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# Effect of increased fertilization on the phytochemical constituents and antioxidant activity of *Jatropha zeyheri* tea under greenhouse conditions

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**ABSTRACT**

Tea fertilization is one of the key factors influencing tea's physiological, physical and chemical qualities. However, the influence of NPK fertilizer on phytochemical constituents and antioxidant activity of *Jatropha zeyheri* indigenous tea has not been documented. Therefore, this study aimed at assessing whether different fertilizer application rates would affect the phytochemical constituents and antioxidant activity of *J. zeyheri* tea leaves under greenhouse conditions. Six treatments constituting NPK fertilizer rates (0, 2, 4, 8, 16 and 32 g) were arranged in a randomized complete block design, with five replications. After 130 days, leaves were harvested and oven-dried for 72 h at a temperature of 60°C. The dried leaves were ground through a 1 mm sieve using a grinder before analysis. After laboratory preparations, phytochemicals and antioxidant activity were analyzed using the UV-visible spectrophotometer. Data were subjected to ANOVA using the Statistix 10.0 software. Treatments had a highly significant effect ($P \leq 0.01$) on antioxidant activity and total flavonoid content contributing 46 and 73% in total treatment variation (TTV), respectively. In contrast, NPK fertilizer had a significant effect ($P \leq 0.05$) on total phenols and tannins, contributing 71 and 59% in TTV, respectively. *Jatropha zeyheri* tea leaves antioxidants and phytoconstituents exhibited positive quadratic relations with increasing fertilizer levels. Fertilizer requirements for phytoconstituents and antioxidant activity were optimized at 3.97 g fertilizer/plant. In conclusion, antioxidant activity, total phenol, total tannin and flavonoid contents were affected by increasing NPK fertilizer rates.

**Key words:** Fertilizer, indigenous tea, plant parts, secondary metabolites, tea quality

## INTRODUCTION

Researchers have devoted most of their attention to herbal and medicinal plants such as natural remedies due to their bioactive compounds (Attarzadeh *et al.*, 2019). *Jatropha zeyheri* Sond commonly known as Sefapabadia in Sepedi, Xidomeja in Xitsonga and Thundamali in Tshivenda among South African people (Luseba and Van der Merwe, 2006; Ndou, 2019), is a perennial hairy plant with a deep-rooted rhizome and branched leafy stems (Ndou, 2019), the species belongs to the Eurphorbiaceae family. *Jatropha zeyheri* is utilized by traditional practitioners to treat sexually transmitted infections and urinary tract infections (Van Wyk and Gericke, 2007). Van Wyk and Gericke (2007) reported various health benefits contained by this indigenous plant; for instance, the roots are traditionally used to treat sexually transmitted urinary tract infections. Tea leaves contain thousands of bioactive constituents, such as polyphenols, amino acids, volatile compounds, and alkaloids that exhibit a range of promising pharmacological properties (Yadav *et al.*, 2020). Due to its substantial antioxidant property, tea inhibits the development of various cancers by regulating oxidative damage of biomolecules, endogenous antioxidants, and pathways of mutagen and transcription of the antioxidant gene pool (Attarzadeh *et al.*, 2019).

Overall, good manufacturing of high-quality medicinal plants is dependent on precise awareness of plant nutrient requirements. Research has shown that yield and quality can be improved with fertilization (Mudau *et al.*, 2005, 2006), pruning and

application of growth regulators (Maudu *et al.*, 2012). Among these agronomic practices, N, P and K fertilizers were reported to have a pronounced effect on leaf total polyphenol content in *Camellia sinensis* (Owuor and Jain, 2001) and bush tea (Mudau *et al.*, 2005). Application of N, P and K was also found to improve the accumulation of carbohydrates for plant growth (Sitienei *et al.*, 2013) and to increase photosynthetic rates (Ibrahim *et al.*, 2013). This resulted in the biosynthesis of carbon-based secondary metabolites, such as flavonoids, phenolic acids and tannins, known as total polyphenols, which are antioxidant in nature (Ibrahim *et al.*, 2013).

For healthy growth and optimal yield, nutrients must be available to plants in the correct quantity, proportion and usable form at the right time. To fulfil these requirements, chemical fertilizers are needed. The fertilizer impact on the vegetative growth of various crops is well documented. However, the effect of NPK fertilizer on the phytoconstituents of *J. zeyheri* indigenous tea remains undocumented. Few studies were conducted on wild *J. zeyheri* indigenous leaves, such as the influence of the time-based hot air-drying method on total polyphenols, total antioxidants and tannins (Mutshekwa *et al.*, 2019), the effect of time of harvest on mineral elements (Sehlapelo *et al.*, 2021), different harvesting times on phytochemical constituents and antioxidant activity (Sehlapelo *et al.*, 2020) and effect of other *J. zeyheri* plant parts (rhizome, stem and leaves) on phytochemical constituents and antioxidant activity (Mamabolo *et al.*, 2020).

However, there is no documented information on the response of phytochemical constituents to different NPK fertilizer rates under greenhouse conditions. Therefore, this study aimed at assessing whether different fertilizer application rates would affect the phytochemical constituents and antioxidant activity of *J. zeyheri* tea leaves under greenhouse conditions.

## MATERIALS AND METHODS

### Treatments and Research Design

The experiment comprised six treatments 0, 2, 4, 8, 16 and 32 g of NPK fertilizer 2:3:2 (22), replicated five times in a randomized complete block design (RCBD).

### Description of the Study Site

A greenhouse experiment was conducted at Aquaculture Research Unit (ARU), University of Limpopo, Limpopo Province, South Africa (23°53'10" S, 29°44'15" E) in the summer of 2021. The ambient day/night temperatures averaged 28/21°C, and the relative humidity was 70%. The soil at the site was predominantly Hutton sandy loam, containing 65% sand, 5% silt, 30% clay and 1.6% organic carbon, with electrical conductivity (EC) 0.149 dS/m and pH ($H_2O$) 6.5. The study was carried out during early-October 2021 to mid-April 2022.

### Procedures

*Jatropha zeyheri* tea seeds were collected in the wild at Khureng Village, Zebediela, Lepelle-Nkumpi Municipality (24°33'53" S, 29°23'4" E) in Limpopo Province of South Africa. A seed viability test was performed before planting. Seedling trays were used, and after emergence to 5 cm, *J. zeyheri* seedlings were hardened off for a week through intermittent withholding of irrigation water outside the greenhouse. *J. zeyheri* seedlings were transplanted at a two-leaf stage into 25 cm diameter plastic pots. Each pot was filled with heated-pasteurised sandy soil and Hygromix at a 3 : 1 (v/v) ratio and placed in a spacing of 0.30 × 0.30 m inter- and intra-row spacing. After seven days, treatments were applied when seedlings adapted to the newly introduced environment. Chlorine-free tap water was used per 25 cm pot with 500 ml. Seven days after transplanting, each plant was fertilized with NPK 2 : 3 : 2 (22) according to their respective treatments and 1 g 2 : 1 : 2 (43) Multifeed (Nulandies, Johannesburg). Pests were scouted and monitored daily, whereas diseases were managed using Malasol and cutworm bait during the seedling stage as per label instructions.

### Data Collection

To successfully determine phytochemical constituents and antioxidant activity, 1 g of ground powdered plant materials were extracted with 10 ml of acetone. The filtrates were filtered into pre-weighed vials, and the solvents were evaporated at room

temperature (25°C). The mass extracted was determined.

**Total antioxidant activity:** The free radical scavenging activity of the plant extracts was quantified using 2, 2-Diphenyl-picrylhydrazyl (DPPH) (Sigma-Aldrich) method reported by Gyamfi *et al.* (1999) with slight modifications. Briefly, different concentrations of the plant extract (250-15.63 µg/ml) were prepared to a volume of 1 ml of the solution. L-Ascorbic acid was used as a standard by preparing the same concentration range as the plant extracts. To these 1 ml solutions, 7 ml of 0.2 mmol/l DPPH solution dissolved in methanol was added and vortexed thoroughly. All the prepared mixtures were incubated in the dark for 30 min. The blank was prepared in the same manner as the experimental solutions. However, 1 ml of acetone was added instead of the plant extracts. The control solution was prepared by adding 2 ml of 0.2 mmol/l DPPH to 1 ml of distilled water. After the elapsed time, the solutions were analyzed with a UV/VIS spectrophotometer (Thermo Scientific). The absorbance of the solutions was read at 517 nm, and the percentage antioxidant potential was calculated using the equation below.

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} \times 10$$

**Determining total phenolic content:** The total amount of phenols in each plant extract was established using the Folin-Ciocalteu method. Extract infusion of 0.1 ml was diluted in distilled water with 0.9 ml, then assorted with 1 ml of Folin-Ciocalteu mixture and shaken well (Wang *et al.*, 2011). After incubation for 5 min, 1 ml of sodium carbonate (7%) was complemented to the mixtures, and the mixtures comprised 25 ml of distilled water. Preparation of the standard followed the use of a sequential water-down of quercetin (0.08 to 1.25 mg/ml) in place of the extract. The mixtures were incubated for 90 min at room temperature in a dark environment. The standard and test absorbance solutions were determined against a blank reagent using a UV/visible spectrophotometer (Beckman Coulter-DU730, California, United States) at 765 nm. The total content of phenols was expressed as mg of GAE/g of the extract (Hlahla *et al.*, 2010).

**Total tannin content:** The Folin-Ciocalteu assay was adopted to establish the total tannin content of the plant extracts. In a volumetric flask (10 ml), a volume of 0.1 ml of the leaf extract was mixed with 7.5 ml of distilled water, into which 0.5 ml of the Folin-Ciocalteu phenol mixture was added. Approximately 1 ml of 35% solution of sodium carbonate was added, and the reagent was diluted with 10 ml of distilled water. The mixture was vortexed well and stored in a dark environment at room temperature for 30 min. The standard absorbance and sample tests were established against the blank mixture at 725 nm using a UV/visible spectrophotometer (Beckman Coulter-DU730, California, United States) (Borokini and Omotayo, 2012).

**Total flavonoid content:** Approximately 1 ml of extract was mixed with 4 ml of distilled water, followed by adding 0.3 ml of 5% sodium nitrite. After incubating for 5 min, 0.3 ml of 10% aluminum chloride was added. This process was followed by the addition of 2 ml of 1 mol sodium hydroxide after incubation for another 5 min. The mixture was added to 10 ml with distilled water and left to stand for 30 min after which the absorbance was recorded at 510 nm. The standard was prepared using a serial dilution of quercetin (0.031 to 0.5 mg/ml) in place of the extract. The flavonoid content was shown as mg of QE/g of extract (Borokini and Omotayo, 2012).

**Data Analysis**

Data were subjected to analysis of variance (ANOVA) using the Statistix 10.0 software. When the treatments were significant at the probability level of 5%, the associated mean sum of squares was partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Fisher's Least Significant Difference Test ($P \leq 0.05$). The variable with significant ($P \leq 0.05$) treatment means were further subjected to lines of the best fit using phytochemicals and antioxidant activity responses to different fertilizer application rates and modelled by the regression curve estimations resulting in a

quadratic equation. $Y = b_2x^2 + b_1x + a$; however, Y = phytoconstituents response and x = fertilizer application rate with $-b_1/2b_2 = x$ value for optimum application rate.

## RESULTS AND DISCUSSION

NPK fertilizer rates had highly significant effects ($P \leq 0.01$) on antioxidant activity (AA) and total flavonoids content (TFC), contributing 46 and 73% in TTV, respectively. In contrast, total phenolic content (TPC) and total tannin content (TTC) were significantly affected ($P \leq 0.05$) by NPK fertilizer rates contributing 71 and 59% in TTV, respectively (Table 1). Relative to untreated control, NPK fertilizer rates improved accumulation of phenols, tannins and flavonoids by 10-141, 16-20 and 14-135%, respectively (Data not shown). NPK fertilizer mixture at high application rates reduced phenols, tannins and flavonoids by 21, 19-61 and 56%, respectively. Phenols, tannins and flavonoids over increasing concentrations of NPK fertilizer exhibited positive quadratic relations, with the phytoconstituents reacting to the dosage indicating to be dependent on the rate applied on *J. zeyheri* plant, consequently affecting the leaves (Fig. 1). The models were explained by 85, 98 and 91% of phenols, tannins and flavonoids, respectively

**Table 1.** Partitioning mean sum of squares for antioxidant activity (AA), total phenolic content (TPC), tannins content (TTC) and total flavonoids content (TFC) to increasing NPK fertilizer rates under greenhouse conditions (n = 30)

| Source | d. f. | AA (µg/ml) | | TPC (mg GAE/g) | | TTC (mg GAE/g) | | TFC (mg QE/g) | |
|---|---|---|---|---|---|---|---|---|---|
| | | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) |
| Replication | 4 | 0.40033 | 43 | 4.4730 | 8 | 0.0203 | 23 | 3.3590 | 19 |
| Treatment | 5 | 0.42044 | 46*** | 39.513 | 71** | 0.0525 | 59** | 12.825 | 73*** |
| Error | 20 | 0.09666 | 11 | 11.592 | 21 | 0.0166 | 18 | 1.413 | 36 |
| Total | 29 | 0.91743 | 100 | 55.578 | 100 | 0.0894 | 100 | 17.597 | 100 |

***Significant at $P \leq 0.05$ and ***Highly significant at $P \leq 0.01$.

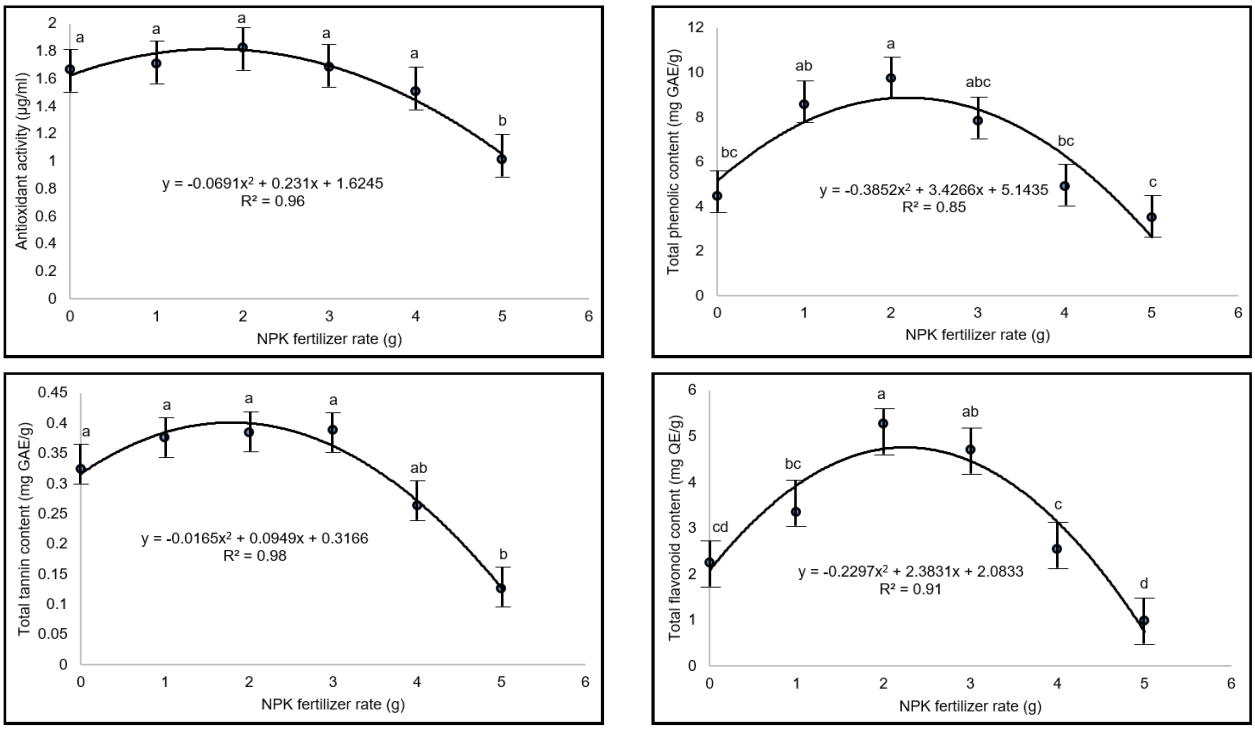


Fig. 1. Response of antioxidant activity, total phenolic content, total tannin content and total flavonoid content of *J. zeyheri* leaves to increasing rates of NPK fertilizer under greenhouse conditions (n=30).

(Table 2). Phytoconstituents over increasing NPK fertilizer rates were displayed by the existence of positive quadratic relations, which enhanced the use of $x = -b_1/2b_2$ for calculated NPK fertilizer application rates of AA, TPC, TTC and TFC, which were 3.34, 4.45, 2.88 and 5.19, respectively (Table 2). The optimum NPK fertilizer application rate was optimized at 4.17 g (Table 2).

In the current study, increasing NPK fertilizer affected the phytochemicals content, which increased with an increase in application rates. Similar observations were made when the addition of fertilizer supplements resulted in significantly increased concentrations of total polyphenols in *Athrixia phylicoides* leaves in all seasons (Mudau *et al.*, 2006). *Moringa oleifera* treated with NPK and compost (50+50%) in combination, depicted higher phenolic and flavonoid content as compared to the control and all other treatments (Sarwar *et al.*, 2020). These findings are in accordance with Ahmed *et al.* (2013), who cited that the application of bio-fertilizer alone or with a combination of nitrogenous fertilizers increased carbohydrate and flavonoid contents in the plant species.

In contrast, Mutua *et al.* (2021) reported that Pepino (*Solanum muricatum*) plants which were not supplied with any fertilizer (control) recorded the highest TPC compared to the other fertilizer rates. The increase in total phenol content was observed highly at low NPK fertilizer rates. The results of the current study are in harmony with the findings of Ibrahim *et al.* (2011), which showed that the accumulation of phenolic content in plant tissues increased under low fertilizer application conditions. Argyropoulou *et al.* (2015) also reported that the synthesis of secondary metabolites was stimulated by nitrogen deficiency, and this enhanced the accumulation of TPC of sweet basil (*Ocimum basilicum* L.). It was reported that normal nitrogen levels promoted the biosynthesis of flavonol glycosides through gene regulation and the accumulation of substrate carbohydrates in *Camellia sinensis*. In contrast, nitrogen deficiency and excess nitrogen had inhibitory effects (Dong *et al.*, 2019).

In the current study, phytochemicals tested reacted to the NPK fertilizer rates indicating to be dosage dependent. Previous studies had analyzed the phenolic content of onions among different cultivars (Metrani *et al.*, 2020); however, information on how fertilizers affect the phenolic content was scarce. The accumulation of polyphenols was the highest under lower fertilizer treatment, although there was no significant difference in the phenolic content between the N1 and N2 treatment groups on *M. oleifera* (Sarwar *et al.*, 2020). Previous studies had evaluated the antioxidant activities of different varieties of Allium species using different extraction methods (Štajner *et al.*, 2008; Chang *et al.*, 2013).

The current study's findings indicated that antioxidants and phytochemicals responded to NPK fertilizers. Inappropriate application of NPK fertilizer could result in a reduction in phytochemical accumulation. In the current study, stimulatory effects were observed when NPK fertilizer was applied at low rates under greenhouse conditions. The optimum NPK fertilizer for enhanced antioxidant activity and phytoconstituents was established at 3.97 g NPK fertilizer mixture/ plant under the greenhouse.

## CONCLUSION

The results from this study showed that antioxidant activity, total phenol, total tannin and flavonoid contents were affected by NPK fertilizer rates. Increasing the NPK fertilizer rate improved the accumulation of

**Table 2.** Quadratic relationship, coefficient of determination and computed optimum of NPK fertilizer rate (g) for antioxidant activity (AA), total phenolic content (TPC), total tannin content (TTC) and total flavonoid content (TFC) under greenhouse conditions (n=30)

| Plant variable | Quadratic equation | $R^2$ | X | P ≤ |
|---|---|---|---|---|
| Antioxidant activity | $Y = -0.0691x^2 + 0.2310x + 1.6245$ | 0.96 | 3.34 | 0.01 |
| Total phenolic content | $Y = -0.3852x^2 + 3.4266x + 5.1435$ | 0.85 | 4.45 | 0.05 |
| Total tannin content | $Y = -0.0165x^2 + 0.0949x + 0.3166$ | 0.98 | 2.88 | 0.05 |
| Total flavonoid content | $Y = -0.2297x^2 + 2.3831x + 2.0833$ | 0.91 | 5.19 | 0.01 |
| | Optimum NPK fertilizer application rate | | 3.97 | |

Calculated optimum NPK fertilizer application rate (x) = $-b_1/2b_2$, where for $b_1 = 0.2310$ and $b_2 = -0.0691$.

phytochemical constituents at low application rates and suggested having stimulatory effects at low amounts. In contrast, inhibitory effects were observed at high NPK fertilizer rates. Antioxidant activity, TPC, TTC and TFC exhibited positive quadratic relations that phytochemical constituents were fertilizer dose-dependent. The findings further suggested that AA, TPC, TTC and TFC of *J. zeyheri* leaves could be stimulated at 3.97 g NPK fertilizer mixture/plant under greenhouse conditions, which was translated to 40 kg NPK mixture/ha.

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