Assessment of the Mineral Elements in the Soil of a Bixa Field and their Distribution in various Plant Tissues on Application of Organic and Inorganic Fertilizer Amendments

Pole F.N[1].*, Ambuko J[2], and Wasilwa L.A[1]. Gweyi-Onyango J[3]
[1]Kenya Agricultural and Livestock Research Organization (KALRO)
[2]Department of Crop production, University of Nairobi (UoN)
[3]Department of Agricultural science and Technology, Kenyatta University (KU)
***Correspondent Email**: finyange@gmail.com

Abstract

Bixa *orellana L.* is a shrub that is commercially known as 'annato'. Its main product is organic dye, which is present in the seed coat, commercially called 'annatto'. It is widely used in the cosmetic and the food industry for giving red to orange-yellow colours to foodstuffs and cosmetic products. Bixa farming in Kenya has declined since the 1970's due to the use of cheap synthetic materials as food and cosmetic colours, which have proved to be carcinogenic. This has triggered a high demand for natural colours from bixa. However, the quality of bixa remains low due to suboptimal application of fertilizers. The objective of this study was to determine the mineral elements in the soil and their distribution in tissues of bixa plants treated with organic and inorganic fertilizer amendments for increased bixa quality. A study was undertaken at the Bixa Company farm at Shimoni in Kwale County in 2018 and 2019. Bixa plants of the same age were applied with different levels of organic (poultry manure at 5 tons/ha and 10 tons/ha) and inorganic fertilizers (NPK 17:17:17 at 100 kg/ha and 150 kg/ha) and a combination of the two (2.5 tons/ha plus 50kgs/ha NPK and 5 tons/ha plus 75kgs/ha NPK) with a control in a randomized complete block design with three replications. Bixa roots, stems and leaves were analyzed for the distribution of both macro and micro nutrients. The data obtained was subjected to ANOVA using SAS version 8.2. Significant means were separated using LSD at $\alpha = 0.05$. The study revealed that application of manure at the rate of 10t/ha and NPK at the rates of 100kg/ha and 150kg/ha significantly ($p < 0.05$) **improved soil fertility and nutrient levels in the various plant tissues compared to the other treatment combinations. More nutrients were found in the leaves followed by stems and then the roots. At the same time the quality of the bixa produced was higher in the three treatments than the rest.**

Key words: Bixa, organic and inorganic fertilizers, mineral elements, plant tissues

Introduction

Bixa *Orellana L.* is a shrub that is commercially known as 'annato'. It originated from the tropical region of the Americas (Baer, 1976). Central and South American natives originally used the seeds to make red body paint as well as lipstick. For this reason, the Achiote as is commonly referred to by the Spanish is sometimes called the lipstick tree (Lauro, *et.al*, 2000). The main product that is obtained from *Bixa orellana* is an organic dye which is present in the seed coat, commercially called 'annatto' in English, 'rocou' in French, 'achiote' in Spanish and 'orlean' in German (Khare, 2007). In Latin American cuisine, annatto is not only used to give an attractive red colour to meat, fish and rice dishes but also to impart distinctive flavor notes (Byrd 1986).

The crop has a number of uses in the food, cosmetic, pharmaceutical and the textile industries respectively. In the cosmetic industry, annato is used for the production of nail gloss, hair oil, lipstick, soap and household products like floor wax, furniture polish, shoe polish, brass lacquer and wood stain (Van Wyk *et.al*, 2005). In the textile industry, annatto has been used for the dyeing of cotton fabrics, wool and silk in particular, thereby giving an orange-red colour which becomes more yellow if the dye is mixed in the dye-bath with wood-ash or sodium carbonate, and then dyed fabric is passed through a weak solution of tartaric or citric acid (Green, 1995). Sometimes annatto is used in mixtures with other vegetable dyes such as curcumin from turmeric in order to make more appealing colours (Jansen, 1981). The seeds and leaves are used in the manufacture of traditional medicine for the treatment of fever, dysentery, kidney diseases and poisoning by cassava (Preston, *et.al*, 1980). The seeds are edible and nutritive but slightly purgative and said to be effective against fever, dysentery, kidney diseases and poisoning by cassava (Preston, *et.al*, 1980).

The major commercial producers of *Bixa orellana* in the world are countries in South America (Brazil and Peru), Central America (Costa Rica, Nicaragua and Panama), the Caribbean (Grenada, Haiti and St. Lucia), Africa (Kenya and Mozambique), India and Sri Lanka. Peru is the largest exporter of annatto seed annually with about 4000 tons and Brazil being the largest producer with about 5000 tons per year. However, almost all the Bixa produced in Brazil is consumed locally (Smith, 1995). Kenya exports about 1500 tons of annatto seed and extracts annually after Peru, thereby becoming the second largest exporter, particularly to Japan and the European Union countries. Côte d'Ivoire and Angola are also exporters (Smith, 2003). In Kenya, *Bixa orellana* is grown by smallholder farmers, particularly in the coastal regions of Kwale, Kilifi and Lamu Counties (Muhindi, 2006).

The main market for annatto is the United States with 3000 tons per year, followed by Western Europe (2500 tons) and Japan (1500 tons). Almost 70% of the product is used in the importing countries to colour cheese (Anand, 1983). Trade in annatto extracts (instead of dried seeds) has increased strongly since the 1980s, with the water soluble norbixin extract being largest in volume, followed by vegetable oil extracts, and solvent-extracted bixin in third place (Ellison, 1999). Kenya is among the world leading countries in bixa (annatto) production, an industrial and cash crop that does well in the coastal region of the country (Smith, 1995). Its market share for food colours is expected to gain increasing importance since most of the synthetic substances have proved to be carcinogenic (Jasen *et.al* 2005). However, bixa farming has continued to decline since the 1970's. Most farmers have cut down their trees while others have left them in the bushes unattended as a result of the lower prices offered for the commodity which is attributed to competition with cheap synthetic materials that were introduced in the market (Muhindi, 2006).

The quality of the bixa produced has also gone down as the level of bixin (the most essential product) has greatly reduced. The limited use of synthetic food colours has however triggered a high demand for bixa in the world market. This has been demonstrated by the sharp increase in prices for the product at the farm gate where farmers are currently selling the produce to the Kenya Bixa Company at a price of KES 42.00 per kilogram in 2013 to KES 75.00 in 2019. In a bid to revitalize the Bixa sub-sector, a study was undertaken in Kwale County with the main objective of determining the distribution of mineral elements in the soil and plant tissues of bixa plants treated with organic and inorganic fertilizer amendments.Plants, like animals and humans need all the essential nutrients, water, light and energy to synthesize food. If any of the essential nutrients are not available or low in the soil, the plant functions will be upset and characteristic symptoms will develop. Soil testing will therefore provide information on the level of total or available nutrients in order to formulate suitable fertilizer recommendations to correct any nutrient deficiencies or amendments to rectify any toxicity problems. The use of organic and inorganic fertilizers and the harvesting of bixa at the right physiological maturity as well as the drying of the crop in ideal environmental conditions has been known to improve productivity and quality of bixa seed thereby increasing the bixin quality (Elias et.al, 2002).

Materials and Methods

Study area

The study was conducted at the Kenya Bixa Company nucleus farm located at Shimoni in Lungalunga Sub-county of Kwale County, 65 kilometres South of Mombasa in 2018 and 2019. The site is 50 m above sea level and lies at latitude 3^0 05'N and 38^o 33'E. The rainfall pattern is bi-modal with peaks in May and November during the long and short rain seasons respectively. Average annual rainfall is 900 mm. The mean monthly maximum and minimum temperatures are 32^oC and 21^oCrespectively. The soils are red sandy loams that are deep and well drained. The main economic activities are coconut, cashewnut and bixa farming, fruit farming and mixed farming of both crops and livestock. The farm has an already established bixa plantation, with trees in different stages of development.

Experimental Procedure

Trials were therefore established using the already existing bixa plants of three varieties namely the red pointed pod, pink rounded pod and the green pointed pod respectively. Bixa plants of the same size and age per variety were identified by measuring the tree canopies. For each variety, a block of 315 trees was identified and marked out. In each of the three blocks, 21 plots made up of 15 bixa trees each were also measured and marked out at a spacing of 4 x 4 metres. This gave a total of three blocks and 63 plots. Treatments were then administered in the three blocks in a RCBD with three replicates at the time when the plants had started flushing. The treatments comprised of two levels of NPK fertilizer (100, 150kg/ha) and 2 levels of poultry manure (5 tons, 10 tons/ha) as well as two levels of manure-NPK combination at half the rates of the manure and fertilizer levels (2.5 tons manure plus 50 kg NPK/ha and 5 tons manure plus 75kg NPK/ha) and a control. Weeding of the blocks was undertaken to ensure that they were weed free at all times. Pruning was also carried out to remove all the dead and entangled branches from the trees so as to enable enough light penetration and free air circulation into the plants.

Determination of the mineral elements in soil treated with organic and inorganic fertilizers

Before the application of the various treatments of organic and inorganic fertilizer amendments in the bixa plots within the three blocks, soil samples were collected for analysis in order to determine the initial levels of nutrients available for plant use in the respective plots. The purpose was to give an idea of the additional nutrients required to effectively support plant growth and improve the yields. The soil samples in each blockwere collected in a diagonal manner and around the canopies of the bixa plants. In each plant to be sampled the soil was collected at three different points around the canopy. A soil auger was used to collect the samples to a depth of 15 cm since the feeder roots are located around that depth. All the soils sampled in each blockwas put together in a clean bucket and taken to a drying shade where it was spread on a polythene sheet. Once dry (to a moisture content of 13%), the soil was thoroughly mixed and passed through a 2mm sieve. A representative sample of 1 kilogram was collected from the main sample in each blocksince the soils were homogenous and put in a polythene bag and properly labelled. All the 3 samples were then be taken for analysis in the laboratory at the KALRO National Research Laboratories (NARL) in Kabete.

The prescribed soil amendments as per the various treatments in the plots within the blocks were then administered after weeding and pruning the trees. The different fertilizer and manure rates were applied around the bixa tree canopies by digging a trench that is one foot wide and half a foot deep. The soil amendments (organic and inorganic fertilizers respectively) were then sprinkled in the trenches around the trees and thereafter the trenches were covered using the dugout soil. The developing bixa pods were given the necessary management practices until they attained maturity stage. After the bixa was harvested, another set of soil samples was taken in each plotwhereby one sample was collected for each treatment for all the seven treatments. The samples were prepared in a similar manner as for the ones taken before the application of the amendments. They were then taken for analysis at the same laboratory (NARL) in Nairobi. The samples were analyzed for the following nutrients using the modified mehlic1 laboratory technique: Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Sulphur (S), Zinc (Zn), Boron (B), Iron (Fe) and the soil pH in order to gather some information on the levels of nutrients available in the soils and theirdistribution in the different plant tissues as per the respective treatments. The trial was conducted for two seasons (in the short rains of 2018 and long rains season of 2019 respectively.

Determination of the mineral elements in the tissues of plants treated with organic and inorganic fertilizers

Just like the soil analysis, the mineral elements in the plant tissues were analysed for nutrient distribution by collecting plant tissue samples. The samples were collected when the bixa pods had attained maturity but just before harvesting. In all the three blocks, roots, leaf and stem samples were collected from all the bixa plants in every plot and preserved separately. The leaves to be analyzed were collected from the recently matured fully expanded leaf. In each plant, six leaves were collected to make a total of 60 leaves per plot. For the stem tissues, a section of the bark of the plant stem measuring 5 x 10 cm was collected from each plant at a height of 50 cm from the ground, while the roots were collected by digging the soils around the canopy of the bixa plant at three different locations within the canopy and the roots were cut out from the tree. A total of six root pieces of 15 cm in length were cut from each treatment.

Each of the collected samples were placed in a labelled, open paper bag and later on put in a water tight bag. They were then taken to the closest laboratory or area for processing within 24 hours. In this case, the samples were taken to KALRO-Mtwapa laboratory where the initial processing took take place. Care was taken to ensure that there was no mix up of samples. In the laboratory, the samples were dried at 65 degrees centigrade in a forced draught oven for 24 hours for the leaf samples and 48 hours for the stem and root samples respectively. Thereafter, each of the samples was chopped into small pieces and ground in a stainless steel mill fitted with a screen less than 1mm in diameter. The finely ground samples were then packaged in a plastic container or bag that was properly labeled. All the ground samples were then taken for analysis of the total N, P, K, Ca, S, Zn, Band

Fe at the National Agricultural Research Laboratories (NARL) at Kabete in Nairobi. In each block 1 sample was collected for the 3 different plant tissues per each of the 7 treatments, making a total of 21 samples per block.

Statistical data analysis

Data for soil, roots, stem and leaf samples collected were keyed into excel spreadsheet and subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS) version 8.2 to determine whether the variables being tested were significant at 5% probability level. Significnat means were separeted using Least Significance Difference (LSD)at 5% probability level.

Results and Discussion

Determination of the mineral elements in soil treated with organic and inorganic fertilizers

Results of the initial soil analysis in the three blocks indicate that all the three fields have similar soil fertility status. The soil reaction (pH) was found to be satisfactory for tree crops growth. Nitrogen, phosphorus, potassium and zinc were deficient and the soil organic carbon content was also very low. The *Bixa orellana*plantprefers organically rich and well-drained soil with a pH value that ranges between 5.5 and 7.5 (Table 1).

Table 1: Initial laboratory soil analysis results

	Initial soil analysis data					
Field	Block I		Block II		Block III	
Lab. No/2018	8002		8003		8004	
Soil depth (30 cm)	Value	Class	Value	Class	Value	Class
Fertility results						
• Soil pH	5.92	Medium acid	5.78	Medium acid	6.02	Slightly acid
• Total Nitrogen %	0.08	low	0.07	low	0.09	low
• Total Org. Carbon %	0.69	low	0.63	low	0.81	low
Phosphorus ppm	15	low	5	low	25	low
Potassium me%	0.12	low	0.08	low	0.06	low
Calcium me%	5.2	adequate	4.6	adequate	5.8	adequate
Magnesium me%	1.14	adequate	1.00	adequate	1.25	adequate
Manganese me%	0.21	adequate	0.22	adequate	0.50	adequate
Copper ppm	1.10	adequate	1.30	adequate	1.00	adequate
Iron ppm	39.9	adequate	28.7	adequate	13.6	adequate
Zinc ppm	1.01	low	0.77	low	0.78	low
Sodium me%	0.12	adequate	0.10	adequate	0.08	adequate

Where, me% stands for miliequivalent; ppm stands for parts per million; all the nutrients analysed are given in figures of the available minerals.

The following recommendations were given after the soil analysis:

(i) Application of 20 kilograms of well decomposed manure or compost mixed well with 100 grams of N: P: K 17:17:17 or N: P: K 19:19:19 fertilizer per tree in split applications one or two weeks after theonset of the rains.

(ii) To correct zinc deficiency spray growing trees with a foliar feed containingzinc.

Results of soil analysis after application of fertilizer amendments

The results of the analysis that was carried out on the soil after the application of the various fertilizer ammendments per treatment revealed that there was significant ($p < 0.05$) improvement on the soil fertility on each fertilizer amendment rate applied per treatment compared to the control (Table 2) and the subsequent discussions on each of the mineral element.

Table 2: Soil analysis after application of organic and inorganic fertilizer amendments

Treatment	Soil pH	Org. Carbon	N	P	K	Ca	Mg	Mn	Cu	Fe	Zn	Na
NPK@150kg/ha	7.647a	1.650b	0.580b	5544b	1204.3a	20.68a	7.378a	96.590b	2.063a	775.8a	1.3133ab	2368a
NPK @100kg/ha	6.437b	1.740b	0.603ab	5794ab	1154.6a	20.12a	7.234ab	102.89b	1.672b	737.0a	1.4123a	2294a
Manure only 10t/ha	6.383b	2.487a	0.633a	6012a	1116.6a	19.78a	6.433b	127.64a	1.615b	705.8a	1.5135a	2270a
Manure 5t+npk@75kg	6.200c	1.397c	0.510c	4352c	885.9b	13.27b	4.850c	57.970c	1.562bc	444.5b	1.2858b	1498b
Manure @5t/ha	6.140c	1.237d	0.472cd	4265c	806.7b	12.73b	4.830c	57.840c	1.537bc	427.0b	1.2553b	1476b
Manure@2.5t+npk@50kg/ha	6.073c	1.187d	0.448d	4089c	753.3b	10.59b	4.061c	44.110d	1.313c	392.5b	1.1546bc	1372b
Control	5.907d	0.710e	0.080e	15d	0.1c	5.2c	1.130d	0.310e	1.133d	27.4c	0.8533c	0c
Means	6.398 b	1.486 c	0.475 cd	4295 c	845.9 b	14.62 b	5.131 b	69.621c	1.556 bc	501.4 b	1.2554 b	1611 b
LSD	0.1339	0.1489	0.0447	392.5	165	5.092	0.8021	13.45	.2507	219	0.0913	468.7
CV	1.2	5.6	5.3	5.1	11	19.6	8.8	10.9	9.1	24.6	39.6	16.4
P-Value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Soil pH

The results indicate that the level of pH greatly improved from medium acid to slightly acidic and alkaline conditions across all the three blocks after the application of fertilizer amendments, with a mean value of 6.398 compared to the initial 5.92. Application of both organic and inorganic fertilizers greatly improved the soil P^H across all treatments (Table 2).

Total organic carbon(%)

The results show that the level of organic carbon improved from low to moderate, with the application of manure at the rate of 10 tonnes/ha producing significantly higher($p < 0.05$) level than the rest of the treatments across the blocks(Table 2).

Total nitrogen (%)

The results show that the application of fertilizers and manures increased the amount of nitrogen in the soil from low to high levels with the highest percentage being recorded in the treatments of manure application at the rate of 10 tonnes/ha.

Phosphorus (ppm)

The application of both organic and inorganic fertilizer amendments greatly increased the quantities of phosphorous in the soil across the blocks and across all treatments to a situation whereby there were adequate quantities of the nutrient available for the plants. Again the treatments of manure application at the rate of 10 tonnes/ha produced the highest quantities than the rest (Table 2).

Potassium (me%)

Results of the analysis indicate that the application of organic and inorganic fertilizers increased the amount of potassium in the soil across all treatments. The treatments of manure application at the rate of

10tonnes/ha and those of NPK 100kg/ha together with NPK 150kg/ha produced significantly higher ($p < 0.05$) amounts of potassium compared to the rest.

Calcium (me %)
The results indicate significantly higher amounts of calcium produced at the application rates of NPK 100kg/ha, NPK 150kg/ha and that of manure at the rate of 10 tonnes/ha respectively. In general, the application of fertilizer amendments increased the quantities of calcium available for bixa plants in the soil to adequate levels.

Magnesium me%
Application of the organic and inorganic fertilizers increased the quantities of magnesium in the soil across all treatments. However, the treatments ofmanure application at the rate of 10 tonnes/ha and those of NPK 100kg/ha together with NPK 150kg/ha produced significantly higher ($p < 0.05$) amounts of potassium compared to the rest(Table 2).

Manganese (me %)
The results indicate that the levels of manganese in the soil increased with increased levels of fertilizer and manure application across the blocks and treatments. The treatments of manure application at the rate of 10 tonnes/ha produced significantly higher ($p < 0.05$) quantities of manganese compared to the rest.

Copper (ppm)
Generally, the quantities of copper in the soil increased with the application of both organic and inorganic fertilizers. The application of NPK fertilizer at the rate of 150kg/ha produced significantly higher ($p <0.05$) quantities of copper compared to the rest. At the same time manure application at the rate of 10 tons/ha and that of NPK at 100kg/ha produced higher results but were not significantly different ($p > 0.05$) to one another(Table 2).

Iron (ppm*)*
Significantly high ($p < 0.05$) quantities of iron were produced in the treatments of manure application at the rate of 10 tons/ha and those of NPK fertilizer at the rates of 100kg/ha and 150kg/ha respectively. Adequate levels of iron were also recorded in the other treatments across the sites.

Zinc (ppm)
The quantities of zinc greatly increased from low levels to moderate and adequate levels respectively with increased application rates of organic and inorganic fertilizers. The treatment of manure application rate of 10 tons/ha and that of NPK 100kg/ha produced significantly higher ($p < 0.05$) quantities of zinc compared to the rest of the treatments(Table 2).

Sodium (me %)
The levels of sodium increased with increased rates of fertilizer and manure application. Significantly higher p <0.05) quantities of sodium were recorded in the treatments of manure application at the rate of 10 tons/ha and those of NPK fertilizer at the rate of 100kg/ha and 150kg/ha respectively.

The results agree with the findings of Elias *et.al*, (2002) who reported similar findings when he used fertilizers to increase growth rate and yields of bixa trees in agroforestry systems on an Amazonian Ferralsol(Table 2).

Mineral elements in the tissues of plants treated with organic and inorganic fertilizers
Results of the mineral elements in the tissues of bixa plants under the different treatments of organic and inorganic fertilizers indicate that there were varying levels of different

elements in the different plant tissues for both the macro and micro-nutrients (Figure 1).

After application of the fertilizer ammendments in the various treatments within the blocks, results of the tissue ananlysis indicate that there was a significant increase ($p < 0.05$) of the nutrients in the leaves compared to that of the stem and the roots (Figure 2). The increase of nutrients in the leaves could be attributed to the fact that the manufacture of food for the plant takes place in the leaves and hence the need to have more nutrients in that part of the bixa plant.

Distribution of the different nutrient elements in the plant tissues under different treatments

The results indicate that the different nutrients were distributed in the roots, stems and leaves of the bixa plants in varying levels as per the treatment (Table 4).

Table 3: A general guide to macro and micronutrients' critical levels

Nutrient Type	***Ratings***				
	Very High	*High*	*Medium*	*Low*	*Very low*
Soil pH	7.9-8.4	6.6-7.2 / 7.3-7.8	6.1-6.5	4.6-5.5	< 4.5
Electrical Conductivity (mS*/cm)	>2.0	0.8-2.0	0.4- 0.8	0.15-0.4	< 0.15
Soil organic matter (Carbon and Nitrogen)					
Carbon	> 20	10-20	4-10	2-4	< 2
Nitrogen	>1.0	0.6-1.0	0.3-0.6	0.1-0.3	< 0.1
Phosphorous: Olsen-P (mg/kg)	> 50	30-50	20-30	10-20	< 10
Sulphur (mg/kg)	> 150	50-150	15- 50	5-15	< 5
Boron	-	2-5	1-2	0.5-1	<0.5
Zinc	-	5-15	0.8-5	0.3-0.8	<0.3
Iron	-	-	> 4.5	2.5-4.5	<2.5
Copper		5-15	0.3-5	0.1-0.3	<0.1
Manganese		50-500	2-50	1-2	<1.0

Source:*A Training Manual and Guide on Soil and Plant Sample Collection, Preparation and Interpretation of Chemical Analysis (K. Thiagalingam**:** November 2000)*

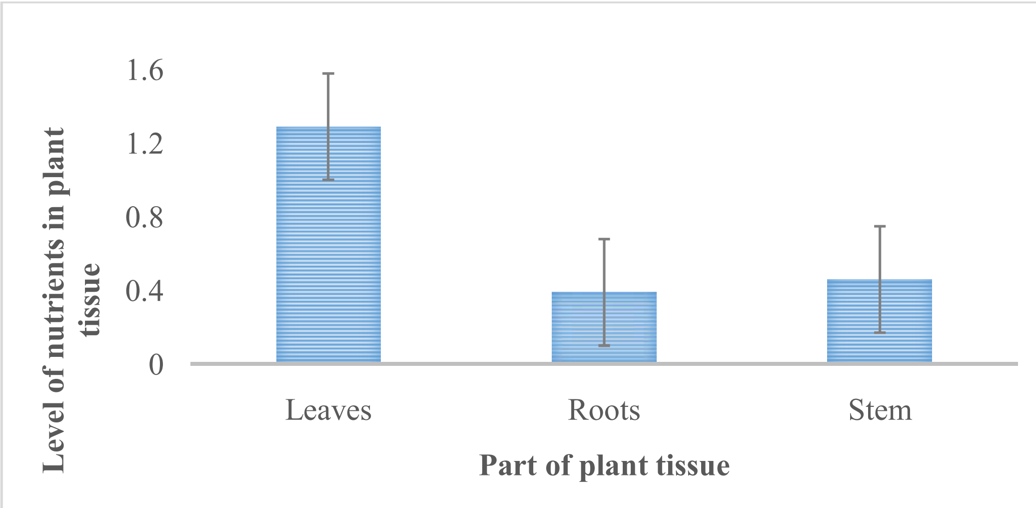

Figure 1. Initial nutrient levels in plant tissues before application of ammendments

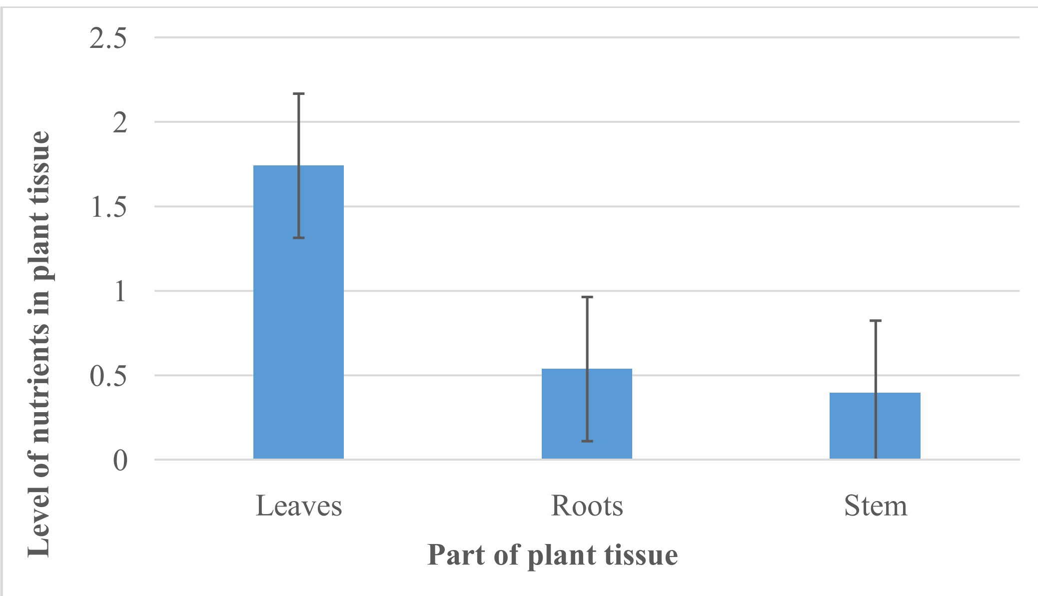

Figure 2. Nutrient levels in plant tissues after application of fertilizer ammendments

Performance of the individual mineral elements in the different plant tisues of bixa plants

The results on the levels and distribution of various nutrients in the roots, stems and leaves of the bixa plants under different treatments of manure and fertilizer amendments are as described below:

Nitrogen (N)

The results indicate that the levels of nitrogen in the plant roots, stem and leaves were significantly different($p < 0.05$) from one another, with the highest amount of the nutrient (1.74%) available in the leaves and the lowest (0.4%) being recorded in the stem (Table 4). The treatment of manure application rate of 10 tons/ha produced significantly ($p < 0.05$) high levels of nitrogen in the leaves than the rest of the treatments. In general, the application of fertilizer amendments increased the levels of nitrogen in the plant across all treatments.

Table 4: Distribution of nutrient elements in tissues of bixa plants

Nutrient Type	Mean nutrients levels in plant tissues			CV	LSD	p-value
	Roots	***Stem***	***Leaves***			
Nitrogen (N)	0.5371b	0.3972c	1.7415a	12.1	0.10034	<.001
Phosphorus (P)	4536a	3256b	4529a	38.6	1474.5	0.028
Potassium (K)	894b	809c	1620a	14.5	149.1	0.005
Calcium (Ca)	16.92b	17.1b	29.34a	14.2	2.784	0.024
Magnesium (Mg)	5.379b	4.162c	6.641a	6.641a	0.7101	0.713
Iron (Fe)	672.3a	337.7b	317.4b	44.8	184.5	0.009
Manganese (Mn)	69.16c	80.87b	113.04a	21.2	17.29	<.001
Zinc (Zn)	0.00907b	0.00992a	0.00943a	77.7	0.00685	0.609
Sodium (Na)	1733a	1174b	934c	18	214.7	<.001

The results confirm the fact that nitrogen is so vital because it is a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide (i.e., photosynthesis), hence the more reason why it was more in the leaves than the other plant tissues. At the same time, nitrogen plays a significant role as a major component of amino acids, the building blocks of proteins. Without proteins, plants wither and die.Nitrogen is an important component of many essential structural, genetic and metabolic compounds in plant cells and an elementary constituent of numerous important organic compounds including amino acids, proteins, nucleic acids, enzymes, and the chlorophyll molecule (Brady and Weil, 2010).

Of all the essential nutrients, nitrogen is the one that is most often limiting for crop growth. Nitrogen is the nutrient which normally produces the greatest yield response in crop plants, promoting rapid vegetative growth and giving the plant a healthy green color. Roots take up nitrogen in its inorganic forms, nitrate (NO_3) and ammonium (NH_4^+) ions. Once inside the plant, NO_3 is reduced to the NH_2 form and is assimilated to form the organic compounds (Peoples, et.al, 1995).The results agree with the findings of Caldeira, et.al, (2017) who reported that the amount of nitrogen tends to be more in the leaves and especially young leaves while determining the levels and redistribution of nutrients in the leaves of *Bixa arborea* Huber. and *Joannesia princeps* Vell. in a forest restoration area in the Amazon.

Phosphorus (P)

Results of the analysis indicate that the amount of phosphorous was highly significant ($p < 0.05$) between the leaves and the stem but not significantly ($p > 0.05$) different between the roots (Table 4). The application of fertilizer amendments (both organic and inorganic) greatly increased the levels of phosphorus in the bixa plants. The treatment of manure application at the rate of 10 tons/ha produced significantly ($p < 0.05$) higher levels of phosphorus (540 mg'kg) in the plat tissues as compared to the rest of the treatments. However, they were not significantly ($p > 0.05$) different from those of the fertilizer application rates of NPK only at 100kg/ha and that of NPK only at 150kg/ha, respectively.

While working on annatto trees (bixa orellana) to determine the mineral nutrition, growth and yields in agroforestry on an Amazonian Ferralsol, Elias *et.al*, (2002) revealed thatthe foliar nutrient concentrations of annatto showed a significant seasonal pattern, with much higher concentrations of Nitrogen and Phosphorus and slightly higherconcentrations of Potassiumcompared to the roots and stem when the foliage was still young after the post-harvest pruning.The presence of high levels of Phosphorus in leaves and roots confirms its importance for the general health and vigor of all plants. Some specific growth factors that have been associated with adding phosphorus to the crop include stimulated root development, increased stalk and stem strength, improved flower formation and seed production, more uniform and earlier crop maturity, increased nitrogen-fixing capacity of legumes, improvements in crop quality, and increased resistance to plant diseases.

Phosphorus is an essential nutrient, both as a component of several key plant structural compounds, and as a catalyst in numerous key biochemical reactions in plants. Phosphorus is particularly important for its role in capturing and converting the sun's energy into useful plant compounds (Foth, 1990).It is a component of various enzymes and proteins as well as a vital component of DNA, the genetic "memory unit" of all living things and also a component of RNA, the compound that reads the DNA genetic code, to build proteins and other compounds essential for plant structure, seed production, and genetic transfer (Kilmer et.al.,1968). The structures of both DNA and RNA are held together by phosphorus bonds. Phosphorus is a vital component of adenosine triphosphate (ATP), the "energy unit" of plants. ATP forms during photosynthesis has phosphorus in its structure, and participates in processes from the beginning of seedling growth through to seed formation, and maturity (Ferguson, and De-Groot, 2000).

Potassium (K)

The results on the distribution of potassium in the tissues of bixa plants indicate that there were significant differences ($p < 0.05$) in the nutrient quantities observed in the roots, stem and leaves respectively. More quantities of potassium 1620percent miliequivqlentwere found in the leaves while the stem had the least (Table 4). The treatments of manure only at 10 tonnes/ha and that of NPK only at 100kg/ha produced significantly higher ($p < 0.05$) amounts of potassium in the plant parts than the rest of the treatments. In general, the application of fertilizer amendments increased the levels of potassium in the plant tissues.

The results agree with those of Xinxiang Xu, et.al, (2020) who reported that Potassium content in the roots, stems, and leaves of seedlings increased with the increased supply of Potassium when conducting an experiment to determine the effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. However, the results also showed that the accumulation of potassium was more in the leaves, follwed by the stem and the roots respectively in that order.

Potassium, along with nitrogen and phosphorus, is one of the three essential plant macronutrients, and is taken up by crops from soils in relatively large amounts. It increases yields and improves the quality of agricultural produce. Potassium also enhances the ability of plants to resist diseases, insect attacks, cold and drought stresses and other adverse conditions (Cakmak, 2005). It helps in the development of a strong and healthy root system and increases the efficiency of the uptake and use of nitrogen and other nutrients. In addition, potassium has an important role in livestock nutrition (Amtmann et.al. 2008).

The importance of potassium stems from its multiple roles in the plant as it is involved in the activation of more than sixty enzymatic systems in the plant cell, and in the synthesis of proteins, vitamins, starch and cellulose which ensure normal plant metabolism, plant growth and formation of strong tissues (Wang, et.al. 2013). Potassium helps in photosynthesis, the process through which the sugars and energy that the plant needs for its development are formed and converted. Potassium also controls the opening and closing of the leaf stomata, which regulate the water status in the plant (Leigh, et.al., 1984). It plays an essential role in the formation of starch and in the production and translocation of sugars. Potassium is, therefore, of special value to carbohydrate-rich crops such as sugarcane, potato and sugar beet. The production of starch and sugar in legumes boosted by potassium benefits the symbiotic bacteria living on the roots and thus improves the fixation of nitrogen (Datnoff, et. al.2007).

Potassium not only increases yields but also enhances crop quality. It improves the nutritive value of grains, tubers and fruits by increasing the contents of protein and oil in seeds of starch, in tubers and seeds, as well as vitamin C and sugar in fruits. With an adequate supply of potassium, cereals produce plump grains and strong stalks. Potassium also improves the flavor and color of fruits and increases the size of tubers and fruits. In addition, it increases the resistance to various injuries during storage and transportation, thus extending shelf life (Zhao, et.al.., 2001).

Calcium (Ca)

The results indicate that the amount of calcium was significantly high ($p < 0.05$) in the leaves with 29.34 percent miliequivalent (me%) and the least being 16.92me % in the roots of the bixa plants tissues. However, there were no significant differences ($p > 0.05$) on the calcium quantities between the roots and stems tissues (Table 4). The treatments of Manure only at 10 tons/ha and that of NPK 100kg/ha produced significantly high ($p < 0.05$) levels of calcium than the rest of the treatments (Figure 4). At the same time, the results revealed that the application of fertilizer amendments greatly increased the levels of calcium across all the treatments.

Calcium plays an extremely important role in producing plant tissues and it enables plants to grow better (Blatt, 2000a).At the same time, Calcium is responsible for holding together the cell walls of plants. It is also crucial in activating certain enzymes and to send signals that coordinate certain cellular activities. It is calcium that is key to normal root system development. Calcium also increases resistance to outside attack and increases the feed value of forage crops to livestock (Allen, *et. al.*2000).This explains why it is present in large quantities in the leaves of the bixa plant.

Magnesium (Mg)

The results indicated that there were significant differences ($p < 0.05$) in the levels of magnesium in the different plant tissues of roots, stems and leaves (Table 4). The highest level of magnesium (6.641me %) was recorded in the leaves while the stems was the least with 4.142 me%. This reaffirms the fact that magnesium is mainly required for the process of photosynthesis which does take place in the leaves. The results are also in line with the findings of Ishfaq et.al, (2022) who reported that the optimum concentration of magnesium in the leaves of woody plants such as bixa is more compared to the other plant tissues. The treatments of the manure application rate of 10 tons/ha and those of NPK 100kg/ha and NPK 150kg/ha produced significantly higher ($p < 0.05$) amounts of magnesium.

Magnesium is required by all crops to help in the capture of the sun's energy for growth and production through photosynthesis. Photosynthesis takes place in chlorophyll, the green pigment in plants, and magnesium is the central atom of the chlorophyll molecule, with each molecule containing 6.7% magnesium (Graham, and Webb, 1991). It also plays an important role in activating enzymes involved in respiration, photosynthesis and nucleic acid synthesis. It aids in phosphate metabolism, serving as a carrier of phosphate compounds through the plant. Magnesium facilitates translocation of carbohydrates (sugars and starches) and enhances the production of oils and fats (Mehmet et.al, 2015).

Iron (Fe)

The results of the analysis on distribution of nutrients in the plant tissues indicate that there were significant differences ($p < 0.05$) in the levels of iron in roots, stem and leaves respectively, with more quantities of iron (672.3 Ppm) being present in the roots (Table 4). At the same time, the treatments of manure application at the rate of 10 tons/ha, NPK 100kg/ha and that of NPK 150kg/ha produced significantly higher ($p < 0.05$) amounts of iron than the rest of the treatments. However, the application of fertilizer amendments increased the quantities of iron to adequate levels across all the treatments.

Iron is an essential micronutrient for almost all living organisms because it plays critical roles in metabolic processes such as Di-nucleic acid (DNA) synthesis, respiration, and photosynthesis. Further, many metabolic pathways are activated by iron, and it is a prosthetic group constituent of many enzymes. An imbalance between the solubility of iron in soil and the demand for iron by the plant are the primary causes of iron chlorosis. Although abundant in most well-aerated soils, the biological activity of iron is low since it primarily forms highly insoluble ferric compounds at neutral pH levels (Agarwal et.al, 2006).

Iron on the other hand plays a significant role in various physiological and biochemical pathways in plants. It also serves as a component of many vital enzymes such as cytochromes of the electron transport chain, and it is thus required for a wide range of biological functions. In plants, iron is involved in the synthesis of chlorophyll, and it is essential for the maintenance of chloroplast structure and function (Albano and Miller, 1993).

Manganese (Mn)

Results of the analysis indicate that there were significant differences ($p < 0.05$) in the levels of manganese in the roots, stems and leaves of bixa plants. More of the manganese nutrient (113.04 me%) was observed in the leaves followed by the stem and roots in that order (Table 4). The treatment of NPK application at the rate of 100kg/ha produced significantly higher ($p < 0.05$) levels of manganese compared to the rest.

Manganese is an essential plant mineral nutrient, playing a key role in several physiological processes, particularly photosynthesis. Deficiency of manganese is a widespread problem, most often occurring in sandy soils, organic soils with a pH above 6 and heavily weathered, tropical soils. It is typically worsened by cool and wet conditions (Alloway 2008). Numerous crop species have been reported to show high susceptibility to manganese deficiency (Mn) in soils, or a very positive response to Mn fertilization, including cereal crops, legumes, stone fruits, palm crops, citrus, potatoes, sugar beets and canola, among others. The impact of Mn deficiencies on these crops includes reduced dry matter production and yield, weaker structural resistance against pathogens and a reduced tolerance to drought and heat stress (Brennan, 1992).

Zinc (Zn)

Results of the analysis indicate that the levels of distribution of the zinc nutrient in thestem was significanly different (p <0.05) from the roots but was not significantly different(p>0.05) from theleaves. However, there was a slightly higher amount of the nutrient (0.00992 Ppm) compared to the leaves (Table 4). The treatments of manure application at the rate of 10 tons/ha and that of NPK 100kg/ha produced significantly higher (p <0.05) levels of zinc compared to the rest of the treatments. In general, the quantities of zinc produced across the treatments were very low and inadequate for plant growth.

Sodium (Na)

Results of the nutrient analysis indicate that the levels of sodium in the roots, stems and leaves were significantly different (p <0.05) from one another, with the highest level (1733 me%) being recorded in the roots (Table 4). The treatments of manure application at the rate of 10 tons/ha and that of NPK 100kg/ha produced significantly higher levels (p <0.05) of sodium compared to the rest of the treatments.

Sodium is not an essential element for plants but can be used in small quantities, similar to micronutrients, to aid in metabolism and synthesis of chlorophyll. Although not essential for most plants, sodium (Na+) can be beneficial to plants in many conditions, particularly when potassium (K+) is deficient. As such it can be regarded a 'non-essential' or 'functional' nutrient (Behmer, et.al, 2005). At low levels, Na+ is not only harmless but can be very useful, particularly in low levels of potassium. This is because, in hydrated form, sodium (Na+) and potassium (K+) ions are chemically and structurally very similar (Amtmann and Sanders, 1999). In that regard, many of the roles that K+ plays in plant cells, including some of the metabolic ones, can therefore be fulfilled by Na+.

Conclusion

The study revealed that the application of both organic and inorganic fertilizer amendments in the soil improves fertility by increasing the levels of different nutrients (macro and micronutrients) required by the bixa plants for healthy growth and increased production of quality bixa. The optimum nutrient levels were realized in the treatment in which manure was applied at the rate of 10t/ha and that of NPK applied at the rates of 100kg/ha as well as 150kg/ha respectively.In regard to the distribution of nutrients in the bixa plant, the study revealed that a majority of the nutrients were found in the leaves followed by stems and then roots respectively.The treatments of manure application at the rate of 10 tons/ha and those of NPK 100kg/ha and 150kg/ha produced significantly high levels of nutrients across all the sites.

Recommendations

The study proposes the need for bixa farmers to be sensitized on the importance of applying fertilizer amendments to their bixa plants. This will greatly ensure increased crop health as well as the yields and quality of bixa. The recommended fertilizer application rates for optimum yields are manure at the rate of 10 tons/ha or the application of NPK fertilizer at the rate of 100kg/ha.

References

Agarwal S, Sairam RK, Meena RC, Tyagi A and Srivastava GC (2006). Effect of excess and deficient levels of iron and copper on oxidative stress and antioxidant enzymes activity in wheat. *J. Plant Sci*., 1: 86-97.

Albano JP and Miller WB (1993). Iron distribution and the occurrence of a physiological disorder in marigold. *Hort. Sci*., 28: 550.

Allen, GJ, Chu SP, Schumacher K, Shimazaki CT, Vafeados D, Kemper A, Hawke SD, Tallman G, Tsien RY, Harper JF *et al.*2000**.** Alteration of stimulus specific guard cell calcium oscillations and stomatal closing in *Arabidopsis det3* mutant. *Science*289: 2338–2342.

Amtmann A., Troufflard S., Armengaud P. (2008); The effect of potassium nutrition on pest and disease resistance in plants. *Physiol. Plantarum*. 133:682–691.

Amtmann, A. and Sanders D (1999). Mechanisms of Na $^{(+)}$ uptake by plant cells. *Advances in Botanical Research* 29, 75–112.

Behmer, S. T., Lloyd, C. M., Raubenheimer, D., Stewart-Clark, J., Knight, J., Leighton, R. S., Harper, F. A., Smith, J. A. C. (2005). Metal hyper accumulation in plants: mechanisms of defense against insect herbivores. *Funct. Ecol.* 19, 55–66.

Blatt, M.R. **(**2000*a*)**.** Cellular signaling and volume control in stomatal movements in plants. Annual Review of Cell and Developmental Biology16: 221–241.

Brady, N., and Weil, R. (2010). “Nutrient cycles and soil fertility,” in *Elements of the Nature and Properties of Soils, 3rd Edn*, ed V. R. Anthony (Upper Saddle River, NJ: Pearson Education Inc.), 396–420.

Brennan, R.F., (1992). The role of manganese and nitrogen nutrition in the susceptibility of wheat plants to take-all in western Australia. *Fertilizer Res* 31:35-41.

Byrd G. A. (1986). Tropical: color cyclopedia of exotic plants and trees for warm-region horticulture--in cool climate the summer garden or sheltered indoors; 3rd ed. East Rutherford, N.J.: Roehrs Co.

Cakmak I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J. Plant Nutr. *Soil Sci*168:521–530.

Cakmak, I. (2000). Role of zinc in protecting plant cells from reactive oxygen species. *New Phytol*., 146:185–205.

Datnoff, L.E. et al., 2007. Mineral Nutrition and Plant Disease. The American Phytopathological Society. ISBN 0-89054-346-1.

Ferguson, Richard. Krista De-Groot, (2000). Editor. *Nutrient Management for Agronomic Crops in Nebraska*, EC 01-155-S. University of Nebraska-Lincoln Extension Service.

Foth, H. (1990). Chapter 12: “Plant-Soil Macronutrient Relations,” in *Fundamentals of Soil Science*, *8th Edn*, ed John Wiley and Sons (New York, NY: John Wiley Company), 186–209.

Graham, R.D, and Webb, M.J., (1991) Micronutrients and plant disease resistance and tolerance in plants. In Micronutrients in Agriculture (J. J. Mortvedt, F. R. Cox, L. M. Shuman and R. M. Welch, eds.), pp. 329–370. SSSA Book Series No. 4, Madison, WI.

Hotz C, BrownKH. (eds) (2004). Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control. Food and Nutrition Bulletin, 2004;25(Supplement2): S91-S204

Jansen, P.C.M. and Cardon, D. Eds (2005). "*Bixa Orellana L*.". PROTA VOL. 3: Dyes and tannins/Colorants ettanins.

Kilmer, V.S., S. Younts, and N. Brady (1968). Editors, *The Role of Potassium in Agriculture*. American Society of Agronomy, Madison, WI.

Lauro, G. J. and Jack. F. F (2000). "Annatto". Natural Food Colorants Science and Technology. IFT Basic Symposium Series. New York: Marcel Dekker. p. 115. ISBN 0-8247-0421-5.

Leigh, R. A.; Wynn Jones, R. G. (1984). "A Hypothesis Relating Critical Potassium Concentrations for Growth to the Distribution and Functions of This Ion in the Plant Cell" (PDF). *New Phytologist*. 97 (1): 1–13.

Mehmet Senbayram, Andreas Gransee, Verena Wahle, and Heike Thiel (2015). "Role of magnesium fertilizers in agriculture: plant–soil continuum," *Crop and Pasture Science* 66(12), 1219-1229.

Muhindi, Felix M., (2006). "Baseline Study to Establish the Gaps in Bixa Production and Marketing," Mimeo, Mombasa, Paper Presented at Bixa Stakeholders' Workshop at YWCA, Likoni, June 2006.

Peoples, M. B., Herridge, D. F., and Ladha, J. K. 1995. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant Soil* 174:3–28.

Smith, J. (Editor) (1995) *Food Additive User's Handbook.* Kluwer Academic/Plenum Publishers, New York.

Smith, J. (Editor) (2003) *Food Additives Data book.* London: Blackwell Science, Oxford, England.

Van Wyk and Ben-Erik (2005). Food Plants of the World. Portland, Oregon: Timber Press, Inc. ISBN 0-88192-743-0.

Wang, Min; Zheng, Qingsong; Shen, Qirong; Guo, Shiwei (April 2013). "The Critical Role of Potassium in Plant Stress Response". *International Journal of Molecular Sciences*, 14 (4): 7370–7390.

Zhao, Duli; Oosterhuis, D.M.; Bednarz, C.W. (2001). "Influence of Potassium Deficiency on Photosynthesis, Chlorophyll Content, and Chloroplast Ultrastructure of Cotton Plants". *Photosynthetica*, 39 (1): 103–109.

Elias M. E. A., G. Schroth, J. L. V. Macêdo, M. S. S. Mota and S. A. D'Angelo (2002). Mineral nutrition, growth and yields of annatto trees (bixa orellana) in agroforestry on an Amazonian Ferralsol. Cambridge University press.

Anand, N., (1983). The market for annatto and other natural coloring materials, with special reference to the United Kingdom. Tropical Development and Research Institute, London. pp. 10–16.

Khare, C.P. (2007). Indian Medicinal Plants. New York: Springer Science + Business Media, LLC. ISBN 978-0-387-70638-2.

Jansen, P.C.M., (1981). Spices, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance. Agricultural Research Reports 906. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands. 327 pp.

Preston, H.D. and Rickard, M.D., (1980). Extraction and chemistry of annatto. *Food Chemistry* 5: 47–56.

Ellison, D., (1999). Cultivated Plants of the World. London: New Holland (1st ed.: Brisbane: Flora Publications International, 1995).

Green, C.L., (1995). Natural colorants and dyestuffs. Non-wood forest products 4. FAO - Food and Agriculture Organization of the United Nations, Rome, Italy.

Alloway, B.J. (2008). Zinc in Soils and Crop Nutrition. 2nd Edition, IZA and IFA, Brussels, Belgium and Paris, France.

Caldeira, M. V. W. ; Moreira, F. L. ; Oliveira Gonçalves, E. de ; Campanharo, Í. F. ; Castro, K. C. (2017).Levels and redistribution of nutrients in the leaves of *Bixa arborea* Huber. and *Joannesia princeps* Vell. in a forest restoration area.*Journal of Scientia Foresails*,.45(114): 305-317.

Xinxiang Xu, Xin Du, Fen Wang, Jianchuan Sha, Qian Chen, Ge Tian, Zhanling Zhu, Shunfeng Ge, and Yuanmao Jiang (2020).

Effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. Journal of Plant Science. Sec. Plant Nutrition. Volume II.

Elias M. E. A., G. Schroth, J. L. V. Mace√ Do, M. S. S. Mota and S. A. D'Angelo (2002). Mineral nutrition, growth and yields of annatto trees (bixa orellana) in agroforestry on an Amazonian Ferralsol. *Journal of Experimental Agriculture*, 38:277-289

Ishfaq. M., Y. Wang, M. Yan, Z. Wang, L. Wu, 3 C. Li, and X. Li (2022). Physiological Essence of Magnesium in Plants and Its Widespread Deficiency in the Farming System of China. *Journal of Frontiers in plant Science*.

Blank Page for typesetting purpose