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Soil Mineral Status, Plant Ionome and Agro-Morphological Traits of *Schkuhria Pinnata* (L.), An Antimalarial Herb: Implications for Cultivation

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ABSTRACT

Background: *Schkuhria pinnata L.*, is an antimalarial plant that is highly threatened by the destructive harvesting methods and its collection largely relies on wild sources, that are also exposed to over-exploitation and habitat destruction.
Aim of the study: The study aimed at figuring out where *S. pinnata* grows best and what its growth requirements are; in order to promote the informed cultivation practices and soil selection as a viable alternative to wild harvesting. The agronomical soil nutrient status of *S. pinnata*, and how it relates to the agro-morphological traits and plant ionome, clues on fertiliser formulations for soils where *S. pinnata* does not shrive were reported.
Methods: A randomised complete block design was employed in agronomical experimental plots in different agro-ecological zones that host Bushenyi, Ntungamo and Kasese districts. Standard procedures for soil and plant analysis were used to analyse soil physicochemical and plant ionome parameters while agro-morphological traits were physically evaluated.
Results: Results demonstrated that soil physicochemical characteristics differed significantly across the study sites ($p \leq .05$). *S. pinnata* significantly performed better in slightly acidic to neutral soils (pH between 5.87-7.25) in Kasese than in other sites. *S. pinnata* harvested from Kasese had the largest total leaf area (mean = 31.43 ± 2.41cm^2) and the highest plant biomass (mean = 7.65 ± 0.64 g).
Conclusion: The study concluded that *S. pinnata* grew best in slightly acidic to neutral, sandy loam, non-saline soils of Kasese in Western Medium-High Farmland

INTRODUCTION

Cultivation of medicinal plants is a conservation strategy that provides a sustainable supply of medicinal materials.[1] Increased demand for medicinal plant ingredients has exerted pressure on scientific research for medicinal plants' growth requirements like soil nutrients. This provides alternative sources of medicinal stocks other than natural wild sources.[2] Soil plays a vital role in plant nutrition as it reserves nutrients and water in which plants grow and develop.[3] Soil nutrient requirements by plants vary depending on quantity needed, thus, soil nutrients are categorised as macro and trace nutrients.[4] Potassium (K), phosphorus (P), calcium (Ca), nitrogen (N), and magnesium (Mg) are categorised under macronutrients, whereas manganese (Mn), copper (Cu), zinc (Zn) and iron (Fe) among others are considered trace nutrients.[4] Previous studies showed that parent bedrock undergoes weathering through physicochemical processes to provide soil nutrients. This key source is supplemented by mining, ores, industrial wastes and atmospheric deposits.[3,5] Nutrients in soil exist in a solid state under optimal soil pH (potential of hydrogen), moisture content, temperature, and soil electrical conductivity.[6]

The nutrients are released into the soil solution by desorption and dissolution processes.[7] Plants primarily obtain nutrients from the soil solution in the ionic state.[8] Under the influence of soil-plant interaction, dissolved nutrients reach the root *mycorrhizal* surface by mass flow and diffusion mechanisms.[8] Soil nutrients are later translocated through apoplastic and symplastic pathways to the aerial parts of the plant.[9] Although the uptake of a particular nutrient mainly depends on its presence in the growing media[8] and buffer capacity[9], the translocation of plant ionome is influenced by other underlying aspects such as soil temperature, soil pH[10], soil moisture[11], root architecture and activity, humus, elemental toxicity, salinity levels[6] and plant genotype.

[8,12] Plant ionome and agro-morphological traits form a fundamental tool for evaluating the efficient utilisation of soil nutrient available in the soil.[4] Additionally, the analysis of the plant ionome (i.e., the elemental composition of plant tissue) is based on the theory that the concentration of elemental nutrients in a plant is an indicator for soil capacity to provide that nutrient.[13] Basing on the variability of each nutrient in the soil, the plant ionome is best expressed as a translocation ratio that accurately provides the translocation efficiency of a given nutrient.[14]

Schkuhria pinnata (Lam.) Kuntze ex Thell, family Asteraceae[15], is a South American native plant.[16] It is normally distributed in dry areas at an elevation of ≤ 3000 metres above sea level.[17] *S. pinnata* has also been reported to be grown in African countries including Kenya, where it is being used to treat malaria among the Kikuyu community[18] and in Cegere sub-county, Apac district in Northern Uganda. However, the literature on how this plant reached African countries and Uganda in particular is scarce. According to plant database[15], *S. pinnata* grows to a height of 30 to 70 Centimetres (cm) and has deep finely divided leaves. The upper leaf surface is normally grooved and both leaf surfaces are pitted with numerous small glands.

Currently, collection of *S. pinnata* medicinal materials for traditional use largely relies on wild sources.[19] It mostly involves uprooting the whole plant[20,21] at any developmental stage from wild habitats.[19] Unfortunately, the wild source is exposed to; over-exploitation, habitat destruction and harsh climatic conditions. These have led to a high genetic pool depletion rate[22,23], minimal yields and unreliable quantity and quality supply.[24] Therefore, a sustainable production practice through cultivation is a forecasted promising approach for increasing the plant's medicinal stock.[25]

Cultivation and domestication of *S. pinnata* were reported[19] in Apac district, northern Uganda where 2 traditional practitioners were found to have cultivated *S. pinnata* plants in their home backyards. However, information concerning the soil under which *S. pinnata* was grown remains unknown. Such scenario, therefore, warranted the evaluation of soil nutrient status required for *ex-situ* growth and development of *S. pinnata*. In this study therefore, we evaluated the soil nutrient status and correlated the soil physicochemical characteristics with plant ionome and agro-morphological traits, to generate significant information on ecological plant growth requirements for cultivation and domestication of *S. pinnata.*

MATERIALS AND METHODS

Study Sites and Design

Agronomical experiments were conducted between October 2019 and April 2020 in agro-ecological zones of South Western Highlands in Ntungamo District and South Western Medium–High farmlands in Bushenyi and Kasese districts. Although, Kasese and Bushenyi study sites are located in the same agro-ecological zones, they greatly differ in weather conditions and altitudes (Table 1), thus influence plant growth performance differently.

Seed Collection and field *S. pinnata* Plant Growth

Agronomical experimental plots in triplicates were designed in randomised complete blocks.[27,28] At each site, measured experimental plots (12 x 15 metres) were set up at one metre apart. Only mature black achenes were harvested from health and mature plants growing at the National Agricultural Research Organization in Uganda at 0^0 25′ 14.0″ N, 32^0 32′ 26.0″E, 1300 m above sea level.

They were identified and authenticated with accession number 50926 at Makerere University herbarium. About 500 grammes (g) of *S. pinnata* achenes were air-dried from until the moisture content was 9.3%. The dried achenes were later stored in airtight container for 1 month[29] before sowing. In each plot, 300 seeds were sown at a depth of 1 to 3 millimetres (mm) in 20 rows, at a spacing of 30 Centimetres (cm). The rows were 60cm apart. The plant's growth was monitored under natural environmental conditions, weeding was done after every 2 weeks. The germination dates for the plants were recorded. On day 43 from the date of germination, at 9.00 a.m., the plants were harvested for agro-morphological and plant ionome studies.

Field Soil and Plant Tissue Sampling

Soil sampling was conducted using a whole-plot composite method in a zig-zag pattern.[14,27] At 1 to 15 cm deep, about 10 subsoil samples were collected, mixed thoroughly to form a composite sample.[14] This was repeated for each plot.

For plant ionome studies, a sack of fresh aerial parts i.e., leaves, stems and flowers at flowering stage was harvested. The study targeted the flowering stage because *S. pinnata* methanol extracts at this stage demonstrated the maximum antimalarial activity against chloroquine-sensitive *Plasmodium berghei* on Swiss albino mice.[30]

Soil and Plant Tissue Sample Preparation

Soil samples were air-dried at 25°C, physically pulverised and sieved through a 2 mm mesh to obtain fine powdered clean soil samples weighing 500g, which were later repackaged and clearly labelled. The plants' aerial parts were oven dried at 45°C to 9.3% moisture content, pulverised with an electric blender, sieved and 500g packed and labelled. The dry soil and plant samples were transported to the Makerere University, Department of Agriculture, and Analytical laboratory for water, soil and plant analysis.

Determination of Soil Physicochemical Characteristics and Plant Ionome

A broad spectrum of agronomical physicochemical characteristics of soil were analysed as described in the working manual for laboratory methods of soil and plant analysis.[31] Thirteen (13) soil physicochemical characteristics i.e., soil pH, organic matter, nitrogen, available phosphorus, potassium, calcium, magnesium, sodium, electrical conductivity, iron, manganese, zinc, copper and soil texture were analysed. For plant ionome, 10 nutrients i.e., nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc and copper were analysed.

Determination of Soil pH and Electrical Conductivity

Finely grounded soil (20.0g) was added to 50 mL of deionised water and the mixture was then poured into a

plastic bottle, mixed thoroughly using a high-speed electric shaker. The mixture was allowed to stand for 30 minutes, then mixed again for 2 minutes, after which measurements of soil pH were taken on soil-water solution at 1:2.5 w/v with a pH metre (JENWAY 3310; France, Paris). The mixture's electrical conductivity readings were taken using the electrical conductivity digital meter (JENWAY 4310; France, Paris).

Determination of Soil Texture by Hydrometer Method

Accurately weighed 50 g of air-dried soil of each sample was put into a 500 mL plastic beaker. The soil was saturate with 200 mL of distilled water. To the saturated soil suspension, 10 mL of 10 % Calgon solution was added and allowed to settle for 10 minutes. The suspension was made to the mark with distilled water and transferred into a plastic bottle and mixed for 12 hours with an electric shaker. The suspension was then transferred into 2000 mL graduated cylinder and a hydrometer inserted. More water was added to 1130 mL, and the hydrometer removed. The cylinder was covered tightly with a rubber bung and the suspension mixed further by inverting the cylinder carefully 10 times and the time noted. 3 drops of amyl alcohol were added to the soil suspension to remove the froth. The hydrometer was gently placed into the column and after 40 seconds, hydrometer reading was taken and the temperature of the suspension measured.

The mixing of the suspension by inversion of the cylinder was repeated for more 10 times and later allowed the suspension to stand for 2 hours and after which, both hydrometer and temperature readings were taken. Thus, the soil particle size was analysed by dispersion of soil particles into fractions of sand (2.00 - 0.05 mm), silt (0.05 -0.002 mm) and clay (< 0.002 mm), and later estimated as percentage sand, silt and clay contents, and assigned the soil textural category according to Marshall's textural triangle and their respective textural classes[31] generated.

Determination of Soil Carbon Content in Organic Matter

This was conducted according to Walkley and Black method[32], following wet oxidation using concentrated sulphuric acid (Analytical grade, Sigma-Aldrich, USA) and potassium dichromate (Analytical grade, Sigma-Aldrich, USA). For each soil sample, 0.6g of soil was added to 10 mL of 0.2M potassium dichromate and 5 mL of 98% sulphuric acid. The mixture was put in a pre-heated block maintained at 145^0C for 30 minutes and cooled to room temperature. The digest was transferred to a 100 mL conical flask, and 0.3 mL of the indicator solution was added and the resultant mixture thoroughly mixed using a magnetic rod. The resultant mixture was then titrated with 0.2M ferrous ammonium sulphate solution (Analytical grade, Sigma-Aldrich, USA). The end-point was achieved when the colour changed from greenish to brown. The titre values were recorded and corrected for the mean of 2 reagent blanks (T), and organic carbon was calculated according to the equation (1) below;

$$\text{Organic carbon \%} = \frac{T \times 0.2 \times 0.3}{\text{Sample Size}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where T is the titration volume.

Extraction of Soil Exchangeable Cations

To extract exchangeable cations i.e., potassium, calcium, sodium, magnesium into soil solution, 5.0 g of finely grounded air-dried soil sample was added to 100 mL of IM ammonium acetate (Analytical grade, Sigma-Aldrich, USA) at pH 7.0. The mixture was shaken vigorously using electric shaker for 30 minutes, filtered using a filter paper to obtain a clear soil solution.

Extraction of Soil Available Phosphorous, Nitrogen and Trace Nutrients by Digestion Process.

Dry powder of soil (0.5g) was digested in digestion mixture of 5mL salicylic acid (Analytical grade, Sigma-Aldrich, USA) and selenium-sulphuric acid (Analytical grade, Sigma-Aldrich, USA), at room temperature. The mixture was heated at 110°C for 1 hour and cooled to room temperature. 3 drops of 30% hydrogen peroxide (Analytical grade, Sigma-Aldrich, USA) were added, each at an interval of 10 seconds. Hydrogen peroxide acts as an anti-foam by oxidising the organic matter while Selenium powder lowers the boiling point and acts as a catalyst for the process. Concentrated sulphuric acid (Analytical grade, Sigma-Aldrich, USA) completes the digestion at elevated temperatures. After complete digestion at 330°C, the digest was cooled to 25°C, diluted to 50 mL using de-ionised water and filtered through Whatman filter paper to obtain a digest solution, on which available phosphorus, nitrogen and trace elements were analysed.

Extraction of Plant Ionome

Plant ionome extraction was conducted according to standard routine procedures.[31] Accurately weighed 0.5g of dry powder of plant tissue was digested in a digestion mixture (5mL) of salicylic acid and selenium sulphuric acid (selenium 3.5g: 1L sulphuric acid) at room temperature. The mixture was heated at 110°C for 1 hour and cooled to room temperature. 3 drops of 30% hydrogen peroxide were added, each at an interval of 10 seconds. After complete digestion at 330°C, the digest was cooled to 25°C and diluted with deionised water.

Analysis of Soil Physicochemical Characteristics and Plant Ionome

Analysis of Exchangeable Cations in the Soil and Plant Tissue. Analysis of potassium, calcium and sodium concentrations was conducted by adding 5.0 mL of either soil solution or plant digest to 1.0 mL of 26.8% lanthanum chloride (Analytical grade, Sigma-Aldrich, USA) in a 50 mL volumetric flask, and made to the mark point with 1M ammonium acetate solution. The calcium, potassium and sodium concentrations in the resultant samples were analysed using a flame photometer (JENWAY PFP 7; France, Paris), at wavelength of 622 nm, 766 nm and 589 nm respectively.

Magnesium concentration was determined by adding 2 mL of either soil solution or plant digest to 5 mL of 15.21g/L of strontium chloride (Analytical grade, Sigma-Aldrich, USA) in a 50 mL volumetric flask and made to the mark point with 1M ammonium chloride solution (Analytical grade, Sigma-Aldrich, USA).Either soil solution or plant digest was sprayed into the flame of atomic absorption spectrophotometer (Agilant Technologies, GTA 120; California, USA), at 285.2 nm.

Analysis of Phosphorous in either Soil or Plant Digest

2 drops of 0.5% p-nitrophenol indicator solution (Analytical grade, Sigma-Aldrich, USA) were added to digest solution (10 mL) in a 50 mL volumetric flask. Then, 5mL of ammonium molybdate/ ammonium vanadate mixed reagent (Analytical grade, Sigma-Aldrich, USA) was added and made to 50 mL with distilled water and shaken to mix. The resultant solution was allowed to stand for 30 minutes and the absorption of the solution was finally determined using Ultraviolet/Visible spectrophotometer (JENWAY 6405; France, Paris) at 882 nm.

Analysis of Nitrogen either in soil or Plant Digest

Colorimetrically, nitrogen was determined using concentrated sulphuric acid, selenium powder plus salicylic acid i.e., Kjeldahl method (Carolina *et al*., 1991). Either soil or plant digest (0.5 mL) was added to 5 mL of N1. NI was made by dissolving 3.4 g of sodium salicylate (Sigma, Aldrich, USA), 2.5 g of sodium nitrate and 2.5 g of sodium tartrate (Sigma, Aldrich, USA) in 75mL of distilled water and mixture vortexed. Later, 5ml of N2, which was made by dissolving 30g of sodium hydroxide (Sigma, Aldrich, USA) in 10 mL of sodium hypochlorite and made to litre with distilled water was added. Finally, the absorption of the complexed analyte was determined using Ultraviolet/ Visible spectrophotometer (JENWAY 6405; France, Paris) at a wave length of 655 nm.

Analysis of Trace Elements in either soil or Plant Digest

The trace elements i.e., zinc, manganese, copper and iron in the digest were analysed using the flame atomic absorption spectrophotometer(Agilant Technologies, GTA 120; California, USA), on ethylene diamine tetra acetic acid (EDTA) (Sigma, Aldrich, USA), chelating agent extracts at a wavelength of 213.9, 324.7, 248.2, 248.3 nm, respectively.

Notably, Flame Atomic Absorption Spectrophotometer (FAAS), ,which uses acetylene gas is used to analyse almost all the soil nutrients including Ca, Mg, Zn, Mn, Cu, Fe, K and Na. However, in the present study, the use of FAAS was limited to analysis of Mg, Mn, Cu, Fe and Zn due to high costs associated with acetylene gas compared to butene gas, which is used in other apparatus such as flame photometer. Also, the flame photometer that was used, only had filters for Ca, Na, K and Li. Thus, not used to analyse all the nutrients studied despite its cheaper operating costs. Extraction and analysis of available phosphorus and nitrogen involve the use of coloured reagents which complex with the analyte, thus, are perfectly detected by the Ultraviolet/ Visible spectrophotometer. Quantitatively, the elemental values obtained were expressed as the translocation ratio[14] below.

Translocation ratio=Concentration of plant nutrient A in plant tissue/Concentration of nutrient A in soil solution

Determination of Agro-Morphological Traits

Agro-morphological traits including leaf area, plant height, taproot length and plant biomass were physically measured at flowering from 30 randomly sampled representative plants.[34,28] To determine the total leaf area, the leaf length (L) was measured from the insertion of the petiole up to the apex and the widest width (W) perpendicular to the rib alignment[28]; for the leaves on the plant, then multiplied by a Correction Factor (cf) of 0.7.[27] Taproot length (cm) was determined by measuring the uprooted plant from the soil surface level to the end of the taproot using a ruler. Plant height (cm) was measured from the soil surface up to the end of the main plant stem using a ruler.[28] Plant biomass was determined by first washing off the soil of the uprooted plant, oven dried at 40-65°C and weighed to achieve a constant mass on scale.[14]

Statistical Analysis

Study data was computed and expressed as mean ± standard error. Principal Component Analyses (PCA) for soil and plant ionome characteristics were performed to provide insights on whether the study sites of different agro-ecological zones would be clustered differently in SASS JMP version 11. To study the mean variability of soil physicochemical characteristics, ionome translocation ratio and agro-morphological traits across study sites,one-way variance analysis (ANOVA) for parametric and normal distributed data was later run at 0.05 level. Pair-wise comparisons to show significant difference among the means were conducted using Tukey post-hoc method, in SPSS, version 21. Pearson's product-moment correlation was later run to relate soil physicochemical characteristics with plant ionome and agro-morphological traits at significant levels of 0.01 and 0.05.

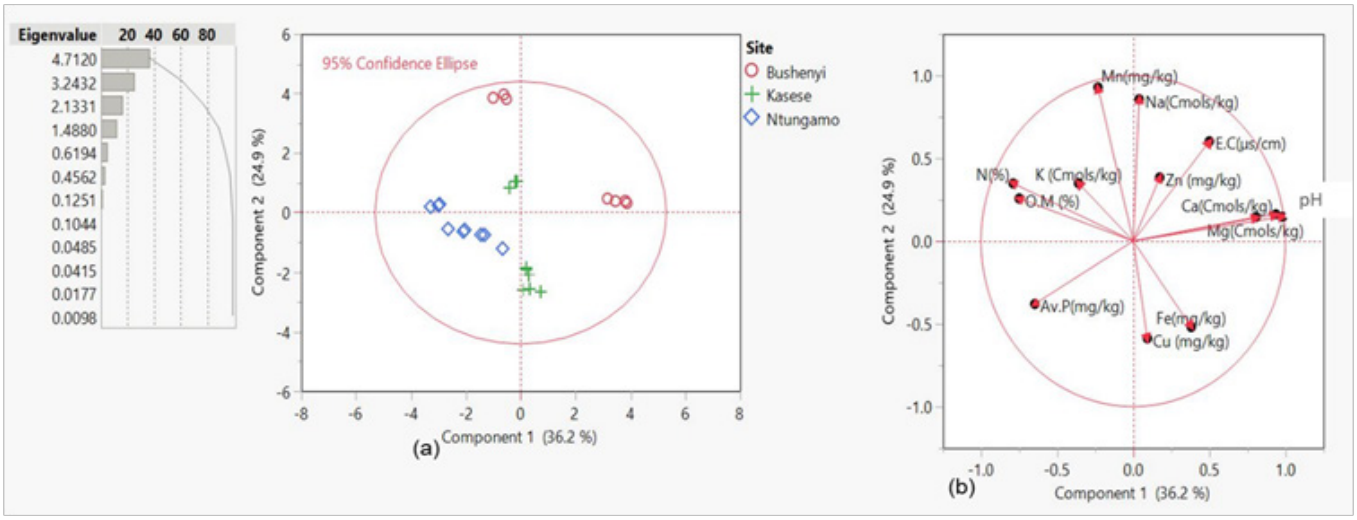
RESULTS AND DISCUSSION

Variability of Soil Physicochemical Characteristics

In the present study, the principal component analysis of soil physicochemical characteristics across the 3 study sites, showed a significant level of variability, explained by 36.2 and 24.9% of the corresponding Principal Components (PC1) and (PC2) respectively (Figure 1). Variability in soil physicochemical characteristics was attributable to the difference in the anthropogenic activities carried out in the neighbourhood of the sites, including house constructions and livestock farming. For instance, livestock residues which are rich in organic matter could have caused variations in soil variability, particularly in soil pH. Our findings are supported by the work done by Gisilanbe *et al.*,(2018) along the 3 slope positions in Ganye, North-Eastern Nigeria where they found that environmental factors such as leaching and erosion processes[35] cause variations in soil characteristics. According to the review done by Rietra *et al.*, (2017), on "Effects of nutrient antagonism and synergism on yield and fertiliser use efficiency" agronomical experiments were identified to be affected by the confounding factors including environmental temperature, rainfall among others. Thus, based on the fact that the design of the current study was agronomical, there is need for further studies that would focus on laboratory-controlled experiments and determine the variability in soil mineral status for comparison purposes.

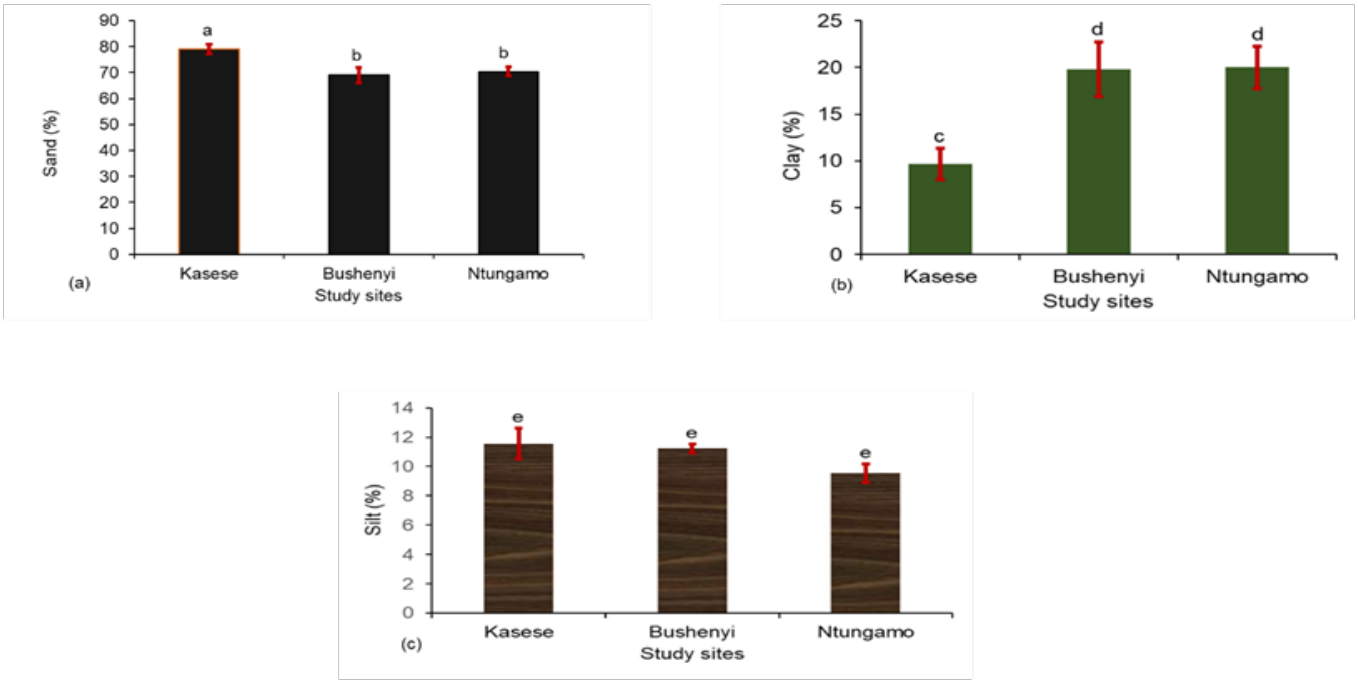
The overview of soil analysis using one-way Anova test revealed that all the soil physicochemical characteristics significantly differed across the study sites ($p \leq .05$), except potassium ($p = .247$), manganese ($p = .053$) (Table 2). According to Rao *et al.*, 2020, soil pH is a measure of acidity, which is a portion of the hydrogen ions that are

FIGURE 1: Principal Component Analysis of Soil Physicochemical Characteristics across Study Sites



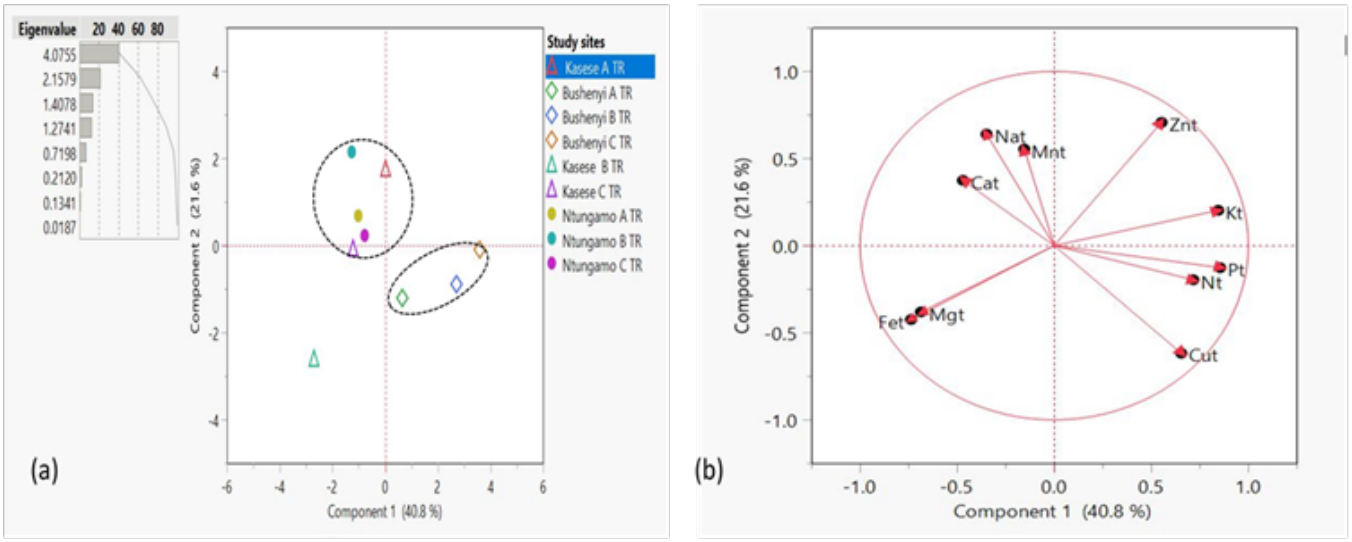
(a) Scores on the two principal components (PC1 and PC2)
(b) Loading plots (PC1 and PC2)

FIGURE 2: Variation of Soil Particle Composition across the Study Sites of Kasese, Bushenyi, and Ntungamo



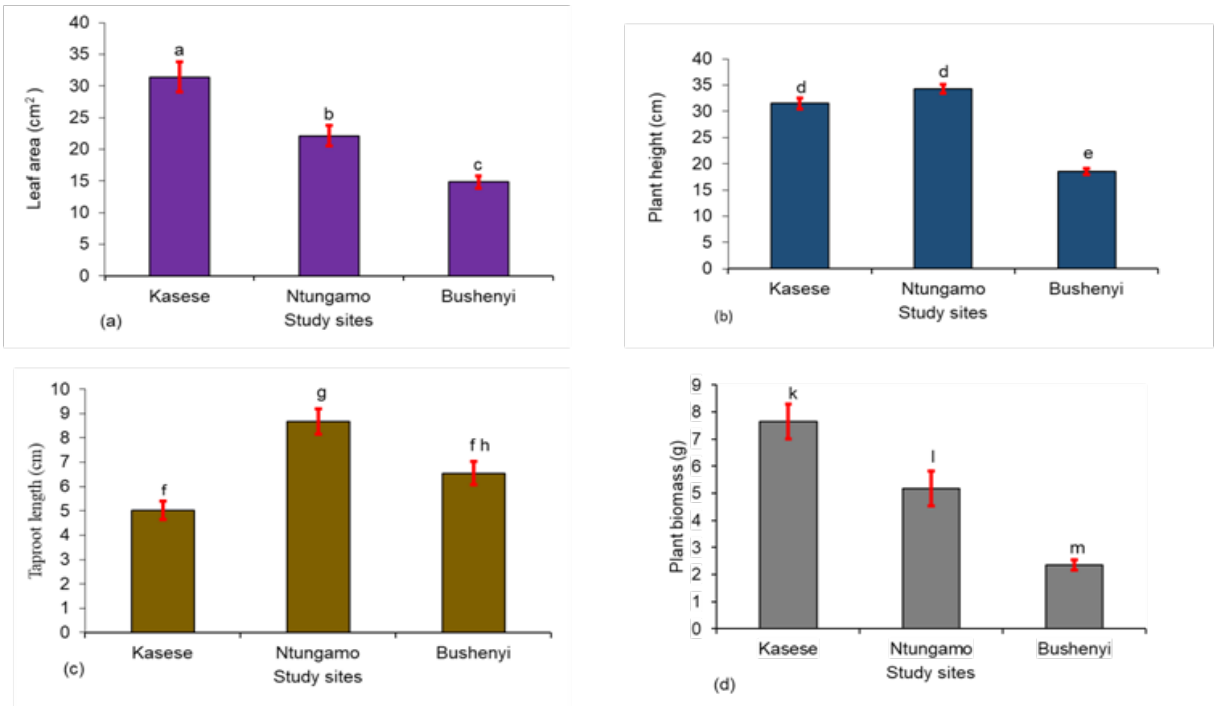
(a) sand, (b) clay, (c) silt.
Mean values superscripted with different letters, which are significant at 0.05 level, those superscripted with the same letters are non-significant

FIGURE 3: Principal Component Analysis of Plant Ionome of S. Pinnata at Flowering Developmental Stage



"t" of elementary symbols represents "tissue". "TR" represents "translocation ratio". Potassium (Kt), nitrogen (Nt), phosphorus (Pt), sodium (Nat), calcium (Cat), magnesium (Mgt), iron (Fet), zinc (Znt), manganese (Mnt), copper (Cut).

FIGURE 4: Variation of Agro-Morphological Traits of Flowering S. Pinnata Grown in Kasese, Ntungamo, and Bushenyi



Mean values subscripted with different letters were considered significant at 0.05 levels (n = 30)

active in the soil solution. In the present study, soil from Kasese had a mean pH value of 6.87 ± 0.05, characterised as slightly acidic and slightly neutral pH. However, this pH value was slightly higher than that of Ntungamo (mean = 6.11±0.09), categorised as acidic and it was lower than that of Bushenyi (mean = 7.78 ± 0.22), which was alkaline. The one-way ANOVA test showed a significant difference in pH mean values among study sites with F (2, 24) = 33.84 and *p*-value of .001. Tukey multiple comparison also showed a significance of p =.003 for Kasese versus Ntungamo and p = .001 for Bushenyi versus either Kasese or Ntungamo. The soil pH across the 3 study sites was highest in Bushenyi, followed by Kasese and lowest in Ntungamo. The soil at Bushenyi was more alkaline compared to other sites. This may be accounted by the observed high electrical conductivity caused by high concentration of salt ions including calcium and magnesium. Also, this is evidenced by the positive correlation between soil pH and calcium (r = 0 .955) , pH and magnesium (r = 0.814; $p \leq .01$) (Table 3). The present finding is in agreement with another study conducted in areas of Perth in Western Australia by Warton and Matthiessen, (2005), where they noted that parent bedrocks are constituted of varying concentrations of calcium salts, including calcium carbonate that causes a liming effect on the soil, which may be responsible for the increase in soil pH.[37] The soil from Ntungamo was slightly acidic, attributed to high nitrogen content, further supported by negative correlation between soil pH and nitrogen (r = -0.699; p = .01). This scenario may be explained by work done by Fageria and Baligar, (2004) in a study under "Nutrient availability", that nitrogen is incorporated in nitrogenous compounds like ammonium compounds which undergo nitrification processes that are accompanied by proton release, thus, reducing soil pH.[13] Furthermore, in a nitrogen fertilisation experiment by Verma *et al.*,(2015), the application of nitrogen containing fertilisers to the soil was also found to cause a decrease in soil pH.[38] The low soil pH in this study was also evidenced by the negative correlation between soil pH and organic matter (r = -0.675; p = .01) and available phosphorus (r = -0.704; p = .01). Interestingly, at Kasese where calcium, magnesium, nitrogen, organic matter and phosphorus were found to be in moderate levels, soil pH was slightly acidic and slightly neutral. Therefore, we conclude that the variability in soil pH could be due to anthropogenic activities and environmental factors such as composition of the parent bedrock, abiotic and biotic conditions of the sites. Also, the information gathered about pH status in this study is for informing the most suitable soil pH for *S. pinnata* growth, to enhance the formulation of an appropriate fertiliser with a target pH value that would reduce toxicity occurrence and optimise nutrient levels to the plant.

In this study, the amount of Organic Matter (OM) to be 3.73± 0.22 % in soil from Ntungamo was higher than that at Kasese (1.80 ± 0.27 %) and Bushenyi (2.28 ± 0.33 %). The statistical analysis indicated a significant difference in mean percentage values of organic matter across the sites with F (2, 24) = 12.70; p =.001 (Table 2). Also, Tukey post-hoc analysis showed significance of p = .001 for Ntungamo versus Kasese and p = .004 for Ntungamo versus Bushenyi. In a study done by Bhatti et al., (2016) at Punjab in India, similar findings were reported. In that study, the organic matter content was reported to be low and ranged between 2.73% to 4.17%[39], which is comparable to our findings in Ntungamo and Bushenyi. The soil in Punjab was considered to be slightly acidic, a similar pH status in Ntungamo and Kasese. Therefore, the anthropogenic drivers including intensive agriculture with the use of agrochemicals seen at Punjab could also have caused the variation in organic matter in our study. The source of organic matter is known to be the death and decomposition of living tissues by microbial activity of extracellular enzymes that releases phosphorus and nitrogen among other nutrients.[40] Thus, it is more likely that increase in organic matter may increase the availability of nitrogen as evidenced by positive correlation between organic matter and nitrogen (r = 0.889; p = .01) (Table 3). Similarly, soil from Ntungamo was the richest in nitrogen (mean = 0.24 ± 0.02%) followed by that of Kasese (0.12 ± 0.15 %) and poorest at Bushenyi (0.14 ± 0.03%). The mean values of soil nitrogen were statistically different across the sites with F (2, 24) = 7.46; p = .003 (Table 2). Tukey post-hoc comparisons showed a significant difference of p = .003 for Ntungamo versus Kasese and p =.004 for Ntungamo versus Bushenyi. According to the work done by Neina, (2019) on the role of soil pH on plant nutrition and soil remediation, plants obtain nitrogen in form of nitrogenous compounds such as nitrates which are often found incorporated in organic matter and are bio available through mineralisation.[41] Thus, the observed difference in nitrogen levels across sites may implicate several environmental factors that enhance biological processes that unlock the nutrients from organic matter. Interestingly, nitrogen and organic matter were also positively correlated (r = 0. 889, p= .01) (Table 3). Thus, based on nutrient-source relationship, organic matter serves as a primary source of organic nitrogen in the soil[13], an implication that increasing availability of organic matter increases nitrogen concentration. According to Achen *et al.*,(2014), the availability and balance of nitrogen in the soil implicates nitrification and de-nitrification processes.[42] Therefore, factors such as microbial reactions that influence such processes might have been different across the sites in this study.

In the present study, the mean values of available phosphorus in soil were statistically different with F (2, 24) = 21.56; p = .001. Soil phosphorus was highest in soil from Kasese (mean = 56.30 ± 0.50 Cmols/kg), followed by that at Ntungamo (mean = 54.98 ± 0.62Cmols/kg) and was the lowest at Bushenyi (12.32 ± 0.74Cmols/kg) (Table 2). Pair-wise comparison showed a significant difference value of p = .001 for Bushenyi versus either Ntungamo or Kasese. The possible explanation for this situation provided by Reitra *et al.*, (2017), that the high concentration of calcium ions in the soil are responsible for the reduced phosphorus availability, as calcium is well known to form a calcium phosphate complexes of very low solubility in soil solution, thus, reducing its availability.[4]

On the other hand, calcium in soil from Bushenyi (mean = 10.26 ± 1.25Cmols/kg) was higher than that of Kasese (mean = 4.90 ± 0.19 Cmols/kg) and Ntungamo (mean = 2.44 ± 0.15Cmols/kg). Statistically, calcium level at Bushenyi was significantly different from that of Kasese and Ntungamo with F (2, 24) = 29.50; p = .001 (Table 2).

The multiple comparison showed that the mean values of calcium across the sites significantly differed with p = .001 for Bushenyi versus either Kasese or Ntungamo. Often, the source of soil nutrients is majorly the existing parent bedrock, which undergoes weathering through physicochemical processes. This fact was confirmed by a study conducted by Kabrick and colleagues, (2011) as they identified soil pH as a factor that influenced the distribution of exchangeable cations including calcium in Ozark highland forest soils.[43] Therefore, the same factorcould also explain the variation of calcium across the study sites. Similarly, in our study, variation in calcium concentration was seen to correlate positively to soil pH (r = 0.955; p = .01), which may be accounted by the finding of Warton and Matthiessen, (2005) that the increasing effect of soil pH on the solubilities of calcium ions, increases its availability in soil solution.[37] Also, calcium correlated positively with magnesium concentration (r = 0.925; p = .01) (Table 3). This particular relationship between calcium and magnesium may be linked to their chemical behaviour that relates to the combining powers. In support to our findings, Addis and Ahebaw, (2017) noted that cations of the same combining powers are likely to have the common chemical origin.[44] On the other hand, calcium levels were found to correlate negatively with organic matter (r = -0.554; p = .01), nitrogen (r = - 0.395; p = .01) (Table 3) and available phosphorus (r = -0.645; p = .01) in this study. In this regard, a recent study done by Wangalwa *et al.*, (2021) on occurrence of *Citropsis articulata* (Willd.ex Spreng) Swingle & Kellerm, of family Rutaceae in 3 tropical forests of Uganda, noted a similar scenario between calcium and phosphorus.[45] This observation may indicate that calcium has a potential to cause a decrease in the availability of nitrogen and phosphorus, inducing their nutritional deficiencies in *S. pinnata*.

Magnesium was highest in soil from Bushenyi (mean = 2.25 ± 1.17Cmols/kg), followed by that at Ntungamo (mean = 0.81 ± 0.05 Cmols/kg) and was the least in soil from Kasese (mean = 0.60 ± 0.42 Cmols/kg). The one-way Anova test showed that magnesium concentration in Bushenyi soil was significantly higher than that of Kasese and Ntungamo with F (2, 24) = 15.25; p = .001 (Table 2). The pair-wise tukey's post-hoc test indicated a significant difference of p = .001 for Bushenyi versus either Kasese or Ntungamo. Likewise calcium, magnesium was found to vary across the sites, which may also be due to the nature of the parent bedrock. Variation in magnesium concentration may be responsible for the variation seen in soil pH (r = - 0.814; p = .01) across sites. Also, there are evidences from the correlations that magnesium is likely to reduce the availability of nitrogen (r = - 0.435;p = .05), phosphorus (r = - 0.617; p = .01) and potassium (r = - 0.467; p= .01). Consequently, this may lead to nutritional deficiencies in *S. pinnata*, compromising its medicinal value.

There was a significant difference among the mean values of sodium across the sites with F (2, 24) = 8.74; p = .001. Sodium in the soil samples from Bushenyi was found to be the highest (mean = 1.34 ± 0.29 Cmols/kg), followed by that at Kasese (mean =0.46 ± 0.05 Cmols/kg) and Ntungamo (mean = 0.44 ± 0.04 Cmols/kg). Tukey post-hoc analysis further indicated that sodium in the soil samples from Bushenyi was significantly higher than that from Kasese (p = *.004*) and Ntungamo (p = *.003*) (Table 2).

The electrical conductivity of the soil from Bushenyi (mean = 171.17 ±1.98 µS/cm) was higher than that at Kasese (mean = 129.52 ± 1.81 µS/cm) and Ntungamo (mean= 74.42 ± 0.24 µS/cm). Statistically, there was a significant difference among the electrical conductivity mean values with F (2, 24) = 17.89; p =*.001*. Multiple comparison conducted using Tukey post-hoc test showed significant difference values of p =*.006* for Kasese versus Ntungamo, p = *.043* for Kasese versus Bushenyi and p =*.001* for Ntungamo versus Bushenyi. According to Rao *et al.*, (2020), electrical conductivity (EC) of a solution is a measure of the ability of the solution to conduct electricity.[36] Notably, the EC indicates the presence or absence of salts but does not indicate which salts might be present.[46] Thus, the variation in electrical conductivity across study sites might have been due to environmental conditions including rainfall, moisture, microbial reactions together with the nature of the parent bedrock. Although Pearson's product-moment correlation in this study only indicated a positive relationship between electrical conductivity and exchangeable cations i.e., calcium (r = 0.520) and sodium (r =0.634) at $p \leq .05$), a different study conducted by Salem *et al.*, (2020) noted a positive correlation between electrical conductivity and micronutrients i.e., iron (r = 0.85), zinc (r = 0.81), manganese (r = 0.90) at $p \leq .05$ (Table 3). Therefore, variation in EC across the sites could be due to varied concentrations of both macro and micronutrients of the parent bedrocks of the study sites.

Iron is one of the trace nutrients that is required by plants in small quantities and at the same time expresses plant nutritional deficiencies.[4] In this study, soil from Kasese had the highest amount of iron and presented a mean value of 33.31 ± 1.77 mg/kg, followed by that at Bushenyi (mean = 24.07 ± 1.31 mg/kg) and iron was lowest at Ntungamo (mean = 18.10 ± 8.00 mg/kg). One-way Anova test showed that the concentration of iron significantly differed across sites with F (2, 24) = 4.39; p = *.024* (Table 2). Multiple comparison test did not show significant difference in mean values of iron from Kasese and Bushenyi. However, a significant difference value of p= *.019* for Kasese versus Ntungamo was shown. We attributed the variation of iron to mineralisation processes that release minerals into the soil. Mielki *et al.*, (2016) studied the relationship between iron availability in *Zea mays*' leaf tissue and its nutrient source, the soil organic matter in a semi-hydroponic system. They noted a positive correlation between organic matter and iron accumulation in the leaf tissues.[47] Similarly, the variation in iron concentration across the study sites may be accounted by the difference in organic matter content and influence of both abiotic and biotic factors on mineralisation processes.

Zinc concentration was highest in soil from Kasese (mean = 15.41± 3.32 mg/kg) and lowest in the soil from Ntungamo (mean = 8.16 ± 0.38 mg/kg). These mean values were not statistically different from that of zinc in the soil samples from Bushenyi (mean = 12.51 ± 0.31mg/kg). Thus, the F (2, 24) = 3.54; p=*.046* (**Table 2**). Pair-wise comparison test indicated a significant difference of p=*.037* for Ntungamo versus Kasese. Factors affecting the availability of zinc in soil solution including the pH of the

TABLE 1: Geographical Coordinates and Weather Parameters at the Study Sites

Site	Latitude	Longitude	Elevation (m)	Annual rainfall (mm)	Annual temperature (oC)
Kasese	0o 11′ 30.85″ N,	30o 5′ 24.68″ E	964.8	800	17.7 - 30.2
Bushenyi	00 36′ 59.814″ S	300 39′ 20.442″E	1417.7	1200	14.0 - 26.0
Ntungamo	10 8′ 40.15″ S	300 7′ 38.22″E	1774.0	1780	13.0 - 24.3

TABLE 2: Soil Physicochemical Characteristics of Kasese, Ntungamo and Bushenyi Sites

Soil physicochemical parameter	Study Site			F-value	P-value
	Kasese	Ntungamo	Bushenyi		
pH	6.87 ± 0.05a	6.11±0.09b	7.78 ± 0.22c	33.84	.001
O.M (%)	1.80 ± 0.27d	3.73± 0.22e	2.28 ± 0.33d	12.70	.001
N (%)	0.12 ± 0.15f	0.24 ± 0.02g	0.14 ± 0.03f	7.46	.003
Av. P(mg/kg)	56.30 ± 0.50h	54.98 ± 0.62h	12.32 ± 0.74i	21.56	.001
K (Cmols/kg)	0.83 ± 0.3j	0.72 ± 0.19j	0.65 ± 0.05j	1.49	.247
Ca (Cmols/kg)	4.90 ± 0.19k	2.44 ± 0.15k	10.26 ± 1.25l	29.5	.001
Mg (Cmols/kg)	0.60 ± 0.42m	0.81 ± 0.05m	2 .25 ± 1.17n	15.25	.001
Na (Cmols/kg)	129.52± 1.81q	74.42 ± 0.24r	171.17 ±1.98s	17.89	.001
Fe (mg/kg)	33.31 ± 1.77t	18.10 ± 8.00u	24.07 ± 1.31tu	4.39	.024
Mn (mg/kg)	21.32 ± 2.98v	26.09 ±1.74vw	36.56 ±6.54w	3.34	.053
Zn (mg/kg)	15.41± 3.32x	8.16 ± 0.38y	12.51 ± 0.31xy	3.54	.045
Cu (mg/kg)	22.28 ± 1.63z	0.00 ±00a	1.26 ± 0.04a	14.84	.001

TABLE 4: Translocation Ratio of *S. Pinnata* ionome across the Study Sites

Nutrient	Kasese	Ntungamo	Bushenyi	F-value	p-value
Nitrogen	30.38 ± 7.01b	6.69 ± 1.56a	31. 40 ± 9.24b	3.355	.041
Phosphorus	0.43 ± 0.01c	0.07 ± 0.06d	0.19 ± 0.02e	15.128	.005
Potassium	3.62 ± 0.64f	4.54 ± 0.61f	5.69 ± 1.11f	2.696	.146
Calcium	0.14 ± 0.02g	0.22 ± 0.09g	0.11 ± 0.04g	0.812	.487
Magnesium	0.52 ± 0.25h	0.25 ± 0.06h	0.25 ± 0.12h	2.922	.130
Iron	8.33 ± 2.25i	10.52 ± 2.73i	4.77 ± 1.49i	1.710	.258
Sodium	30.13 ± 7.91j	28.86 ± 5.68j	17.25 ± 1.95j	1.530j	.290
Manganese	12. 54 ± 4.23k	7.15 ± 0.56k	4.75 ± 1.20k	2.437	.168
Zinc	13.78 ± 4.67l	16.32 ± 1.08l	17.56 ± 1.65l	0.432	.668
Copper	0.93 ± 0.37m	0.000 ± 0.00n	2.57 ± 0.18o	28.770	.001

Data were computed as mean ± standard error. Mean values superscripted with different letters in a given row are significant at 0.05 level (n = 9).

Turkey post-hoc comparison: Nitrogen, p =.384 for Kasese Vs Ntungamo, p =.521 for Kasese Vs Bushenyi, p = .046 for Ntungamo Vs Bushenyi. Phosphorus, p =.533 for Kasese Vs Ntungamo, p = 0.005 for Kasese Vs Bushenyi, p =.015 for Ntungamo Vs Bushenyi.

Potassium, p=.588 for Kasese Vs Ntungamo, p = 0.125 for Kasese Vs Bushenyi, p = 0.450 for Ntungamo Vs Bushenyi. Calcium, p= .660 for Kasese Vs Ntungamo, p=.942 for Kasese Vs Bushenyi, p=.479 for Ntungamo Vs Bushenyi.

Magnesium, =.175 for Kasese Vs Ntungamo, p = 0.168 for Kasese Vs Bushenyi, p =.999 for Ntungamo Vs Bushenyi. Iron, p=.114 for Kasese Vs Ntungamo, p=.529 for Kasese Vs Bushenyi, p=.238 for Ntungamo Vs Bushenyi.

Sodium, p=.986 for Kasese Vs Ntungamo, p = 0.321 for Kasese Vs Bushenyi, p = 0.385 for Ntungamo Vs Bushenyi. Manganese, p=.359 for Kasese Vs Ntungamo, p=.158 for Kasese Vs Bushenyi, p =.179 for Ntungamo Vs Bushenyi. Zinc, p=.819 for Kasese Vs Ntungamo, p =.654 for Kasese Vs Bushenyi, p=.952 for Ntungamo Vs Bushenyi.

Copper, p =.022 for Kasese Vs Ntungamo, p =.022 for Kasese Vs Bushenyi, p =.001 for Ntungamo Vs Bushenyi.

TABLE 3: Correlations of soil Soil Physicochemical Characteristics of Kasese, Ntungamo and Bushenyi Sites

Soil parameter	pH	O.M (%)	N (%)	P (mg/ kg)	K (Cmols/ kg)	Ca (Cmols/ kg)	Mg (Cmols/kg)	Na (Cmols/ kg)	E.C (µs/ cm-)	Fe (mg/ kg-)	Mn (mg/kg-)	Zn (mg/ kg)	Cu (mg/k g)
O.M	-0.675**	1											
N	-0.699**	0.889**	1										
Av. P	-0.704**	0.301	0.317	1									
K	-0.293	0.131	0.240	0.269	1								
Ca	0.955**	-0.554**	-0.395**	-0.643**	-0.396	1							
Mg	0.814**	-0.383	-0.435*	-0.617**	-0.467**	0.896**	1						
Na	0.160	0.264	0.365	-0.431*	-0.467*	0.144	0.039	1					
E.C	0.576**	-0.240	-0.204	-0.287	0.204	0.520**	0.316	0.634**	1				
Fe	0.339	-0.276	-0.394	-0.121	-0.204	0.209	0.029	-0.180	0.154	1			
Mn	-0.094	0.393	0.513*	-0.270	0.417	-0.100	-0.138	0.886**	0.460	-0.472*	1		
Zn	0.229	-0.319	-0.237	0.167	0.648**	0.141	-0.026	0.135	0.646**	-0.102	0.240	1	
Cu	0.026	-0.189	-0.249	.0271	-0.065	-0.057	-0.296	-0.219	0.058	0.860**	-0.438*	-0.049	1

TABLE 5: Correlation between Soil Physiochemical Characteristics and *S. Pinnata* Ionome

		Soil physicochemical characteristics												
		pH	O.M (%)	N (%)	Av. P (Cmols/kg)	K (Cmols/ kg)	Ca (Cmols/ kg)	Mg (Cmols/kg)	Na (mg/kg-)	E.C (µs/cm)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Plant ionome	N	0.788*	-0.401	-0.378	-0.812**	-0.420	0.758*	-0.645	0.602	0.576	0.192	0.319	-.069	-.042
	P	-0.663*	0.729*	0.669*	0.582	0.391	-0.573	-0.362	-0.294	-0.370	-0.292	-0.024	.053	-.213
	K	0.203	0.502	0.456	-0.228	0.190	0.312	0.334	0.591	0.553	-0.135	0.561	.244	-.257
	Ca	0.692*	-0.432	-0.414	-0.684*	-0.002	0.514	0.386	0.509	0.723*	0.312	0.297	.334	-.038
	Mg	0.740*	-0.638	-0.589	-0.646	0.139	0.607	0.373	0.446	0.711*	0.131	0.283	.484	-.072
	Fe	-0.125	-0.463	-0.406	0.485	0.397	-0.280	-0.522	0.594	0.014	0.449	-0.372	.417	0.690*
	Na	0.334	0.064	-0.011	-0.739*	-0.366	0.308	0.487	0.215	-0.010	-.097	0.138	-.355	-.548
	Mn	-0.402	-0.094	-0.074	0.402	-0.248	-0.382	-0.460	-0.427	-0.611	0.233	-0.446	-.491	0.559
	Zn	0.731*	-0.125	-0.162	-0.578	-0.255	0.740*	0.627	0.594	0.743*	0.369	0.305	.112	0.154
	Cu	0.070	-0.540	-0.507	0.225	0.004	-0.034	-0.323	-0.262	-0.014	0.589	-0.406	0.059	0.826**

**r denotes correlation coefficient at 0. 01 level, *r denotes correlation coefficient at 0.05 level.

TABLE 6: Correlations between Agro- Morphological Traits and Soil Physicochemical Characteristics

Agro-morpho-logical trait	Soil physicochemical characteristics												
	pH	O.M (%)	N (%)	Av. P (Cmols/kg)	K (Cmols/ kg)	Ca (Cmols/ kg)	Mg (Cmols/kg)	Na (mg/kg-)	E.C (µs/cm)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Leaf area (cm2)	-0.299	0.020	0.183	0.504	0.684*	-0.428	-0.602	-0.328	0.095	0.358	-0.135	0.477	0.602
Plant height (cm)	-0.838*	0.310	0.101	0.869**	0.317	-0.841*	-0.774	-0.617	-0.687	-0.171	-0.356	-0.143	0.234
Taproot length (cm)	-0.429	0.833**	0.131	0.313	0.458	-0.360	-0.084	0.058	-0.021	-0.366	0.306	-0.118	-0.353
Plant Biomass (g)	-0.266	0.094	-0.195	0.500	0.659*	-0.372	-0.494	-0.352	0.110	0.418	-0.183	0.414	0.610*

**r denotes correlation coefficient at 0. 01 level, *r donates correlation coefficient at 0.05 level.

soil solution[48], soil texture, and water availability in the substrates[49] have been identified. Our present study is not in any way different from other studies conducted elsewhere. Therefore, similar factors could account for the varied concentrations of zinc across the study sites.
Copper in the soil from Kasese (mean = 22.28 ± 1.63mg/kg) was the highest, but undetectable from soil at Ntungamo (0.00 ±00 mg/kg), while that at Bushenyi was found to have a mean value of 1.26 ± 0.04 mg/kg. There was a significant difference in mean values of copper across sites with F (2, 24) = 14.84; $p = .001$. Pair-wise comparison test indicated that copper at Kasese significantly differed from that at Ntungamo and Bushenyi ($p = .001$). The immense concentration of copper in soil from Kasese may be linked to mining and industrialisation activities carried out in that particular district. According to Kasese District Integrated Disaster Risk Reduction and Management Plan Report, (2017-2020), Kasese experienced floods from 2013 to 2020.[50] The floods may have washed down the copper residues from Kilembe copper mines to the study site. Our findings agree with what Oves *et al.*, (2016) highlighted that heavy metals are added to the soil through anthropogenic activities including fertiliser application, waste disposal, use of toxic pesticides, coal combustion and smelting.[9] However, there are other drivers for heavy metal contamination. These include volcanicity and weathering of heavy metal enriched parent bedrocks by geochemical processes.[9]

Important to note, concentrations for all soil micronutrients in the present study were found to be below the recommended maximum safe limit levels of Cu-100 mg/ kg, Mn- 2000 mg/ kg, Zn- 300 mg/ kg and Fe- 50,000 mg/ kg by the Food and Agricultural Organisation / World Health Organisation (FAO/WHO).[51] This implied that the soil currently studied was safe for growth of the plant with no suspicion for any possible toxicity occurrence.

An evaluation of soil texture was performed using one-way Anova test and the proportions of sand varied significantly ($p = .008$). Sand percentage composition was highest in the soil from Kasese (mean = 79.00 ± 1.86 %), followed by that from Ntungamo (mean = 70.44 ± 1.66 %) and was lowest at Bushenyi with mean percentage value of 69.00 ± 2.93 % (Figure 2a). This study's results are comparable to previous findings by Bhatti *et al.*, (2016) who studied soil surrounding Sutlet and Beas rivers neighbouring Punjab, an agricultural area in India. Their study reported a sandy soil texture with sand percentage composition ranging between 78.0 and 93.68%.[39] This is similar to our findings in soil from Kasese. Their study also found similar amounts of soil organic matter as what we found in this study. Thus, the variation in sandy nature of the soil in this study can be explained by the organic matter available at the sites.

Also, the percentage composition of clay particles was significantly different ($p = .006$) across the study sites. However, in this particular case, a reverse trend was observed where the soil from Bushenyi presented the highest percentage composition mean value of (mean = 19.78 ± 2.92%), followed by soil from Ntungamo (mean = 20.00 ± 2.24 %), and was lowest at Kasese (9.67± 1.68 %) (Figure 2b).

The silt composition did not show any significant variation across the sites ($p \geq .05$) (Figure 2c). The variability in soil particle sizes may lie on the nature of the parent rock and anthropogenic activities neighbouring the sites. Also, in this study, soil textural classes were determined and we found soil from Kasese to be sandy and loamy, that from Ntungamo to be sandy loam, and finally soil from Bushenyi was sandy clay loam. According to the investigation done by Jimoh *et al.*, (2019) on the effect of soil texture on the phytochemical accumulation and biological activity of *Amaranthus caudatus* L[52], soil characterised as clay loam was found to have the highest accumulation of flavonoids in ethanolic extracts. This implied that the soil textural classes determined in this study could also influence the phytochemicals and antimalarial activity in *S.pinnata*. Therefore, our study may provide a scientific foundation for determination of optimal phytochemical and biological activities, antimalarial in particular in varied soil textures. Together, the soil from Kasese was characterised by slightly neutral and slightly acidic soil pH, moderate levels of phosphorus, calcium, salinity, moderate levels of nitrogen and organic matter, but sand loamy soils. In Ntungamo, soils were characterised by slightly acidic pH, high levels of nitrogen and organic matter, phosphorus, low levels of calcium and salinity and soil texture was sandy loam. On the other hand, soil from Bushenyi was characterised by alkaline pH, high levels of salinity, calcium, moderate levels of nitrogen and organic matter low levels of phosphorus, whereas the soil texture was sandy clay loamy.

S. pinnata Ionome at Flowering

The plant ionome reported in the present study comprised of mobile ions i.e., nitrogen, magnesium, phosphorus, potassium, as well as immobile ions i.e., calcium, copper, zinc, iron, manganese in plant tissues.[13] A principal component analysis showed that translocation efficiency of plant ionome significantly varied across the sites andwas explained; 40.8 and 21.6% of PC1 and PC2 respectively (Figure 3a-b). However, Sauerbeck and Helal, (1990) indicated that translocation of plant nutrients implicates several factors, which may include; root morphological and physiological characteristics, root-soil interaction, rhizosphere conditions and shoot-root relations.[53] According to work done by White, (2011) under "Ion uptake mechanisms of individual cells and roots: Short-distance transport", phenolics and carboxylates are produced by plant roots within the rhizosphere which increase nutrients uptake.[54] Furthermore, Rietra *et al.*, (2017) noted that nutrients are transported across the root membranes by multiple plasma membrane transporters, which may be attributed to the increased nutrient accumulation in the tissue.

One-way Anova test for normally distributed data indicated that translocation ratios for plant ionome did not significantly vary ($p \geq .05$) except for nitrogen, phosphorus and copper ($p \leq .05$) (Table 4). In regard to this, nitrogen was the most effectively translocated element with a ratio (mean = 31.40 ± 9.24) in Bushenyi, followed by nitrogen in Kasese (mean = 20.28 ± 7.01) and the least translocated in Ntungamo (mean = 6.69 ± 1.59). The variation was significantly different with F (2, 6) =3.36; $p = .04$. Pair-wise comparison indicated that nitrogen only significantly varied between Bushenyi and

Ntungamo (p =.046). Although nitrogen concentration was highest in Ntungamo soils, its translocation efficiency in plant tissues was the poorest, which confirms that the presence of a nutrient in the growth medium does not necessarily guarantee its equivalent absorption and translocation in a plant.[6] This implies that nutrient translocation implicates several other factors in addition to nutrient availability in the soil. According to Rietra *et al.*,(2017), soil parameters tend to interact with each other,which may result into either antagonism or synergism of nutrients in plants.[4] In our study, this was evidenced by positive correlation between plant nitrogen and soil calcium (r = 0.758); plant nitrogen and soil zinc (r = 0.740); $p \leq .05$). In addition, soil pH was observed to positively correlate with the nutrient efficiency of calcium (r = 0.692), magnesium (r = 0.740) and zinc (r = 0.731; $p \leq .05$) (Table 5) in *S. pinnata*. Thus, suggesting that increased soil pH may have raised the solubility coefficients of the particular nutrients in the soil solution, thus, a booster for absorption of plant ionome. On the other hand, Sauerbeck and Helal, (1990) identified root morphology, root physiological processes, shoot-root relations entailing how nutrients are absorbed at root level, transported and redistributed into plant organs, root products and exudates, microbial nutrient turnover and action of root enzymes on the available nutrients in the rhizosphere, as factors that affect nutrient translocation efficiency in plants.[53] Therefore, such factors could also have affected the nutrient translocation in the present study.

Phosphorus translocated better in *S. pinnata* grown in Kasese (mean = 0.43 ± 0.00) than Bushenyi (mean = 0.19 ± 0.02) and Ntungamo (mean = 0.07 ± 0.06; p =.005). One-way Anova test showed significant difference with F (2, 6) and p =.005. According to Tukey's post-hoc comparison, there was significant difference of p =.005 for Kasese versus Bushenyi, p =.015 for Ntungamo versus Bushenyi. The difference may probably be due to variations in soil parameters including soil pH, nitrogen and organic matter.

Although copper was significantly abundant in Kasese soils than Bushenyi, it was poorly translocated in plants grown in Kasese (mean = 0.93 ± 0.37), but instead translocated better in plants of Bushenyi (mean = 2.57 ± 0.18). One-way Anova test showed a significant difference with F (2, 6) = 28.77; p =.001. Tukey's post-hoc test indicated p =.022 for Kasese versus either Ntungamo or Bushenyi, p =.001 for Ntungamo versus Bushenyi. The possible explanation for the observed variation could be environmental factors i.e., limited moisture content, soil aeration and biological processes i.e., microbial activity among others. Notably, soils from Ntungamo were found to have undetectable amounts of copper in both soil and plant tissue. Hence, the translocation efficiency for copper could have followed a similar pattern as the abundance levels in the soil. Remarkably, copper availability in soil positively correlated with copper in the tissue (r = 0.826, $p \leq .01$) (Table 5), confirming that copper availability in soil solution granted its uptake by *S. pinnata*, hence, an indicator for the absence of antagonistic interactions of other nutrients with copper.

Variability of Agro-Morphological Traits across Study Sites

In this study, we presented quantitative agro-morphological traits of *S. pinnata*, grown and monitored under different study sites. The variability in agro-morphological traits of a particular plant mostly implicates environmental factors that encompass soil physiochemical characteristics among others. This is supported by the fact that soil nutrients regulate vital processes for the overall growth and development of plants including photosynthesis[55], protein synthesis[56], cell metabolism and cell division[57], and respiration[58], to mention but a few. Thus, the observed variations in agro-morphological traits of *S. pinnata* in this study were determined using one-way Anova test for parametric and normally distributed data and later discussed in relation to variability in soil physicochemical characteristics across the sites. The total leaf area across the sites significantly varied(p =.001). Plants grown in Kasese had the largest total leaf area (mean = 31.43 ± 2.41cm^2), followed by those at Ntungamo (24.07 ± 1.34 cm^2), while those at Bushenyi had the smallest (mean = 14.82 ± 1.01 cm^2) (Figure 4). Pair-wise comparison using Tukey post-hoc test indicated that a significant difference of p =.001 for Bushenyi versus Kasese and p=.004 for Bushenyi versus Ntungamo. However, the comparison did not show any significant difference in total leaf area of plant from Kasese and Ntungamo (p=.065). Although our results only showed positive correlation between leaf area and potassium, other studies including that conducted by Bagale *et al.*, (2018) in a physiological study for strawberries under hydroponic system noted a positive relationship between electrical conductivity and leaf number up to optimal EC level. However, beyond the optimal levels, vegetative growth such as leaf area reduced due to salt stress[6] thus the same situation may explain the present results. Another soil mineral that could have influenced the leaf area in this study is nitrogen. Nitrogen is a component of chlorophyll pigment, that increases photosynthetic rates and leaf area.[42] Also, according to Mikkelsen and Hartz, (2005), lack of nitrogen in plants presents deficient symptoms such as stunted growth, and chlorosis, particularly in older leaves that finally fall off.[59] Therefore, such physiological processes affect leaf area and consequently variations in leaves from one environment to another. Considering soil pH as a 'master of soil variables'[35], it could have influenced the agro-morphological traits in this study including leaf area. A comparison between suitable soil pH range for the cultivation of *Artemisia annua* (Lam.), a medicinal plant of family Asteraceae showed a pH between 4.5 and 8.5[60], whereas that of *S. pinnata* in this study was found to be between pH of 6.5 and 7.5. This indicated that *S. pinnata*'s soil pH range for its growth is likely to be limited as compared to other medicinal plants. This has an implication on its colonisation range, abundance, distribution as well as usage.

The plants grown in Ntungamo were found to be the tallest and had height mean value of 34.27± 0.85cm, while those grown in Bushenyi were the shortest (mean = 18.54 ± 0.61cm). The plants grown in Kasese were intermediates in height (mean = 31.19 ± 1.16 cm) (Figure 4). Analysis using one-way Anova showed that the plant height was significantly different (p =.001). Turkey' multiple comparison test indicated a significant difference of p =.001 for Bushenyi versus either Kasese or Ntungamo

However, the same test did not show any significant difference in plant height of plants from Kasese and Ntungamo ($p = .866$). Lack of soil nitrogen has been identified with stunted growth in most plants.[59] Similarly, the dwarfness observed in plants from Bushenyi could have been due to inadequate amounts of nitrogen in the soil as compared to the rest of the sites. Furthermore, our findings agree with Giel and Bojarczuk, (2010) who found out that high calcium levels and alkaline pH significantly reduces the assimilation of other nutrients such as phosphorus and manganese, limiting proper growth of plants that grow better in non-alkaline soils.[61] Remarkably, this situation was further demonstrated by the negative correlation between the plant height and either soil pH (r = - 0.838) or soil calcium (r = - 0.841) at $p \leq .05$ (Table 6) in the present study. The fact that potassium promotes photosynthetic processes and protein synthesis and its insufficient levels lead to stunted growth[56], then, the variation in plant height could also be linked to soil potassium. According to Hasanuzzaman *et al.*, (2018), potassium reduces the accumulation of reactive oxygen species by activating antioxidant defence machinery in plants, thus, increasing the stress tolerance. However, our Pearson-moment product correlation did not reflect any significant relationship between these 2 variables. Therefore, this discrepancy may be explained by confounding factors including leaching and erosional drivers which are often associated with agronomical studies. Nevertheless, we noted a significant positive correlation between plant height and available phosphorus (r = 0.869, $p \leq .05$) (Table 6). A study conducted by Taliman et al., (2019) on Soybean-Low phytate Line supports our observed relationship with the fact that phosphorus increases the overall plant growth, photosynthesis and dinitrogen fixation of nodules.[62] Therefore, it is more likely that the plant height was influenced by soil pH, soil calcium and phosphorus levels among other soil parameters.

Another agro-morphological trait that demonstrates the health status of a plant is the length of the taproot. In this study, the plants grown in Ntungamo were found to have the longest taproots (mean = 8.67 ± 0.53 cm) followed by those planted at Bushenyi (mean = 6. 35 ± 0.21 cm). Plants grown from Kasese had the shortest taproots (mean = 5.16 ± 0.33 cm). The mean values of the root length were significantly different across the sites ($p = .001$). Comparatively, turkey's post-hoc test indicated the significant difference of $p = .001$ for Kasese versus Ntungamo, $p = .03$ for Kasese versus Bushenyi and $p = .001$ for Ntungamo versus Bushenyi. We suggested that the variation in plant root length could have been influenced by the nature of soil texture which in turn determine the availability of water in the soil. This may further be explained by findings of previous works done by Figueiredo *et al.*, (2016) about the influence of soil texture on nutrient allocation in *Eremanthus erythropappus* (DC) Macleish, where they noted that soil texture increased allocation of potassium, magnesium, phosphorus, sodium and copper into the roots. Similarly, such scenario could have happened in this study, causing the observed variation in root length across the study sites. Also, Pearson-moment product correlation provided evidence for positive relationship between plant root length and soil organic matter (r = 0.833; $p \leq .01$) (Table 6). In support to our observed relationship, a recent study done by King *et al.*, (2020) on soil organic matter as a catalyst of crop resource capture, noted that organic matter reduces the compaction of the soil, improves soil aeration and maintains soil moisture which permit the uptake of soil nutrients required for proper root growth and development.[40] Here, the variation in environmental factors and the key roles of organic matter in the soil could explain the difference in the length of taproots across study sites.

Often, plant biomass is considered among the agro-morphological traits used for selection of cultivars with superior performance potential.[34] In our study, we found that plants from Kasese had the highest mean values of plant biomass (mean = 7.65± 0.64 g), while those from Bushenyi were found to have the lowest plant biomass (mean = 2.03 ±0.18 g). The mean value of biomass of plants from Ntungamo was 5.05 ± 0.45 g (Figure 4).

One-way Anova test showed a significant difference ($p = .001$). When tukey post-hoc test was conducted, a significant difference of $p = .001$ was found to exist between mean value of plant biomass from Kasese and that of either Ntungamo or Bushenyi. On the other hand, the mean values of plant biomass of Ntungamo and Bushenyi were statistically the same ($p = .114$). In this study, we expected significant positive correlations between plant biomass and most of the soil nutrients that regulate the photosynthetic related processes played by; nitrogen in protein synthesis[63], phosphorus in phytic acid uptake[62], potassium in enzyme activation for metabolic reactions[64] magnesium as a constituent of chlorophyll pigment in the plant chloroplasts[65,66], calcium in control of cell division[13] and plant protection through cell wall defence[67], to mention but a few. However, from this study, this was not the case with most of them, except potassium (r = 0.659) and copper (r = 0.610; $p \leq .05$) (Table 6).

The insignificant correlations may be attributable to environmental factors including soil erosion drivers. In line with our argument, Rietra *et al.*, (2017) noted that agronomical experiments are influenced by external factors such as limited and excess water, uncontrolled temperature and fluctuating soil pH. However, they support such agronomical trials to be conducted owing to the facts that results from controlled experiments do not always apply under field conditions. On this, we therefore, conclude that agronomical experiments are as important as those conducted under controlled environments. Also, a controlled laboratory experiment should be considered in further studies.

Generally, plants grown in Kasese were characterised with the largest total leaf area and highest plant biomass while those from Ntungamo were characterised with tallest plants with longer taproots. Exceptionally, plants grown in Bushenyi were the shortest, with the smallest total leaf area. Therefore, the pharmaceutical investors who are always interested in aerial parts (increased total leaf area and highest biomass), should consider growing *S. pinnata* in Kasese where soil pH was slightly acidic and slightly neutral, with phosphorus, calcium, magnesium, electrical conductivity, nitrogen, organic matter in moderate levels.

From this study, therefore, we recommend soil pH between 6.50 and 7.25, low to moderate levels of: nitrogen (0.05-0.20%), organic matter (1.0-3.25%), calcium (4.0-5.0 Cmols/kg), available phosphorus (40.0-70.0 mg/kg), iron (20.0-60.0 mg/kg), copper (9.0-48.0 mg/kg), manganese (10.0-40.0 mg/kg), low to moderate levels of zinc (6.0-15.0mg/kg) to be available for maximum total leaf areas and highest plant biomass. In this study, therefore, phosphorus of about 20.0 to 85.0 mg/kg was associated with highest plant height, whereas for longer taproots, organic matter (2.79-4.74%) should be provided in the growth media. Calcium salts that alter soil salinity must also be maintained within a range of 4.00 to 6.14 Cmols/kg. Thus, the above quantitative measures of soil characteristics should be put into consideration when formulating fertilisers for nutrient-deficient soils for the growth of *S. pinnata*.

CONCLUSION

The physicochemical characteristics of the soils from where *S. pinnata* was grown was associated with the plant ionome and agro-morphological traits. Alkaline soil pH, caused by high concentration of calcium and magnesium available in the soil solution adversely affected the *S. pinnata* growth performance. Soil organic matter, nitrogen as well as availability of phosphorus were found to reduce the soil pH, which resulted to better plant performance. Thus, the present investigation offers a scientific proof for *S. pinnata*'s potential to perform best in soils characterised by slightly acidic and slightly neutral soil pH, sandy loam texture and non-saline at Kasese in Western Medium-High Farmland. For the purposes of designing a suitable fertiliser formula for improved growth of *S. pinnata* and where it does not thrive well, soil pH between 6.5 and 7.5, the synergistic interactions that occurred between calcium and magnesium, soil nitrogen and phosphorus during the nutrient translocation process should be considered.

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