass than apically pruned, middle pruned and base pruned bush tea plants. Pruning at different heights also proved to have little or no effect on quality of bush tea. While only total polyphenols remained higher in unpruned tea plants, no significant differences were observed in tannin and total antioxidant content in unpruned, apically pruned and middle pruned tea plants.

Key words: Pruning, bush tea, chemical composition, growth.

INTRODUCTION

Bush tea (*Athrixia phylicoides* DC.), which belongs to the Asteraceae family, is indigenous to South Africa. There are 14 species in the genus Athrixia, nine of which are found in South Africa (Leistner, 2000). It is a popular beverage used as an herbal tea and as a medicinal plant by traditional African people (Roberts, 1990). It has been used for many years for treating boils, cleansing or purifying blood, bad acne, infected wounds and cuts, skin eruption, and for bathing (Roberts, 1990). Although, the usage of bush tea has declined over time due to the availability of commercially produced teas, the plant still has economic potential as an herbal medicine (Mudau et al., 2007a).

Pruning is one of the most important operations, next to plucking, which directly determines the productivity and quality of tea bushes (Tocklai Tea Research Association (TTRA), 2008). Tea plants are pruned to obtain a given table form and height, to eliminate unnecessary and diseased branches, to rejuvenate the tea plants, and to obtain healthier and better quality tea plants (Yilmaz et al., 2004). In spite of huge crop losses that result from pruning, it is a necessity that has been carried out periodically (TTRA, 2008). Yilmaz et al. (2004) reported less yields in tea harvested 50 cm above the ground in the first year, with yields increasing in the subsequent second and third years. Thus, pruning increases tea yields in the long term. If pruning is not done or is delayed, the size and weight of growing shoots on plucking surface decrease, with loss of vigour of growing apices in the long run (TTRA, 2008). TTRA (2008) distinguished between the following types of pruning: Light pruning where tea bushes are pruned 4 to 5 cm at the top, medium pruning, where tea bushes are cut 45 to 60 cm above ground, heavy pruning where tea bushes are cut 15 to 45 cm above ground followed by collar pruning where all above ground parts of the tea bushes are cut. Light pruning helps to renew the wood, regulate crop distribution and maintain ideal frame height of the bushes (TTRA, 2008). Medium prune helps in rejuvenating the tea bushes that have become old and their yields have started declining (TTRA, 2008). Heavy and collar prune are necessary for complete renewal of tea frame (TTRA, 2008). Collar prune however needs to be carried out only when the root system is strong enough to withstand the shock and initiate new growth, otherwise heavy mortality will result (TTRA, 2008). TTRA (2008) also reported a

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Figure 1. Bush tea seedlings growing on a mist bed.

crop loss of 30-35% after light pruning and 60 to 70% after medium pruning.

Ravichandran (2003) reported that pruning is an essential agronomic practice in the production of leaves for the manufacture of black tea as it leads to enhanced branching and hence a greater number of tender leaves. Satyanarayana et al. (1994) also reported that pruning leads to enhanced branching and hence a greater number of tender leaves. According to them unpruned tea plants produce more dormant buds than growing buds. Therefore, pruning prior to harvest has been considered to have great effects on plant productivity and quality. Asil (2008) reported that in comparative plucking method of black tea based on three lengths (*viz.,* 5, 10 and 15 cm), the best result of green leaves yield and quality were obtained in the 5 cm treatment in spring flush, followed by 10 and 15 cm, respectively. Plucking of leaves increased the concentration of total polyphenols and total antioxidants in green tea (Owour et al., 2000).

Pruning was also found to affect quality of tea. Mahanta and Baruah (2006) reported that all the pigment contents of black tea, except chlorophyll, were found to be higher in pruned tea leaf than unpruned tea, thus enhancing the quality of made tea. Ravichandran (2003) also reported that the precursors responsible for tea quality, such as polyphenols, were found to increase in the first year and thereafter declined in content with time from pruning. According to Ravichandran (2003), the green pigment content and ash content increased and the lipoxygenase activity declined progressively with time from pruning. Data that describe the effect of pruning on growth (yield) and chemical composition in bush tea is lacking, yet pruning is a standard practice in the management of other teas. Therefore, this study was undertaken to determine the effect of pruning at different heights on growth and quality (chemical composition) of bush tea.

MATERIALS AND METHODS

The experimental site and planting materials

The planting materials for the experiment were made up of mature bush tea stock plants and were collected from Mudzidzidzi village, South Africa, next to Tshatshingo Potholes on 27 November 2007 and planted at Madzivhandila College (22^{o} 56' 60S, 30^{o} 28' 60E, altitude 709 m, summer rainfall and dry winter), under 40% shaded nursery. Selected planting materials were true-to-name and type, free of disease and insects, and in the proper physiological state. During cultivation, to stimulate rapid and prolific rooting of cuttings, plants were cut about 7 to 8 cm long and were treated with Seradix No.2 (0.3% IBA) (Bayer Pretoria, South Africa). The cuttings were planted on seedling trays on a mist bed (Figure 1), supplied with a misting system operating through misting nozzles. The mist bed was 3 m long, 1.5 m wide and0.5 m high. Irrigation was done 3 times a day except on rainy days.

Rooted cuttings were transplanted directly into 20 L bags after two and a half months. The medium used during transplanting was pine bark and sand at a ratio of 2:1. In an attempt to achieve

optimum growth, the growing bush tea plants in plastic bags were treated with a split application of NPK at rates of 300, 300 and 200 kg/ha (Mudau et al., 2007b) two weeks after transplanting. Pruning treatments, namely zero or no pruning (control), top-branch pruning, middle pruning and basal pruning were done a month after transplanting. The experiment was harvested for chemical analysis 12 weeks after transplanting.

Experimental design and treatments

Treatments consists of zero or no pruning (control), top-branch pruning, middle pruning and basal pruning arranged in a randomized complete block design with 10 replicates. Zero or no pruning involved leaving individual bush tea plants intact with no pruning from start to end of the experiment. Top-branch pruning involved cutting or pruning of all top branches and stems of individual bush tea plants at the top up to 15 cm length. Middle pruning involved cutting or pruning of all branches and stems of individual plants right in the middle of individual plants. Basal pruning involved cutting or pruning individual plant at the base just above the soil (media) surface.

Data collection

Parameters measured were growth parameters in the form of plant height, number of branches, biomass, and leaf area, and chemical compositions such as polyphenols, tannins and antioxidant activities.

Determination of total polyphenol content

Methanol was used as the extraction solvent for the determination of total phenols. Duplicates of 2 g of tea were extracted using 40 ml of the solvent as follows. An amount of 20 ml of methanol was added to 2 g of sample in centrifuge tubes and the sample were vortex mixed every 10 min for 2 h to improve extraction efficiency. The samples were then centrifuged at 3500 rpm for 10 min (25°C) using centrifuged tubes and decanted. Each sample residue was rinsed once with 20 ml of solvent, vortex mixed for 5 min, centrifuged as above, and decanted. Two supernatants were combined and used for analysis. The Folin Ciocalteau method (Singleton and Rossi, 1965), modified by Waterman and Mole (1994), was used to determine total phenols in the black tea extract. This method was based on the reducing power of phenolic hydroxyl groups (Hahn et al., 1984) which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. In brief, methanol extract (0.5 ml) was added to a 50 ml volumetric flask containing distilled water and mixed. Folin Ciocalteau phenol reagent (2.5 ml) was then added and mixed, followed by 7.5 ml sodium carbonate solution (20 g/100 ml) within one to eight minutes after addition of the Folin Ciocalteau phenol reagent. The contents were mixed and the flask made up to volume with distilled water and thoroughly mixed. Absorbance of the reactants was read after 2 h at 760 nm using UV-visible genesys 20 Spectrophotometer. Catechin was used as standard to prepare a standard curve and results were expressed as mg catechin equivalents/100mg of samples on dry weight basis.

Determination of tannins

The Vanillin HCL method of Prince et al. (1978) was used for the determination of Tannins. This method is based on the ability of flavonoids to react with Vanillin in the presence of mineral acids to produce a red colour that is measured spectrophotometrically. The extracts and reagents were maintained at 30°C in a thermostat-controlled water bath before mixing the reactants. The methanolic extract (1 ml) was added to 5 ml vanillin reagent (4% HCL in methanol and 0.5 ml vanillin in methanol) and mixed. Sample blanks were done with 4% HCL in methanol replacing vanillin reagent. The reactants were maintained at 30°C and absorbance was read at 500 nm after 20 min. Absorbance reading of the blanks was subtracted from those of the samples. Catechin was used as a standard. The results were expressed as mg catechin equivalents/100 mg sample on dry weight basis.

Determination of antioxidant activity

Antioxidant activity of the extracts was determined using Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika et al. (2004). TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the $ABTS^+$ radical cation chromogen in relation to that of Trolox, the water soluble vitamin E analogue which is used as an antioxidant standard. The $ABTS^+$ was produced by mixing equal volume of 8 mM ABTS with 3 mM potassium persulfates prepared in distilled water and allowed to react in the dark for at least 12 h at room temperature before use. The $ABTS^+$ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH_2PO_4, 0.2 M $NaHPO_4$ and 150 mM NaCl in 1 L of distilled water, with pH adjustment using NaOH where necessary. This solution was made fresh for each analysis. The $ABTS^+$ solution (2900 µl) was added to the methanol extracts of tea (100 µl) of Trolox in a test tube and mixed. Absorbance readings (at 734 nm) were taken after 30 min (for the samples) and 15 min (for the standard) of the initial mixing of the samples and standard, respectively. The results were expressed as µM Trolox equivalents /g of sample on dry weight basis.

Data analysis

Data were then subjected to analysis of variance (ANOVA) using PROC GLM (General linear model) procedure of SAS version 8.0 (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Response of bush tea growth to pruning

Results in Table 1 showed a decrease in plant height, number of branches, fresh and dry biomass from zero or no pruning, to apical pruning, to middle pruning up to basal pruning. These results concur with TTRA (2008) observation of increasing crop loss at every degree of pruning from light, medium pruning to heavy pruning. Fresh biomass figures reflected the same 30% loss at apical pruning equivalent to light pruning (TTRA, 2008) and 60% loss at middle pruning equivalent to medium prune as reported by TTRA (2008). Basal pruning which is equivalent to collar pruning (TTRA, 2008) showed the lowest results in all the growth parameters recorded. This was largely attributed to high mortality rate that resulted from the treatment applications. This result suggests that the bush tea plant roots on which the treatment was applied were not strong enough to initiate new growth, as in the case of other established perennial tea such as

Table 1. Growth characteristics of bush tea as affected by pruning at different heights.

Treatment (Pruning methods)	Plant height (cm)	Number of branches/plant	Leaf area/ plant (cm^2)	Fresh biomass/plant (g)	Dry biomass/plant (g)
No or 0 pruning	38.4 ± 2.03a	31.8 ± 3.16a	306.1 ± 29.05a	27.43 ± 2.92a	10.97 ± 1.26a
Apical pruning	30.54 ± 2.03b	28.5 ± 3.16a	199.0 ± 29.05b	18.73 ± 2.92b	8.25 ± 1.26a
Middle pruning	24.48 ± 2.03c	16.5 ± 3.16b	213.4 ± 29.05b	10.88 ± 2.92b	4.17 ± 1.26b
Basal pruning	3.81 ± 2.03d	4.1 ± 3.16c	114.8 ± 29.05c	1.98 ± 2.92c	0.46 ± 1.26c

Figures in a column followed by the same letter are not significantly different the 5% level probability. ($p > 0.05$).

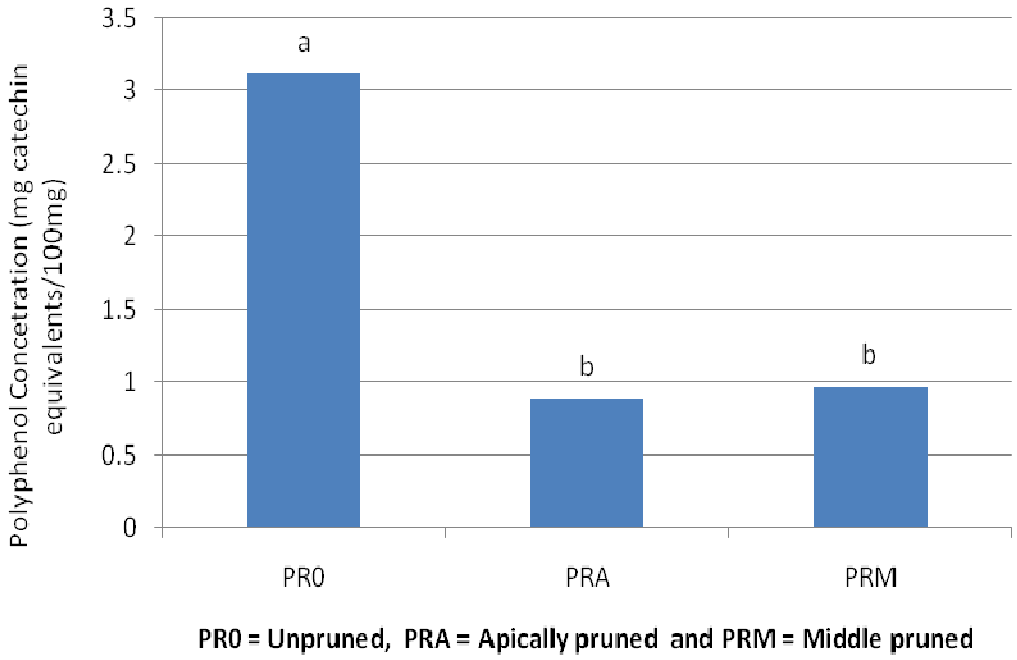

Figure 2. Total polyphenol concentrations of bush tea pruned at different heights. *Means denoted by the same letter are not significantly different at the 5% level probability.

Cammellia sinensis.

Effect of pruning on chemical composition

Concentration of total polyphenols

There was variation in the concentration of total polyphenols between unpruned (PR0), apically pruned (PRA) and middle pruned (PRM) tea plants as shown in Figure 2. The highest total polyphenol concentration was observed in unpruned (PR0) plants with 3.12 mg/g, while the lowest was observed in apically pruned (PRA) plants with 0.89 mg/g. The difference between the highest and the lowest total polyphenol concentration was 2.23 mg/g. Significantly higher total polyphenol in unpruned (PR0) than in pruned (PRA and PRM) tea contradicts results by Mahanta and Baruah (2006), who reported that higher phenolic contents were achieved in pruned black tea.

Tannin concentration

Although, there was no significant difference in tannin content between unpruned (PR0), apically pruned (PRA) and middle pruned (PRM) as show in Figure 3, PRO plants yielded highest tannin concentration of 0.27 mg/g; 0.07 higher than the lowest tannin concentration of 0.2 mg/g yielded by middle pruned (PRM) bush tea plants. Lack of significant difference between unpruned (PR0) and pruned (PRA and PRM) tea contradicts Mahanta and Baruah (2006), who reported higher pigment contents in pruned black tea than in unpruned black tea enhancing the quality of made tea, possibly due to bushes of black tea are larger than those of bush tea and can therefore translocate large reserves to growing points after pruning.

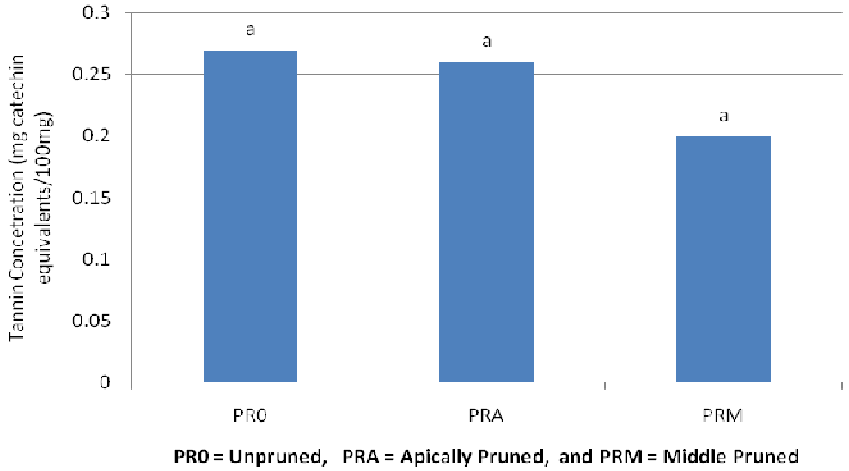

Figure 3. Tannin concentrations of bush tea pruned at different heights. *Means denoted by the same letter are not significantly different at the 5% level probability.

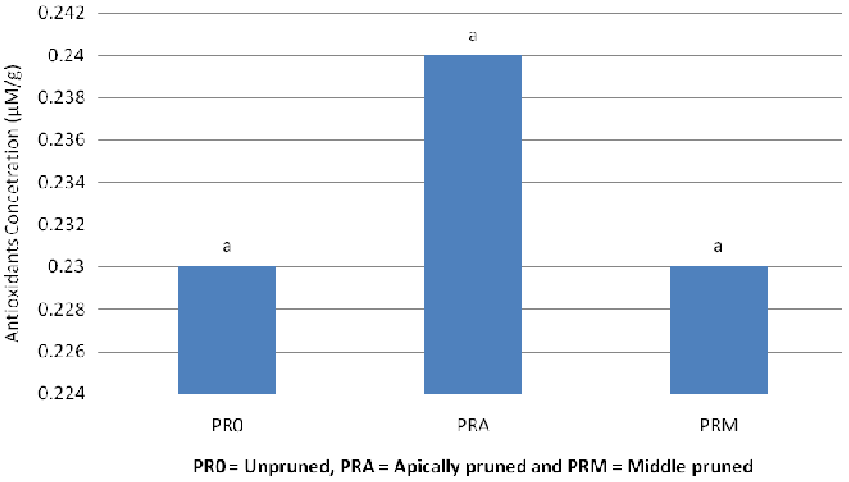

Figure 4. Total antioxidant contents of bush tea pruned at different heights. *Means denoted by the same letter are not significantly different at the 5% level probability.

Total antioxidant contents

There were no significant differences in total antioxidants between PR0, PRA and PRM plants as shown in Figure 4. However, PRA plants yielded highest total antioxidants at 0.24, 0.01 mg/g higher than the lower yielding PRO and PRM plants. Lack of significant difference between unpruned (PR0) and pruned (PRA and PRM) tea contradicts Mahanta and Baruah (2006) who reported higher pigments of carotenoid and anthocynanin content in pruned black tea than in unpruned black tea enhancing the quality of made tea. These results suggest that pruning does not affect concentration of antioxidants in bush tea.

In conclusion, the results showed that pruning of bush tea largely led to crop losses. Compared to apically, middle and base pruned plants, unpruned bush tea plants remained the tallest plants, with higher number of branches, bigger leaf area and a larger biomass. Basal pruning is not viable in bush tea as the treatment showed highly reduced growth. The trial showed that pruning at different heights has little or no effect on quality of bush tea. While only total polyphenols remained higher in unpruned tea plants, no significant differences were observed in tannin and total antioxidant content in unpruned, apically pruned and middle pruned tea plants.

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