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Research Article

Suitability of Blended Minjingu Fertilisers for Flue-Cured Tobacco Production in Tanzania's Sandy Soils

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Abstract

Objective: To evaluate the suitability of minjingu $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% N fertilizer blends for tobacco yield and quality.

Methods: The experiment was laid out in a randomized complete block design in 3 sites during the 2020-2021 farming season at Mtanila (Chunya), Tumbi (Tabora) and Songambele (Urambo). The experiment was done 3 times and used 5 types of treatment as described below: T1=standard $N_{10}P_{18}K_{24}$+standard CAN27%, T2=minjingu $N_{10}P_{18}K_{24}$+minjingu CAN27%, T3=minjingu $N_{10}P_{18}K_{24}$ standard CAN27%, T4=standard $N_{10}P_{18}K_{24}$ minjingu CAN27% and T5=Absolute control. The plot measured was 6m×6m. Tobacco variety (*Nicotiana tabacum* L. cv. K326) seed sourced from the Tobacco Research Institute of Tanzania was used in the experiment. Seedbeds were first raised in a nursery with a spacing of 1.5×20m before being transplanted in field plots after 60 days. Basal application of $N_{10}P_{18}K_{24}$ fertilizer was done a week after transplanting tobacco seedlings, followed by top-dressing CAN27N% fertilizer 2 weeks after application of $N_{10}P_{18}K_{24}$ fertilizer. All agronomic management procedures were strictly observed.

Results: Across the sites, the tobacco plants fertilized with minjingu basal $N_{10}P_{18}K_{24}$ fertilizer and top-dressed with minjingu CAN27% (T2) yielded 1942.59kg ha^{-1} of dry tobacco leaf, which did not significantly ($P \leq 0.001$) differ with the yield of 2033.64kg ha^{-1} from plots fertilized with the standard $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T1). The tobacco plants fertilized with standard $N_{10}P_{18}K_{24}$ and top dressed with minjingu CAN27% produce higher yields of dry leaf (1738.88kg $ha^{-1)}$. This indicates that minjingu CAN27% fertilizer significantly contributed to the increased yield when applied in combination with minjingu $N_{10}P_{18}K_{24}$.

Conclusion: Minjingu $N_{10}P_{18}K_{24}$ and minjingu CAN27% yielded tobacco plants that were not significantly different to those yielded by application of standard $N_{10}P_{18}K_{24}$ and standard CAN27%. Additionally, the minjingu CAN27% contributed significantly to the dry leaf yield and quality of the tobacco.

Keywords: grade index, nicotine, reducing sugar, NPK basal, CAN top-dressing, soil fertility

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1 INTRODUCTION

The impact of Coronavirus 2019 (COVID-19) has shocked the financial and commodity markets worldwide. The tobacco industry has also been highly affected by COVID-19 due to the reduced volume of production following the lockdown, resulting in sharp increases in prices of fertilizers and agrochemicals in Africa and around the world[1-3]. Fertilizers used in Tanzania's tobacco production are of pure quality and are imported from abroad[4]. Tobacco requires high-quality fertilizer applied at the appropriate time and rate based on the soil's fertility status[5]. Nitrogen (N), phosphorus (P) and potassium (K) are the most important nutrients for tobacco, and the yield is influenced by the N content[6,7]. When the soil is deficient in N, the tobacco yield decreases. When the soil contains too much N, it may reduce the tobacco quality by forming harsh tissue and leaf darkening that make curing difficult[7]. Therefore, balancing the N content is the most important factor for improving tobacco quality and yields.

The impacts of COVID-19 can still be felt to this day as the price of imported tobacco fertilizer is still very high. The use of fertilizer that is cheap, locally available and affordable to small scale tobacco growers may provide a solution to the tobacco industry. There has been no locally produced blend tobacco $N_{10}P_{18}K_{24}$ fertilizer for the past decade. However, currently, Tanzania can purposely utilize the blending of minjingu organic hyper-phosphate (MoHP) rock into $N_{10}P_{18}K_{24}$ fertilizer for flue-cured tobacco production. The typical natural MoHP used is composed of 29-30% P_2O_5, 38-40% CaO, 3.2% MgO and small amounts of several other elements such as Fe, Zn, Mn and Cu[8,9]. Since this natural MoHP is rich in P, the blending to NPK fertilizers used N from NH_4 (7.1% N) and NO_3 (2.9% N), while K (K_2O) was derived from muriate of potash-MOP (25%) and sulphate of potash-SOP (75%)[10]. Based on N's importance in tobacco, a top dressing of minjingu CAN27% N was also blended, containing 14% N from NH_4 and 13% N from NO_3 with an additional 1.7% MgO and 3% CaO, and 3% S[10].

The application of organic fertilizer to tobacco resulted in slightly lower leaf yield than the application of readily inorganic fertilizer[11]. A study by Tabaxi et al.[12] observed that tobacco growth and yield were high when inorganic fertilizer was used compared to organic fertilizer. However, the type of fertilizer used did not significantly affect the nicotine and sugar content of the tobacco leaves. On the other hand, mixing equal proportions of organic and inorganic fertilizers increased the total amount of N absorbed in leaves[13]. The minjingu $N_{10}P_{18}K_{24}$ and CAN27% were also blended using organic and inorganic fertilizers to improve fertilizer quality for tobacco products based on Tanzania tobacco fertilizer specifications[10].

In our understanding, the application of the new blended minjingu $N_{10}P_{18}K_{24}$ and CAN27% fertilizers as per Tanzania tobacco fertilizers specifications[10] to flue-cured tobacco in Tanzania has not been researched. Therefore, the primary objective of the current study was to evaluate the potential suitability of minjingu blended NPK and CAN27% for flue-cured tobacco production in 3 sites namely Tabora, Urambo and Chunya, all located in Tanzania. These findings may help tobacco stakeholders make proper decisions for the required cheap and high-quality tobacco fertilizer in Tanzania.

2 MATERIALS AND METHODS

2.1 Site Description and Experimentations

Field experiments were conducted in 3 sites during the 2020-2021 farming season. The first site was Mtanila-Chunya, located 1439m above sea level (a.s.l), with an average temperature of 22.75°C and 995mm of rainfall. The second site was Tumbi-Tabora, located 1151m a.s.l, with an average temperature of 27°C and 950mm of rainfall. The third and final site was Songambele-Urambo, located 1135m a.s.l, with an average temperature of 27.4°C and 1004mm of rainfall.

A flue-cured tobacco variety (*Nicotiana tabacum* L. cv. K326) sourced from the Tobacco Research Institute of Tanzania was used. *Nicotiana tabacum* L. cv. K326 seed was sown in two seedbeds at Mtanila-Chunya and Tumbi-Tabora on the 15th and 16th September 2020 respectively. For each site, one seedbed with a size of 1.5×20m was fertilized with 5kg of standard $N_{10}P_{18}K_{24}$ fertilizer, and the second seedbed was fertilized with 5kg of minjingu $N_{10}P_{18}K_{24}$ fertilizer. Tobacco seedlings were hardened off 2 weeks before transplanting in fields.

The experimental field layout used across the sites was a Randomized Complete Block Design (RCBD) with 5 treatments replicated 3 times. The treatments were T1 (standard $N_{10}P_{18}K_{24}$+standard CAN27%), T2 (minjingu $N_{10}P_{18}K_{24}$+minjingu CAN27%), T3 (minjingu $N_{10}P_{18}K_{24}$+standard CAN27%), T4 (standard $N_{10}P_{18}K_{24}$+minjingu CAN27%) and T5 (Absolute control).

Mature tobacco seedlings were transplanted in experimental sites 60 days after sowing on the 15th, 18th and 22nd November 2020 at Tumbi-Tabora, Songambele-Urambo and Mtanila-Chunya respectively, at a spacing of 1.2m between ridges and 0.50m between plants. Basal application of 30g $plant^{-1}$ for $N_{10}P_{18}K_{24}$ fertilizer (50kg N, 90kg P, and 120kg K ha^{-1}) was done on 22nd November, 24th November and 14th December 2020 (7 days after transplanting) at Tumbi-Tabora, Songambele-Urambo and Mtanila-Chunya respectively, following sufficient moisture levels in soils. Top-dressing of 8g plant^{-1} CAN27% fertilizer (33.75kg N ha^{-1}) was done on 01st December, 08th December and 29th December 2020 (21 days after transplanting) at Songambele-Urambo, Tumbi-Tabora and Mtanila-Chunya respectively.

2.2 Physical and Chemical Properties of the Soils from Experimental Sites

12 soil samples were collected in a zigzag fashion from 12 random points in each of the 3 locations at a depth of 0-30cm before experimentation. Collected samples were thoroughly mixed to make a composite soil sample from each experimental site. The composite soil samples for each site were air-dried and ground to pass through a 2mm sieve for laboratory analysis. Boron (B) was determined by the extractable water method as described by Moberg[14]. Exchangeable Ca, K, Mg and Mn were determined through Atomic Adsorption Spectrophotometer (AAS)[14]. The bray-1 method was used to determine the available P, while the total N content was determined using the Kjeldahl method[14].

2.3 Leaf Harvesting, Determination of Nutrients, Reducing Sugars and Nicotine

A mature middle leaf was sampled from 3 inner row plants at each experimental site, making a total of 9 plants per plot. The length and width of the leaf samples were measured using a tape, and the area of the leaf was determined by multiplying the length, width, and a correction coefficient factor of 0.64[15]. The leaf samples were dried in an oven at 65°C. Dried leaf samples were chopped, ground and sieved through a 0.5mm wire mesh and then analyzed for N, P, K, calcium (Ca) and boron (B) by dry ash and wet digestion method[14]. For dry ashing, 0.5g of the sieved leaf was weighed in crucibles and placed in a muffle furnace for further heating at 600°C for 3 hours. The ash was dissolved through a 6 N HCL and distilled water, each with 10mL. The filtrate through a Whatman filter paper number 42 was used to determine Ca using Atomic Adsorption Spectrophotometer (AAS) and K using a flame photometer. Total N was determined by the Kjeldahl method, while P was determined by the ascorbic acid molybdate blue method. Boron was determined using the diethylene triamine pentaacetic acid extraction (DTPA) method. Reducing sugars were determined by automatic titrator using the method described by Fernández-Novales et al[16]. Nicotine was determined using spectrophotometric analysis[17].

The leaves per plot were harvested weekly following ripening of leaves. The green leaf weight was measured using a digital balance scale and placed in curing barns for 7 days. Cured leaves were weighed using a digital scale to obtain the dry leaf weight.

2.4 Statistical Analyses

The data was analyzed using the Statistica 8.0 software package version 7. Green and dry leaf weight were evaluated based on the interactions among the sites, fertilizer regimen and each factor individually. The two-way ANOVA statistical analyses were performed through RCBD with treatments being sites and fertilizer treatments. A post-hoc Tukey's-HSD multiple comparison tests was used. The significance threshold was set at P=0.05 and P=0.001 for high significance. The treatment means were compared by the standard error of the difference of the mean.

3 RESULTS

3.1 Physical and Chemical Properties of the Experimental Soils

The physical and chemical properties of soils at Chunya, Tabora and Urambo sites (Table 1)[18]. The soil pH was strongly acidic for Tabora soils (5.25) and medium acidic in Chunya (5.79) and Urambo (5.76). The soil was categorized as sandy for Tabora and Chunya while it was loamy sandy for Urambo. Urambo had organic carbon (OC) of 0.79%, Chunya 0.82% and Tabora had the lowest OC at 0.25%. Available Sulphur (S), 8.11-8.33mg kg^{-1}, potassium (K), 0.25-0.34cmol (+) kg^{-1} and phosphorous (P), 44.87-55.23mg kg^{-1} levels were ranked as medium, while calcium (Ca) in the range of 0.14-0.51cmol (+) kg^{-1} and total nitrogen (N) in the range of 0.02-0.04% were low. Soil exchangeable magnesium (Mg) across the site was very low (0.22-0.26cmol (+) kg^{-1}) in Tabora and Urambo and low 0.39cmol (+) kg^{-1}) in Chunya. Extractable boron (B) was low (0.31-0.42mg kg^{-1}) in all soils, while exchangeable Mn (>1.0mg kg^{-1}) was high across all the sites.

3.2 Effects of Fertilizer Treatments on Leaf Area, Green and Dry Tobacco Leaf

The data on the effects of fertilizer treatments on leaf area, green and dry tobacco leaf yields are shown in Table 2. The Urambo site had significantly (P≤0.001) higher leaf area (790.72cm^2), green (19079.00kg ha^{-1}) and dry tobacco leaf yields (1764.07kg ha^{-1}) than Chunya and Tabora sites. There was no significant difference in leaf area, green and dry tobacco leaf yields between Chunya and Tabora.

The tobacco plants fertilized with standard $N_{10}P_{18}K_{24}$ top-dressed with standard CAN27% fertilizer (T1), minjingu $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% fertilizer (T2), and standard $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% fertilizer (T4) had significantly (P≤0.001) higher green leaf yields (20402.90, 19791.32 and 19443.23kg ha^{-1} respectively) than tobacco plants fertilized with minjingu $N_{10}P_{18}K_{24}$ top-dressed with standard CAN27% fertilizer (T3), which had a yield of 1779.85kg ha^{-1}. The lowest green leaf yield was recorded in unfertilized tobacco plants (absolute control T5), which had 7731.19kg ha^{-1} (Table 2).

Table 1. Some Physical and Chemical Characteristics of Experimental Soils

Soil Constituents	Sites		
	Tabora	Chunya	Urambo
pH	5.25	5.76	5.79
Sand	88.39	85.71	84.95
Silt	4.59	4.80	2.91
Clay	6.95	9.25	11.03
Texture Class	Sandy	Sandy	Sandy loam
Organic carbon (OC) %	0.25	0.82	0.79
Nitrogen (N) %	0.02	0.03	0.04
Phosphorous (P) mg kg^{-1}	53.71	55.23	44.87
Sulphur (S) mg kg^{-1}	8.11	8.33	8.12
Potassium (K) cmol (+) kg^{-1}	0.27	0.34	0.25
Calcium (Ca) cmol (+) kg^{-1}	0.14	0.51	0.40
Magnesium (Mg) cmol (+) kg^{-1}	0.22	0.39	0.26
Sodium (Na) cmol (+) kg^{-1}	0.24	0.96	0.01
Boron (B) mg kg^{-1}	0.31	0.40	0.34
Manganese (Mn) mg kg^{-1}	7.30	4.59	13.32

Table 2. Green, Dry Leaf Yields and Leaf Area of Tobacco Produced Using Standard and Minjingu Fertilizers

Sites	Green Leaf Yield (kg ha^{-1})	Dry Leaf Yield (kg ha^{-1})	Leaf Area (cm^2)
Tabora	15820.13±1328.38 b	1412.96±157.38 b	717.64±54.93 b
Chunya	16198.35±1483.89 b	1448.52±139.79 b	731.79±57.32 b
Urambo	19079.00±1342.65 a	1764.07±162.47 a	790.72±46.41 a
Treatments			
T1 - Standard NPK+Standard CAN27%	20402.90±929.58 a	2033.64±64.49 a	834.38±21.68 a
T2 - Minjingu NPK+Minjingu CAN27%	19791.32±1013.88 ab	1942.59±82.16 a	825.75±22.61 a
T3 - Minjingu NPK+Standard CAN27%	17793.85±783.33 b	1515.43±66.77 c	873.85±31.33 a
T4 - Standard NPK+Minjingu CAN27%	19443.23±1003.13 ab	1738.88±80.34 b	823.11±34.24 a
T5 - Absolute Control	7731.19±808.79 c	478.70±58.63 d	376.51±23.10 b
2 WAY ANOVA			
Site (S)	10.01***	31.89***	3.67*
Treatment (T)	52.86***	201.33***	63.45***
Interaction (S×T)	1.62ns	1.22ns	0.72ns

Notes: The same category of evaluated interface sharing similar letter(s) including a, b, and ab do not differ significantly based on their respective standard error (SE) at 5% error rate. Values presented are means±SE $\bar{x}$ (Standard error of means); *P<0.05, ***P<0.001; ns=non-significant (P≥0.05).

Significantly higher dry leaf yields were recorded in plants fertilized with standard $N_{10}P_{18}K_{24}$ top-dressed with standard CAN27% fertilizer (T1), minjingu $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% fertilizer (T2) (2033.64 and 1942.59kg ha^{-1} respectively) (Table 2). The tobacco plants fertilized with standard $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% fertilizer (T4) were next in line, with a yield of 1738.88kg ha^{-1}, followed by those fertilized with minjingu $N_{10}P_{18}K_{24}$ top-dressed with standard CAN27% fertilizer (T3) which attained a yield of 1515.43kg ha^{-1} dry leaf. The absolute control (T5) plants had the lowest dry leaf yield at 478.70kg ha^{-1}.

The lowest leaf area was recorded in the absolute control (T5), which had a leaf area of 376.51cm^2. The rest of the fertilizer treatments did not cause significant differences in leaf area. There was no significant interaction between sites and treatments for the leaf area, green leaf and dry leaf yields (Table 2).

3.3 Effects of Fertilizer Treatments on Concentrations of nutrients in leaves

The nutrient concentrations of tobacco leaves produced in different fertilizer treatments are presented in Table 3. There was no significant difference in the B and P content of leaves across the sites, while there was a significant difference in the N and K content of leaves from each site (*P*≤0.001). The Urambo site had an N content of 2.12% and K content of 1.92%. Furthermore, Urambo (0.34%) and Chunya (0.30%) sites had significantly (*P*≤0.001) higher leaf Ca concentration than Tabora (0.12%).

Significantly higher concentrations B were observed in leaves of the tobacco plants fertilized with the standard $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% fertilizer (T1) (Table 3). Next in line were leaves from tobacco plants fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the minjingu CAN27% fertilizer (T2), followed by leaves from tobacco plants fertilized with minjingu $N_{10}P_{18}K_{24}$ top-dressed with standard CAN27% fertilizer (T3), and then leaves from tobacco plants fertilized with the standard $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% (T4). Leaves from unfertilized tobacco plants (absolute control, T5) had the lowest B concentration at 13.73mg kg^{-1} (Table 3). The site and type of treatment had a significant impact on the concentration of B in the leaf (Figure 1A). The plants treated with minjingu $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% fertilizer (T2) had the highest B content, but this did not differ significantly with those treated with the standard $N_{10}P_{18}K_{24}$ top-dressed with the standard CAN27% fertilizer (T1) across the sites (Figure 1A).

Across the fertilizer treatments, the concentrations of Ca and P in the tobacco leaves did not differ significantly. The absolute control (T5) plants had the lowest N concentration (1.57%) than the other plants (Table 3). However, the site had a significant impact on the leaf concentrations of Ca and N. The leaf Ca concentration was significantly higher for Urambo and Chunya sites and lowest in Tabora, while leaf N concentrations were significantly higher for Chunya and Urambo sites and lowest in Tabora (Figure 1B).

Tobacco plants fertilized with the standard $N_{10}P_{18}K_{24}$ top-dressed with the standard CAN27% fertilizer (T1), minjingu $N_{10}P_{18}K_{24}$ top-dressed with the minjingu CAN27% fertilizer (T2) had significantly higher leaf K concentrations (1.83 and 1.80% respectively) than the other plants (Table 3). There site and type of treatment had a significant impact on leaf K concentration. Tobacco plants in Urambo site fertilized with the standard $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% fertilizer treatment (T1) had the highest leaf K concentrations (2.73%), followed by tobacco plants in Chunya site fertilized with the minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the minjingu CAN27% fertilizer (T2), which had leaf K concentrations of 2.05% (Figure 1B).

3.4 Effects of Fertilizer Treatments on Tobacco Leaf Reducing Sugars, Nicotine and Grade Index

The effects of fertilizer treatments on the content of reducing sugars, nicotine and overall grade index of the tobacco leaves are presented in Table 4. The reducing sugar content was significantly (*P*≤0.001) higher in tobacco plants at the Tabora site (21.65%) than those at the Chunya (17.73%) and Urambo (16.75%) sites. However, tobacco leaves grown in Urambo (1.00) and Chunya (0.94) sites had a significantly (*P*≤0.001) higher tobacco leaf grade index than those grown in the Tabora site (0.70). The leaf nicotine content across the sites did not differ significantly.

Unfertilized tobacco plants (absolute control, T5) had significantly (*P*≤0.001) higher contents of reducing sugar (23.43%) than the rest of the tobacco plants, all of which did not have significant differences in reducing sugar contents among them (Table 4). Therefore, there were no significant interaction effects between sites and treatments. Leaf nicotine content and grade index for tobacco plants fertilized using standard $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T1) were significantly (*P*≤0.001) higher than the rest of the tobacco plants. However, the unfertilized tobacco plants (T5) recorded the lowest nicotine content and grade index. The sites and type of treatment had a significant on the leaf nicotine content (Figure 2). Tobacco plants in the Tabora site had the highest leaf nicotine content reaching 3.65% for plants that were fertilized with standard $N_{10}P_{18}K_{24}$ and was top-dressed with the standard CAN27%N fertilizer (T1), followed by Urambo site for the same treatment (3.19%), with no significant difference between them. A similar trend was observed for both treatments at both sites, with nicotine leaf contents of 2.94% and 2.72% respectively. Overall, by considering all treatments, the Urambo site had the highest nicotine content in tobacco leaves, followed by the Chunya site (Figure 2).

3.5 Soil pH and Residual Nutrient Concentrations after Reaping Tobacco Leaves

Results of the soil pH and residual nutrient concentrations after reaping tobacco leaves are presented in Table 5. The soil pH differed significantly (*P*≤0.001) across the sites. Tabora had strongly acidic soils with a pH of 5.01, followed by Urambo (pH 5.13) and Chunya (pH 5.15). Residual soil B, Ca, and P differed significantly (*P*≤0.001) across the sites and except soil P, which was higher for the Tabora site (42.85mg kg^{-1}), the Chunya site had significantly (*P*≤0.001) higher residual soil B (0.34mg kg^{-1}) and residual soil Ca (1.61cmol (+) kg^{-1}). Urambo was the next with residual soil B of 0.32mg kg^{-1} and residual soil Ca of 1.21cmol (+) kg^{-1}, but it had significantly (*P*≤0.001) higher residual soil K (0.17cmol (+) kg^{-1}). Residual soil total N was higher in Chunya site (0.06%), but did not differ significantly with Tabora soils (0.05%). The lowest residual soil total N was recorded in Urambo (0.04%).

Comparing the fertilizer treatments, the soil that was fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with minjingu CAN27% (T2) had a significantly (*P*≤0.001) higher pH (5.51), followed by unfertilized soil (T5) with

Table 3. Effect of Fertilizer Treatments on Nutrient Tobacco Leaf Concentrations Produced from Tabora, Chunya and Urambo Districts

Sites	Leaf B (mg kg^{-1})	Leaf Ca (%)	Leaf N (%)	Leaf P (%)	Leaf K (%)
Tabora	16.41±0.37 a	0.12±0.01 b	1.49±0.07 c	0.18±0.01 a	1.54±0.10 b
Chunya	16.44±0.48 a	0.30±0.03 a	1.77±0.08 b	0.20±0.02 a	1.92±0.12 a
Urambo	16.29±0.30 a	0.34±0.03 a	2.12±0.04 a	0.21±0.02 a	1.28±0.08 c
Treatments					
T1 - Standard NPK+Standard CAN27%	16.98±0.15 ab	0.28±0.05 a	1.81±0.13 a	0.22±0.04 a	1.83±0.23 a
T2 - Minjingu NPK+Minjingu CAN27%	17.35±0.21 a	0.27±0.04 a	1.86±0.05 a	0.20±0.03 a	1.80±0.10 a
T3 - Minjingu NPK+Standard CAN27%	17.16±0.18 ab	0.26±0.05 a	1.89±0.12 a	0.19±0.02 a	1.46±0.03 b
T4 - Standard NPK+Minjingu CAN27%	16.68±0.20 b	0.23±0.04 a	1.82±0.11 a	0.19±0.02 a	1.46±0.17 b
T5 - Absolute Control	13.73±0.26 c	0.24±0.05 a	1.57±0.15 b	0.18±0.02 a	1.34±0.13 c
2 WAY ANOVA					
Site (S)	0.33ns	28.21***	154.60***	0.64ns	216.06***
Treatment (T)	66.52***	0.66ns	14.61***	0.19ns	62.10***
Interaction (S×T)	2.35*	3.35**	21.58***	1.36ns	80.29***

Notes: The same category of evaluated interface sharing similar letter(s) including a, b, c and ab do not differ significantly based on their respective Standard error (SE) at 5% error rate. Values presented are means ±SE $\bar{x}$ (Standard error of means); *P<0.05, **P<0.01, ***P<0.001; ns=non-significant (P≥0.05).

a pH of 5.03, and then the soil fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T3) with a pH of 5.02. There was no significant difference in the soil pH of the soil that was fertilized using T3 and that which was fertilized using T4 (Table 5). The site and type of treatment had a significant impact on soil pH (Figure 3). After reaping tobacco leaves across the sites, soil pH reduced slightly in comparison to before tobacco cultivation (Table 5, Figure 3). However, the T2 treatment (minjingu $N_{10}P_{18}K_{24}$ top-dressed with the minjingu CAN27% fertilizer) slightly increased the soil pH in Chunya (5.73), Urambo (5.69) and Tabora (5.11). The T4 treatment (standard $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27%) and T3 (minjingu $N_{10}P_{18}K_{24}$) top-dressed with the standard CAN27%) also caused minor increases in soil pH across the sites (Figure 3).

Soil in plots fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the minjingu CAN27% (T2) and plots fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T3) had significantly (P≤0.001) higher residual soil B (0.32mg kg^{-1}), followed by soil in plots fertilized with the standard $N_{10}P_{18}K_{24}$ and top-dressed with minjingu CAN27% (T4) which had residual soil B of 0.30mg kg^{-1}, but did not differ significantly with soil in plots fertilized with T2 and T3 (Table 5). The lowest residual soil B was recorded in soil fertilized with standard $N_{10}P_{18}K_{24}$ top-dressed with the standard CAN27% (T1) (0.29mg kg^{-1}) and unfertilized soil (T5) (0.28mg kg^{-1}). The site and type of treatment did not have a significant impact on soil B.

Plots fertilized with the minjingu $N_{10}P_{18}K_{24}$ and top-dressed with minjingu CAN27% (T2) had significantly (P≤0.001) higher residual soil exchangeable Ca (1.44cmol (+) kg^{-1}) than the other plots (Table 5). The site and type of treatment had a significant impact on exchangeable residual Ca (Figure 4A). The T2 treatment was associated with a significant increase in residual exchangeable Ca reaching 1.69, 1.42 and 1.23cmol (+) kg^{-1} for Chunya, Tabora and Urambo sites (Figure 4A). The residual soil total N did not differ significantly across the treatment plots. However, the site and type of treatment had a significant impact on exchangeable residual total N (Figure 4B). Apart from Tabora site for the plot fertilized with the standard $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T1), the rest of the treatment plots showed an increase in residual N. Unfertilized plots (T5) had a significant (P≤0.001) increase in residual soil N (0.09%).

Residual soil P was significantly (P≤0.001) higher (40.33mg kg^{-1}) in tobacco plots fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T3) than the rest of the plots (Table 5). The following plot for the higher residual soil P (32.88mg kg^{-1}) Next in line was the plot fertilized with standard $N_{10}P_{18}K_{24}$ and top-dressed with the minjingu CAN27% (T4) (residual soil P 32.88mg kg^{-1}). The plot fertilized with the standard $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T1) had a residual soil P of 31.22mg kg^{-1}, and the plot fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the minjingu CAN27% (T2) had a residual soil P of 30.04mg kg^{-1}. The lowest residual soil P was seen in the unfertilized plot (T5) at 29.17mg kg^{-1}. Interaction of treatments and sites showed that the Tabora site had a higher soil residual P than Urambo and Chunya. Excitingly, the plot fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T3) in Tabora

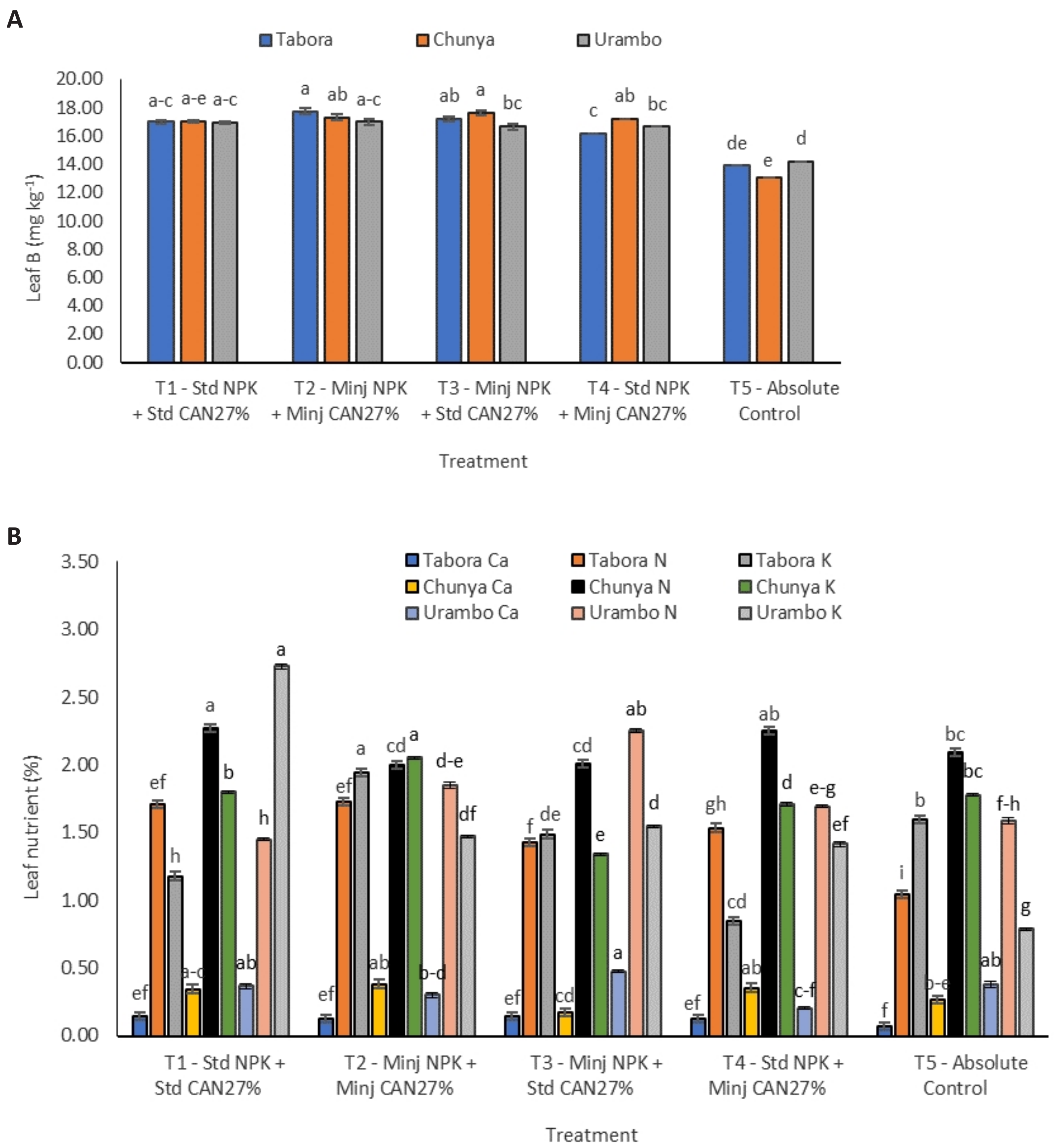

Figure 1. Effect of different fertilizer application on tobacco leaf B, Ca, N and K and concentrations. A: Effect of different fertilizer application on tobacco leaf B concentrations; B: Effect of different fertilizer application on tobacco leaf Ca, N and K concentrations. Note: Letters on top of each column sharing similar letter(s) mean that there is no significant difference.

site had significantly ($P \leq 0.001$) higher residual soil P at 67.45mg kg^{-1}, followed by the plot fertilized with standard $N_{10}P_{18}K_{24}$ and top-dressed with the minjingu CAN27% with 44.32mg kg^{-1} in the same site (Figure 4C). The residual soil K in the plot fertilized with the standard $N_{10}P_{18}K_{24}$ and top-dressed with the standard CAN27% (T1) was significantly ($P \leq 0.001$) higher (0.18cmol (+) kg^{-1}) than the rest of the plots, which did not differ significantly among them. The site and type of treatment did not have a significant effect on residual soil K.

4 DISCUSSION

The micronutrient B is essential for the tobacco plant, influencing protein metabolism and alkaloid production of tobacco. Results for the nutrient analysis before the experiment indicated that the soil had low amounts of extractable soil B and high amounts of Mn across the sites (Table 1). The total N content and exchangeable Ca of the soil were low across the sites, while exchangeable Mg was very low in the Tabora and Urambo sites and low in the Chunya site. The soil S, K and P contents were medium across the site. The applied fertilizer treatments for basal NPK application, which had N, P and K, and trace contents of B, Ca and Mg improved the levels of nutrients in the soil and tobacco growth yields. Application of top-dressing fertilizer CAN27% improved the levels of Ca and N in soils. Another study also observed a significant increase of Ca with or without application of CAN27% top-dressed fertilizer[19]. The soil at the Tabora site was strongly acidic (pH 5.25), while that at Chunya and Urambo was moderately acidic (pH 5.76 and 5.79 respectively) (Table 1). In addition, the Tabora site had the lowest OC (0.25%) in comparison to the Chunya (0.82%) and Urambo (0.79%) sites, indicating that the soils at Chunya and Urambo could

Table 4. Effects of Fertilizer Treatments on Tobacco Leaf Reducing Sugar, Nicotine Content and Grade Index

Sites	Reducing Sugar (%)	Nicotine (%)	Grade Index (GI)
Tabora	21.65±1.37 a	2.20±0.27 a	0.70±0.10 b
Chunya	17.73±0.83 b	2.34±0.15 a	0.94±0.14 a
Urambo	16.75±1.01 b	2.45±0.22 a	1.00±0.14 a
Treatments			
T1 - Standard NPK+Standard CAN27%	16.79±0.87 b	3.26±0.14 a	1.42±0.11 a
T2 - Minjingu NPK+Minjingu CAN27%	16.80±0.70 b	2.77±0.11 b	1.04±0.08 b
T3 - Minjingu NPK+Standard CAN27%	17.75±1.59 b	1.92±0.21 c	0.84±0.10 b
T4 - Standard NPK+Minjingu CAN27%	18.80±1.53 b	2.36±0.26 b	1.02±0.09 b
T5 - Absolute Control	23.43±1.83 a	1.33±0.15 d	0.10±0.02 c
2 WAY ANOVA			
Site (S)	8.50***	1.16ns	6.60**
Treatment (T)	5.79***	23.71***	36.40***
Interaction (S×T)	1.38ns	3.36***	0.81ns

Notes: The same category of evaluated interface sharing similar letter(s) including a, b, and c mean that there is no significant difference based on their respective Standard error (SE) at 5% error rate. Values presented are means ±SE $\bar{x}$ (standard error of means); ***P*<0.01, ****P*<0.001; ns=non-significant (*P*≥0.05).

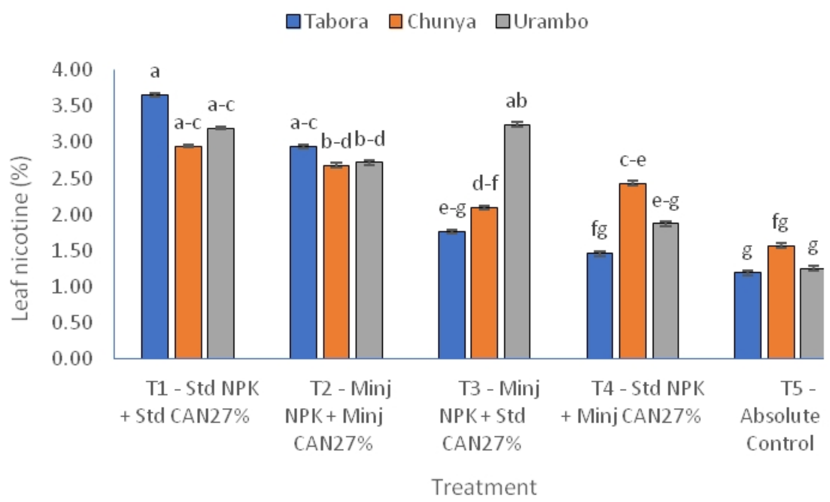

Figure 2. Effect of different fertilizer application on tobacco leaf nicotine content. Note: Letters on top of each column sharing similar letter(s) indicate that there is no significant difference.

have higher contents of organic matter with the capacity to retain more moisture and nutrients than the soil at Tabora. As a result, significant (P≤0.001) green leaf yields (19079kg ha^{-1}), dry leaf yields (1764.07kg ha^{-1}) and leaf area (790.72cm^2) were obtained from the Urambo site, followed by Chunya, which had a leaf area of 731.79cm^2, green leaf yield of 16198.35kg ha^{-1} and dry leaf yield of 1448.52kg ha^{-1} (Table 2).

Tobacco plants planted in soil fertilized with standard $N_{10}P_{18}K_{24}$ top-dressed with the standard CAN27% fertilizer (T1) and minjingu $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% fertilizer (T2) had significantly (P≤0.001) higher dry leaf yields of 2033.64 and 1942.59kg ha^{-1} respectively. These plants also had the largest leaf area. However, they did not differ significantly from the other treatments. The results further indicated that minjingu CAN27% fertilizer (T4) is the best top-dressing fertilizer as it produced a higher dry leaf yield of 1738.88kg ha^{-1} after T1 and T2 (Table 2). The higher dry leaf yields for T1, T2, and T4 were due to the release of nutrients to the soil and the smooth uptake of adequate nutrients from the soil to the tobacco leaf (Table 3). All the leaf nutrients that were measured (N, P, K, Ca and B) had sufficient concentrations based on the description given by Bryson and Mills[20]. The interaction between sites and treatments showed that tobacco plants fertilized with minjingu $N_{10}P_{18}K_{24}$ (T2) had higher amount of B in the tobacco leaf across the sites but did not differ significantly with tobacco plants fertilized with standard $N_{10}P_{18}K_{24}$ (T1). The leaf B for T4 also did not differ enormously from the leaf B for T1 at Chunya and Urambo sites (Figure 1A). Except for the leaf K for T4, the

Table 5. Residual Soil pH and Nutrients after Harvesting Tobacco Leaf Applied with Different Fertilizer Applications

Sites	Soil pH	Soil B (mg kg^{-1})	Soil Ca (cmol (+) kg^{-1})	Soil Total N (%)	Soil P (mg kg^{-1})	Soil K (cmol (+) kg^{-1})
Tabora	5.01±0.05 c	0.26±0.00 c	1.17±0.04 c	0.05±0.01 ab	42.85±3.62 a	0.14±0.01 b
Chunya	5.15±0.09 a	0.34±0.01 a	1.61±0.01 a	0.06±0.00 a	24.98±1.08 c	0.13±0.01 b
Urambo	5.13±0.09 b	0.32±0.00 b	1.21±0.00 b	0.04±0.00 b	30.35±0.92 b	0.17±0.01 a
Treatments						
T1 - Standard NPK+Standard CAN27%	5.00±0.06 c	0.29±0.01 b	1.32±0.06 b	0.04±0.01 a	31.22±1.95 c	0.18±0.01 a
T2 - Minjingu NPK+Minjingu CAN27%	5.51±0.10 a	0.32±0.01 a	1.44±0.06 a	0.05±0.01 a	30.04±2.95 d	0.14±0.01 b
T3 - Minjingu NPK+Standard CAN27%	5.02±0.11 b	0.32±0.01 a	1.30±0.09 cd	0.05±0.01 a	40.33±6.81 a	0.14±0.01 b
T4 - Standard NPK+Minjingu CAN27%	5.03±0.05 b	0.30±0.01 ab	1.31±0.06 bc	0.06±0.01 a	32.88±3.06 b	0.15±0.01 b
T5 - Absolute Control	4.91±0.07 d	0.28±0.01 b	1.28±0.08 d	0.06±0.01 a	29.17±0.90 e	0.13±0.01 b
2 WAY ANOVA						
Site (S)	206***	54.95***	2196.2***	2.40ns	3151844***	12.60***
Treatment (T)	1265***	4.33**	94.1***	1.05ns	450097***	7.82***
Interaction (S×T)	711***	0.58ns	63.1***	2.21*	766934***	1.6ns

Notes: The same category of evaluated interface sharing similar letter(s) including a, b, c, d, e, ab, bc and cd indicate that there is no significant difference based on their respective Standard error (SE) at 5% error rate. Values presented are means ±SE $\bar{x}$ (Standard error of means); *P<0.05, **P<0.01, ***P<0.001; ns=non-significant (P≥0.05).

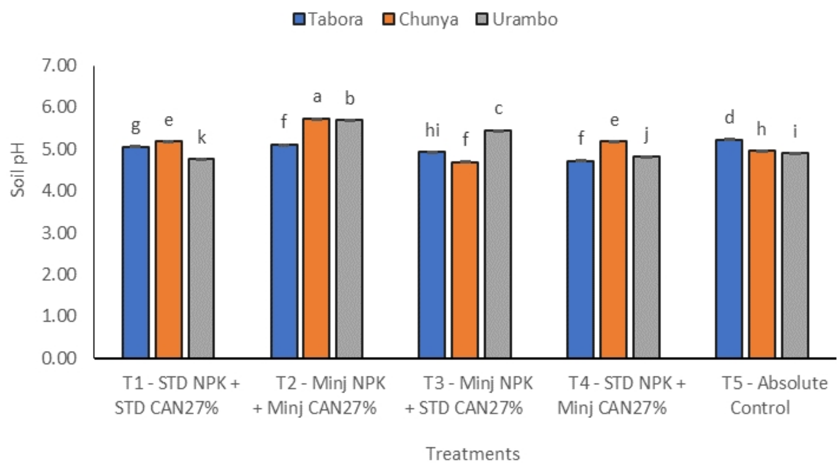

Figure 3. Effect of different fertilizer application on soil pH after reaping tobacco leaves. Notes: Letters on top of each column sharing similar letter(s) indicate that there is no significant difference.

leaf Ca, N, P, and K were significantly (P≤0.001) higher in T1, T2 and T4. However, it did not differ significantly from the rest of the treatments. The leaf Ca and N were recorded highest for T1, T2 and T4 treatments at all 3 sites. However, Ca was slightly low for T4 in Chunya (Figure 1B). The higher dry leaf yield at the Urambo site and the highest leaf K (2.73%) concentrations were a result of the application of the standard $N_{10}P_{18}K_{24}$ fertilizer (T1). Next in line was the Chunya site, which had a leaf K concentration of 2.05% in tobacco plants fertilized with minjingu $N_{10}P_{18}K_{24}$ (T2) (Figure 1B).

Higher leaf B and K were measured in Urambo and Chunya for tobacco leaves fertilized with standard $N_{10}P_{18}K_{24}$ and top-dressed with standard CAN27% (T1), minjingu $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% (T2) and standard $N_{10}P_{18}K_{24}$ top-dressed with minjingu CAN27% (T4). This was strongly associated to reducing sugar and nicotine content (Table 4, Figure 2). Thus, tobacco plants produced in Urambo and Chunya had significant (P≤0.001) dry leaf yields (Table 2) and grade index (Table 4) than tobacco leaf produced from the Tabora site. The low leaf K (1.28%) recorded in Urambo was due to the accumulation of dry leaf matter that reduces K concentration due to a dilution effect[21]. Several studies have suggested that there exists an association between Ca, N and K content and improved production and quality of the tobacco leaf flavor, color, texture, sugar and nicotine contents[5,22-27].

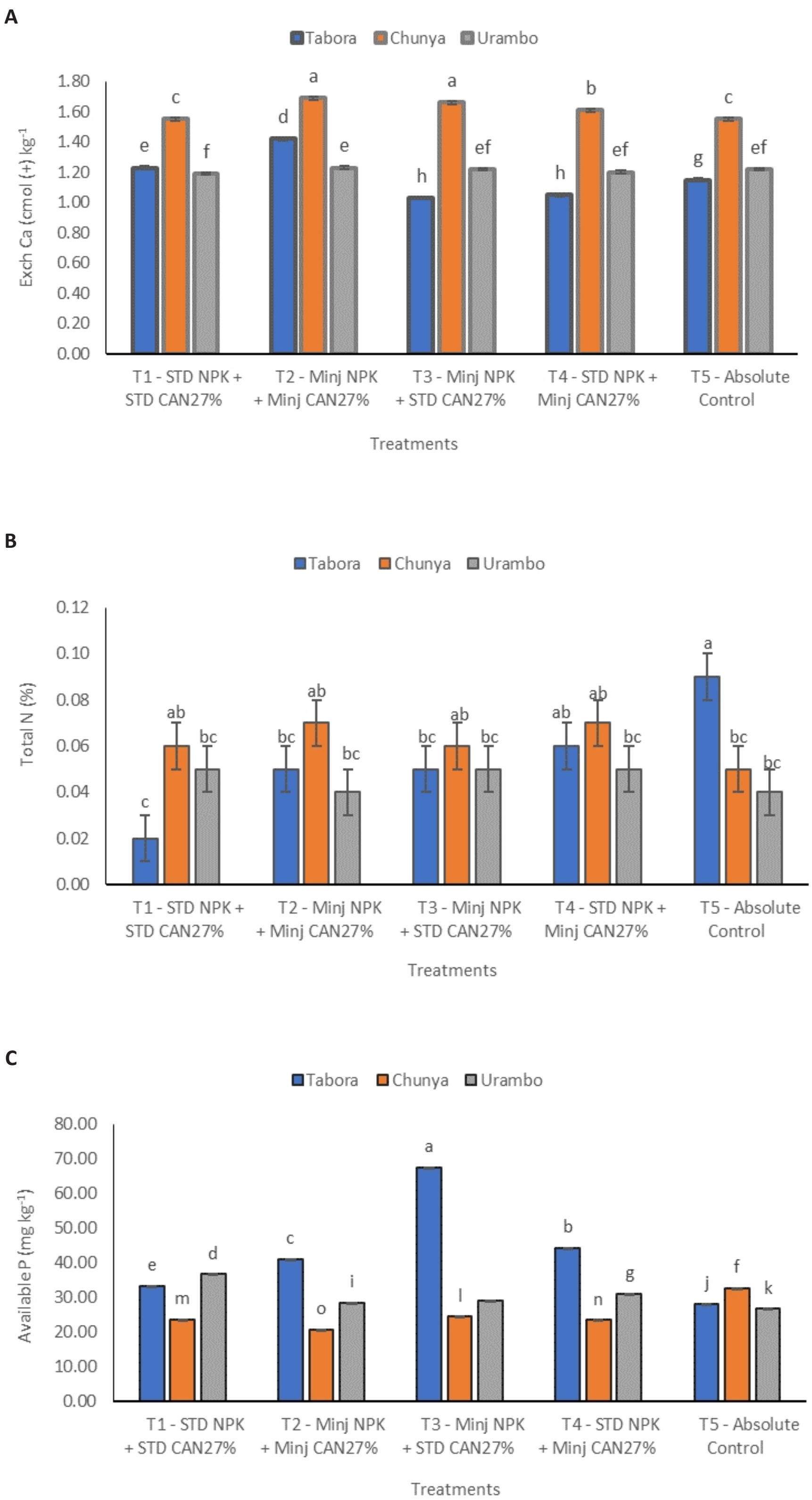

Figure 4. Effect of different fertilizer application on residual soil exchangeable Ca, residual soil total N, and residual soil available P. A: Effect of different fertilizer application on residual soil exchangeable Ca; B: Effect of different fertilizer applications on residual soil total N; C: Effect of different fertilizer application on residual soil available P. Notes: Letters on top of each column sharing similar letter(s) indicate that there is no significant difference.

Tobacco planted either with or without fertilizer application plots was noted to have lowered the soil pH (Table 5, Figure 3). The lower soil pH could result from the H^+ generated by nitrification and the acidification caused by nicotine released by the tobacco roots[19]. However, tobacco plants fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with minjingu CAN27% (T2) were associated with an increase in soil pH (5.51). Tobacco plants fertilized with standard $N_{10}P_{18}K_{24}$ and top-dressed with minjingu CAN27% (T4) also reduced the soil pH to 5.03, and did not differ significantly ($P \le 0.001$) with plants fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with standard CAN27% (T3) which had soil pH of 5.02. Therefore, these results suggest that minjingu fertilizers, particularly CAN27%, can reduce soil acidity. Similar results were obtained by Dai et al.[28], who observed that the combination of organic and inorganic fertilizers reduced the soil acidity.

Minjingu fertilizers, particularly $N_{10}P_{18}K_{24}$, showed indices for having a substantial amount of B as the residual soil B were much higher than the rest of the treatments. However, the residual soil B was still adequate and did not go beyond 1kg ha^{-1}. B concentrations beyond this level have been shown to affect the leaf N and P concentrations[29]. Across the sites, residual soil exchangeable Ca increased significantly ($P \le 0.001$) regardless of whether the tobacco plants were fertilized or not (Table 5, Figure 4A). The increase of residual soil Ca could be due to the increase in soil acidity (Figure 3), which decomposed OC and hence increased Ca[19]. The minjingu CAN27% could also have the potential to release substantial amounts of Ca to the soil as treatments that applied this fertilizer showed higher residual soil exchangeable Ca than those that were not (Table 5, Figure 4A).

Whether fertilized or unfertilized, tobacco plants showed the ability to increase residual total soil N. However, the increased residual total N did not differ significantly across the sites and treatments (Table 5, Figure 4B). This could be because the fertilizers used have an equal ability to supply N to the soil. Tobacco plots fertilized with minjingu fertilizers showed significantly ($P \le 0.001$) higher residual P due to the ability of minjingu $N_{10}P_{18}K_{24}$ to release P slightly for an extended period. However, the residual soil P was lower than the initial soil P (Figure 4C) due to the higher P requirement for tobacco plants for development of roots and improving the color and quality of leaves[19]. The significantly high residual soil P for the tobacco plants fertilized with minjingu $N_{10}P_{18}K_{24}$ and top-dressed with standard CAN27% (T3) in the Tabora site (Figure 4C) could be associated with the mineralization of P_2O_5 in the more acidic soil at the Tabora site. However, similar results could have been expected for T2. Therefore, the laboratory errors or contamination with P residues for T3 cannot be neglected. Furthermore, the residual soil exchangeable K across the sites and treatments were lower than the initial soil K before tobacco production. The lower residual soil exchangeable K could be due to the influence of K on leaf yield and quality of the tobacco[26].

Application of NPK fertilizers was associated with an increase in alpha radioactivity due to the presence of heavy metals such as As, Cd, Ni, Pb and Zn, all of which increase the carcinogenic effect of tobacco[30-34]. However, the current study did not analyze the heavy metals residuals in soils and the tobacco leaf. Therefore, the study recommends that further studies to determine the amount of heavy metals in the tobacco leaf to ensure the quality of tobacco leaf produced.

5 CONCLUSIONS

The basal application of minjingu $N_{10}P_{18}K_{24}$ fertilizer and top-dressed with minjingu CAN27% produced 1942.59 kg ha^{-1} dry leaf yields, which did not differ significantly with 2033.64 kg ha^{-1} tobacco dry leaf yield produced using the basal standard $N_{10}P_{18}K_{24}$ and top-dressed with standard CAN27%. However, the tobacco fertilized with the standard basal $N_{10}P_{18}K_{24}$ had a significantly higher-grade index than tobacco fertilized with minjingu $N_{10}P_{18}K_{24}$ fertilizer and top-dressed with minjingu CAN27%. In addition, the minjingu CAN27% showed its potential in improving dry leaf yields and lowering the soil pH.

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Conflicts of Interest

The authors declared no conflict of interest.

Author Contribution

Lisuma JB performed the statistical analysis, wrote and revised the article; Muna IE designed the study, supervised the trials and revised the article; Furahini AM set up the experiment and collected data at Tumbi, Tabora; Pessa A set up the experiment and collected data at Mtanila, Chunya; Mbao K set up the experiment and collected data at Songambele, Urambo; Malley Z monitored the field sites and revised the article; All authors approved the final version.

Abbreviation List

CAN27%, Calcium ammonium nitrate - 27% N
COVID-19, Coronavirus 2019
K, Potassium
MoHP, Minjingu organic hyper-phosphate
N, Nitrogen
$N_{10}P_{18}K_{24}$, Nitrogen 10%, Phosphorous 18%, Potassium 24%
OC, Organic carbon
P, Phosphorus
RCBD, Randomized complete block design
SE, Standard error
T, Treatment

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