EVALUATION OF THE RHIZOSPHERE SOIL PH AND ELECTRICAL CONDUCTIVITY OF BIXA TREATED WITH DIFFERENT FERTILIZER AMENDMENTS

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Abstract

Bixa *orellana L.* is a shrub that is commercially known as 'annato'. The main product that is obtained from *Bixa orellana* is an 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. Bixa farming in Kenya has declined since the 1970's due to the use of cheap synthetic materials. However, the limited use of synthetic food colours has triggered a high demand for natural bixa. A study was undertaken in Kwale County with the objective of evaluating the Rhizosphere soil pH and Electrical Conductivity (EC) of bixa plants treated with different fertilizer amendments. This was done in Shimoni at the Kenya bixa nucleus farm where bixa plants of the same age were subjected to treatments of different levels of organic (poultry manure at 5 tons/ha and 10 tons/ha respectively) and inorganic fertilizers (NPK 17:17:17 at 100 kg/ha and 150 kg/ha) as well as a combination of the two (manure 2.5tons plus NPK 50 kg/ha and manure 5 tons plus NPK 75 kg/ha respectively) with a control, in a randomized complete block design with 3 replications. Bixa roots from each treatment were dug out and analysed for the rhizosphere soil pH and electrical conductivity respectively. The results revealed that the rhizosphere soil pH improved with the application of both organic and inorganic fertilizer soil amendments from 5.78 to 6.5. The treatment in which manure was applied at the rate of 10 tons/ha produced significantly higher levels of rhizosphere pH than the rest. On the soil rhizosphere EC, the study revealed that the EC significantly reduced with increased use of organic fertilizer amendments to as low as 0.2 (mS*/cm). The application rate of manure at 10 tons/ha recorded the lowest EC levels.

Key words: Bixa, organic and inorganic fertilizers, rhizosphere, electrical conductivity, soil pH

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 of *Bixa orellana* to make red body paint as well as lipstick and hence the name 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). For the Latin Americans, they not only use annatto for giving an attractive red colour to meat, fish and rice dishes but also for imparting distinctive flavour notes (Byrd, 1986).

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

The annual world production of dried annatto seed at the beginning of the 21st century was estimated at about 10,000 tonnes of which 7000 tons entered international trade (Francis, 2000). The major commercial producers of *Bixa orellana* in the world are countries in South America, Central America, the Caribbean, Africa, India and Sri Lanka. Brazil is the largest producer with about 5000 tonnes per year. However, almost all the Bixa produced in Brazil is consumed locally (Smith, 1995). Peru is the largest exporter of annatto seed annually with about 4000 tonnes. Kenya produces and exports about 1500 tonnes of annatto seed extracts annually, thereby becoming the second largest exporter of the crop to Japan and the European Union countries. The main market for annatto is the United States with 3000 tonnes per year, followed by Western Europe (2500 tonnes) and Japan (1500 tonnes). 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 average market price per ton of seed varied strongly; between 1984 and 1990. It fluctuated between US$ 600 and US$ 2300 (Rajendran, 1991). 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. The industrial and cash crop does well in the coastal region of the country (Smith, 1995). In that regard, 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 ((Muhindi, 2006). 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 have been 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 (Macharia, G. (1984). 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 (Cheruiyot *et.al*., 2019).

In a bid to revitalize the Bixa sub-sector, a study was undertaken with the broad objective of determining the Rhizosphere soil pH and Electrical Conductivity of bixa plants treated with different fertilizer amendments. The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms (Giri, *et.al,* 2005). This region is approximately one-millimeter-thick around the root tissues. It contains many bacteria and microorganisms that feed on sloughed-off plant cells, known as rhizo-deposition (Hütsch Birgit, *et.al,* 2002) as well as the proteins and sugars released by roots. The interaction between different organisms living in close physical association, to the advantage of one another influences plant growth by the uptake of water and nutrients. It also helps in the improvement of soil fertility and enhance the degradation of toxic chemicals. Much of the nutrient cycling and disease suppression needed by plants occurs immediately adjacent to the roots due to root exudates and communities of microorganisms (Grayston. *et.al*., 1998). The rhizosphere also provides space to produce allelochemicals to control neighbours and relatives (Grayston *et.al*, 1998). The plant-soil feedback loop and other physical factors are important selective pressures for the communities and growth in the rhizosphere.

Soil pH measurement is useful because it is a predictor of various chemical activities within the soil. As such, it is also a useful tool in making management decisions concerning the type of plants suitable for location, the possible need to modify soil pH (either up or down), and a rough indicator of the plant availability of nutrients in the soil (Bais, et.al.,2006). At the same time, pH influences several soil factors affecting plant growth, such as soil bacteria, nutrient leaching, nutrient availability, toxic elements, and soil structure. In bixa plants, the nutrients are most available to plants in the pH range of 5.5 to 6.5. The Electrical Conductivity (EC) of a solution on the other hand is a measure of the ability of the solution to conduct electricity.

The EC is reported in either milli-ohms per centimetre or the equivalent deci-siemens per meter. When ions (salts) are present, the EC of the solution increases. If no salts are present, then the EC is low indicating that the solution does not conduct electricity well. However, the EC indicates the presence or absence of salts, but does not indicate which salts might be present. For example, the EC of a soil sample might be considered relatively high. No indication from the EC test is available to determine if this condition was from irrigation with salty water or if the field had been recently fertilized and the elevated EC is from the soluble fertilizer salts. In order to determine the source of the salts in a sample, further chemical tests must be performed (Fernando and Miguel, 2016). Excess salts hinder plant growth by affecting the soil-water balance. Bixa plants will therefore not tolerate salt levels in excess of 0.4 (mS*/cm) which can greatly lower productivity.

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. 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 of different ages.

Study design

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. 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.

Data collection

Three bixa plants were selected at random in each of the plots within the blocks. The plants were tagged for ease of identification and data collection. Four holes were dug from the surface to the root zone of each of the bixa plants (0-30 cm) at 4 different locations along the edges of the plant canopy. A piece of the bixa root measuring 20 cm was cut from each of the four locations of the bixa plant while ensuring that the soil attached to the root was intact. The exercise was done both before and after the application of the different fertilizer treatments. The root pieces from each plant were put in a clean polythene bag. Each bag was then labelled with the plot number as well as the tree tag number. All the samples from the different plots were transported to the laboratory for analysis of both the soil pH and electrical conductivity respectively.

Determination of the Rhizosphere soil pH for bixa plants treated with different fertilizer amendments

Two bixa plant roots were removed from each of the polythene bag and rinsed in a jar with 100 millilitres of distilled water. All the root samples from the respective bixa plots with different fertilizer treatments were subjected to the same procedure. The resultant soil-water solutions had their PH levels determined separately by the use of a pH meter, while ensuring that the meter electrodes are rinsed using distilled water before they were used to measure the pH level of the next sample.

Determination of the Rhizosphere soil electrical conductivity for bixa plants treated with different fertilizer amendments

The remaining two bixa plant roots in the respective polythene bags were then removed from each of the polythene bag and rinsed in a jar with 100 millilitres of distilled water. All the root samples from the respective bixa plots with different fertilizer treatments were subjected to the same procedure. The resultant soil-water solutions had their Electrical Conductivity (EC) levels determined separately. The conductivity of the water solution was measured by means of a dip type cell (Cell Constant of 1.0) conductivity meter, while ensuring that the meter electrodes were rinsed using distilled water before they were used to measure the EC level of the next sample.

Statistical data analysis

Data collected were keyed into excel spreadsheet and subjected to analysis of variance (ANOVA) using the Statistical Package of SAS version 8.2. Significance was tested at 5% level. Significant means were separated using LSD at 5% significant level.

RESULTS AND DISCUSSION

Rhizosphere soil pH for bixa plants treated with different fertilizer amendments

Results of the soil analysis in the three blocks indicated that the rhizosphere soil pH improved with the application of both organic and inorganic fertilizer soil amendments. However, the treatments that had organic fertilizers incorporated into the soils produced better results than those that had inorganic fertilizers only. The treatments in which manure was applied at the rate of 10 tons/ha produced significantly higher ($p \leq 0.05$) levels of rhizosphere soil pH than the rest as can be seen in the Table 1.

As a result, the soil pH immediately surrounding the bixa plant roots changed from the condition of medium acidic to slightly acidic towards the neutral condition as can be seen in the figure (Figure 1) below.

Table 1: Mean rhizosphere soil pH values under different treatments of soil amendments

Treatment	Mean pH value
Manure only 10t/ha	6.446[a]
manure 5t/ha	6.250[b]
Manure 5t+npk@75kg	6.120[bc]
Npk only100kg/ha	6.007[cd]
Control	**5.872[de]**
Npk only100kg/ha	6.007[cd]
Manure2.5t+npk@50kg	5.862[de]
Npk only@150kg/ha	5.831[e]
p-value	**0.001**
CV%	**2.6**

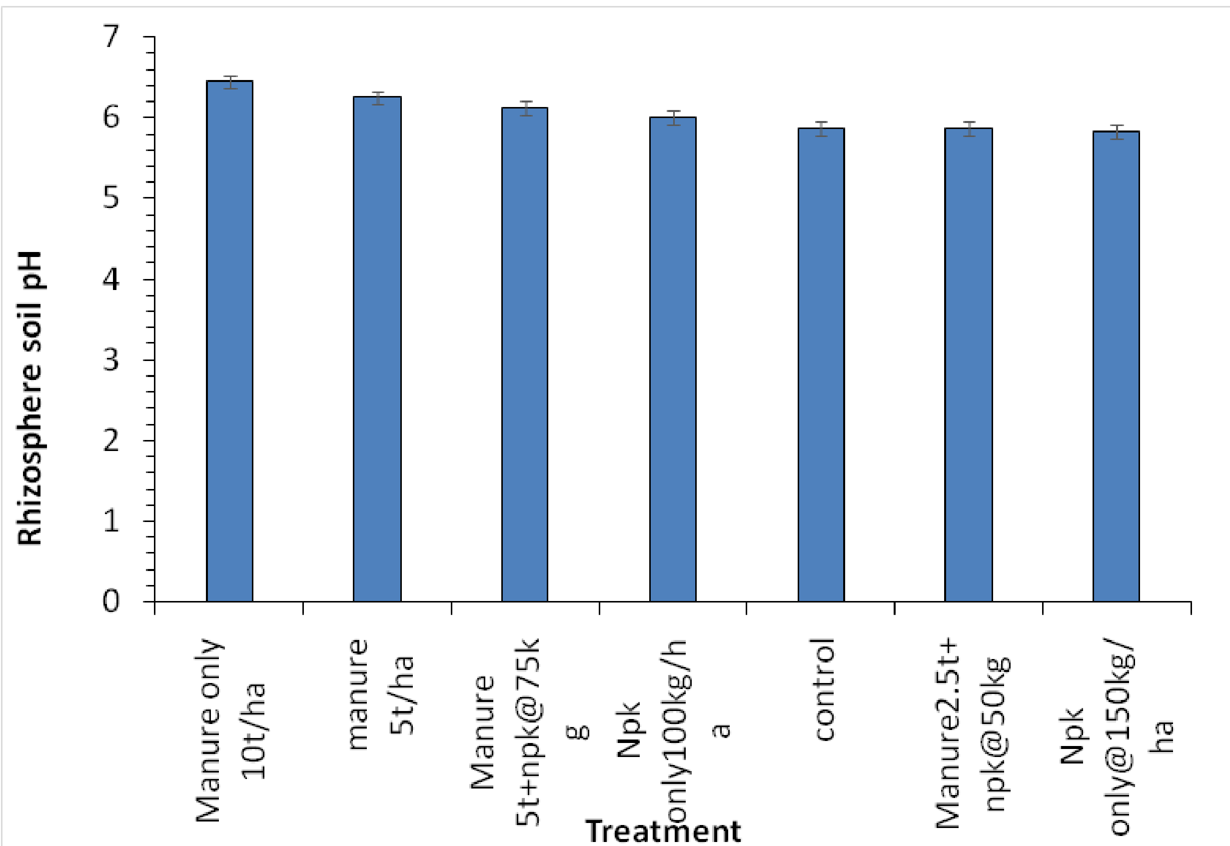

Figure 1: Effect of organic and inorganic fertilizers on the soil rhizosphere pH in bixa plants

The results are in line with the findings of Yanan Li *et.al.* (2020) when conducting studies on the Effects of Different Fertilizer Treatments on Rhizosphere Soil Microbiome Composition and Functions. He revealed that chemical fertilizer (NPK) treatment decreased soil pH, and chemical fertilizer combined with straw (NPK+S0.5) treatment increased ammonium nitrogen(NH4+-N). Thus, organic fertilizers increased N fixation and nitrification, and inorganic N fertilizer accelerated denitrification.

Previous studies have also suggested that organic fertilizer application does not only improves soil organic matter content, preserves moisture and fertility and reduces losses of soil nutrients such as N, P and K, but also helps to maintain soil pH stability (Zhang, et.al, 2010). Manure/straw application is an important way of increasing soil organic fertilization, and crop residues have significant beneficial effects on soils' physical, chemical and biological processes. In addition, an increasing number of research studies have demonstrated that fertilizers with straw significantly enhance bacterial activity (Tayyab, *et.al,* 2018). The soil rhizosphere microbiome has a critical role in plant growth, nutrition, health and breeding (Mendes, et.al, 2017, Yang, et.al, 2017).

The results of this study were in agreement with the results of several other studies that were conducted and observed that organic manure treatment increased soil pH, but chemical fertilizer treatments, such as NPK fertilizer, decreased soil pH (Warren and Fonteno, 1993; Whalen et al. 2000; Liu et al. 2010). The decrease of soil pH by NPK fertilizer may be as a result of leaching of basic cations, such as potassium, calcium, and magnesium from the soil. However, Chang et al. (1990) reported that soil pH decreased by 0.3–0.7 after 11 years of organic manure treatment (with livestock by-products) at base rich soil, and that the decrease was greater when the amount of manure treatment was increased. Generally, organic manure with livestock by-products increases soil pH, but the effects differ depending on the organic matter content, treatment amounts, and soil properties.

Similar to other studies, NPK fertilizer treatment acidified the soil because the urea that was used by the plants as NH4+ first and then the H+ was released into soil, thereby decreasing the soil pH (Magdof et al. 1997). This process reduces most of the activities of bacteria and actinomycetes in the soil (Kaur et al. 2005) and seems to be the cause of soil cations leaching over the long term (Likens et al. 1996; Bailey et al. 2004). The long-term utilization of NPK fertilizer eventually results in deficiencies in other essential nutrients, which may deteriorate the physical, chemical, and biological properties of the soil. The soil directly surrounding the plant generally has a different pH than the surrounding soil. This is due to the fact that as the plant uptakes/secretes different chemicals, it greatly alters the pH of the surrounding soil and in turn affects the microbes that are dependent on that plant. It is therefore important for the bacteria present to be adaptable to changing environments (Hinsinger, 2001).

Rhizosphere soil electrical conductivity of bixa plants treated with different fertilizers

The results of the analysis indicate that the soil electrical conductivity (EC) significantly reduced with increased use of organic fertilizer amendments. Manure application rate of 10 tonnes/ha recorded the lowest EC levels than the other treatments as shown in the table (Table 2) below.

Table 2: Mean rhizosphere soil EC values under different treatments of soil amendments

Treatment	Mean pH Value
Control	**0.5022[a]**
Npk only100kg/ha	0.4467[b]
Npk only@150kg/ha	0.4067[c]
Manure2.5t+npk@50kg	0.3733[cd]
Manure 5t+npk@75kg	0.3411[d]
Manure only @ 5t/ha	0.2933[e]
Manure only @ 10t/ha	0.200[f]
p-value	**0.001**
CV%	**11.1**

The use of inorganic fertilizers slightly reduced the electrical conductivity as compared to the plots where no fertilizer amendments were applied as can be seen in the figure (Figure 2) below. This could be attributed to the fact that chemical fertilizers contain more acids and salts while the organic fertilizers have low levels of acids and hence absorbed excess salts in the soil thereby lowering the electrical conductivity.

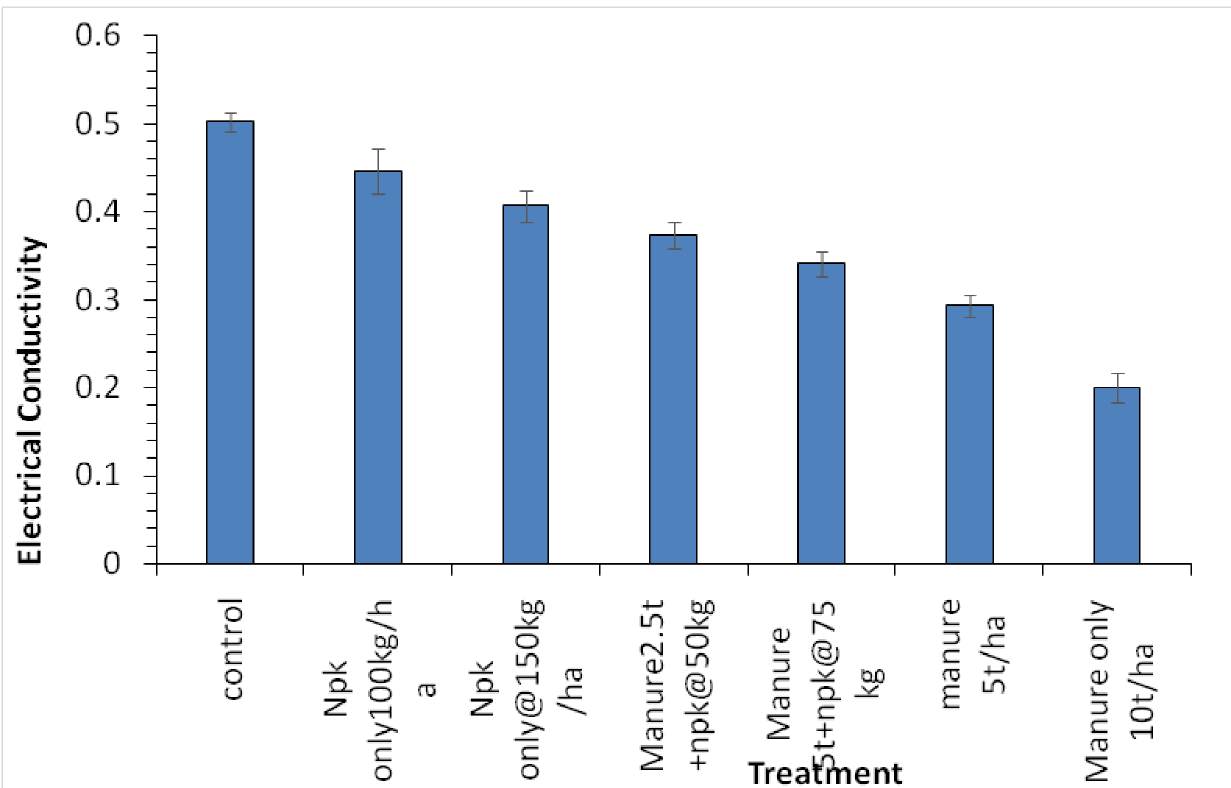

Figure 2: Effect of organic and inorganic fertilizers on the soil electrical conductivity in bixa plants

However, the salinity levels recorded after the application of both organic and inorganic fertilizer amendments were lower than the minimum recommended limits that can affect crop growth. It is reported that most agricultural crops are not affected by soil salinity until ECe levels reach 4.0 dS m_1 (Landis 1988), which is also the minimum ECe value for classifying soils as saline (U.S. Salinity Laboratory Staff 1969).

Excessive salt accumulation in the soil does not only inhibit soil microbial activity and reduce the rhizosphere nutrient activation, but also causes soil acidification that leads to crop nutrition disorders (Xue et.al., 1995). Accordingly, it is an important indicator of changes in soil quality. Dai X et.al. (2013) reported that the lowest EC values are generally found in the soils under rotation. At the same time, the application of organic manure proved to significantly inhibit the accumulation of salt in the soil, thereby lowering the EC levels.

Conclusion

The study revealed that the rhizosphere soil pH improved with the application of both organic and inorganic fertilizer soil amendments. However, those treatments that had organic fertilizers incorporated into the soils produced better results than the ones that had inorganic fertilizers only. The treatment in which manure was applied at the rate of 10 tonnes/ha produced significantly higher levels of rhizosphere pH than the rest. In regard to the soil rhizosphere electrical conductivity (EC) the study revealed that the soil electrical conductivity (EC) significantly reduced with increased use of organic fertilizer amendments. The manure application rate of 10 tonnes/ha recorded the lowest EC levels than the rest. Application of inorganic fertilizers to the bixa plants slightly reduced the electrical conductivity as compared to the plots where no fertilizer amendments were applied.

Recommendations

The study proposes the need for bixa farmers to be sensitized on the importance of applying organic fertilizer amendments to their bixa plants. This will greatly ensure increased soil pH and reduced electrical conductivity, thereby improving yields of quality bixa. The recommended organic fertilizer application rate for optimum results of both rhizosphere soil pH and electrical conductivity is 10 tonnes/ha of fully decomposed manure.

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