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Effects of Increasing Doses of Auxins (A.I.A.) and NPK Fertilizer (17-17-17) on Nursery Initiation of *Pennisetum purpureum* Rejects and Biological Tillage on Degraded Soil, Kisangani

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Abstract. Our study was initiated to evaluate the effects of increasing doses of auxins (A.I.A.) and NPK fertilizer (17-17-17) on nursery initiation of *Pennisetum purpureum* shoots and organic tillage on degraded soil, in Kisangani.

The crop was installed under real conditions in Kisangani on an area of 42.9 m^2. The agronomic parameters observed were the rate of cuttings recovery, the tillering index of the shoots, the soil-root interface under the emerging grass stand and the dynamics of earthworms.

We adopted a randomized complete block design, following the provisions for agroforestry experiments.

Results showed that organo-mineral fertilization of the seedling beds under the conditions of our trials clearly influenced rhizogenesis and metabolic activity of the vegetative apparatus of the nursery seedlings. This suggests that a residual effect related to the nutrient content of the plant material would accompany the transplantation of well-fed nursery offshoots into the field. And the application of auxins and NPK, under the conditions of use defined during our experiments, did not clearly influence the root density nor the soil-root interface.

Key words: auxins, NPK, *Pennisetum purpureum*, rhizogenesis, degraded soil, fertilizers

Introduction

Auxin is mostly produced in photosynthetic organs such as young leaves from which transport directs auxin from source organs to sink organs. However, every plant tissue is potentially capable of producing auxin. This is particularly the case in the primary root where biosynthetic genes are expressed with some similarity in expression profiles with a map of auxin contents at the cell level obtained using state-of-the-art auxin assay techniques (cell sorting followed by chromatography coupled to mass spectrometry) (Petersson *et al*., 2009).

Although the knowledge of the biosynthetic pathways is not exhaustive, the continuous discovery of new genes allows us to know more and more about the production steps. To date two distinct pathways have been characterized; one dependent and the other tryptophan dependent (Ljung, 2013).

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NPK compounds and NPK blends are multi-nutrient fertilizers containing a certain percentage of 3 main nutrients nitrogen (N), phosphorus (P) and potassium (K) .These products are intended to facilitate the complete fertilization of a crop, from a single application. The raw material sources of each nutrient can vary depending on the final production process of the NPK fertilizer.

Wilhelm KNOP German agricultural chemist, in 1861, the precise nutrient requirements of green plants necessary for their growth. It is about 4 elements corresponding to the following letters of his name: K: Potassium; N: nitrogen; O: oxygen; P: phosphorus

Except for oxygen, the 3 components have become the basis of chemical fertilizers in the form of soluble salts that can be directly assimilated, making it possible to obtain good yields but with significant risks of leaching into the water table in watercourses.

Pennisetum purpureum is a tall perennial grass with high forage production potential that is used to form rich pastures and agroforestry fallows (Pyame, 2015; Ntamwira, 2021). However, its establishment by cuttings in the field requires frequent replanting for costly replanting, particularly during dry spells that have become common in the middle of the rainy season (Pyame *et al*., 2021a).

Would nursery multiplication of propagation material on substrates provided with low doses of growth hormones lead to more abundant and less costly production of shoots and stump fragments, to the point of interfering with soil fertility and biological tillage? This general question is broken down into three specific research questions below:

1. Would the application of increasing microdoses of hormones (auxin AIA) and NPK fertilizers in the nursery affect the rate of cuttings recovery and the tillering coefficient of induced shoots?
2. Would nursery application of increasing microdoses of hormones (auxin AIA) and NPK fertilizer affect the soil-root interface (bulk density, porosity, root density) under the emerging grass stand?
3. Would nursery application of increasing microdoses of hormones (AIA auxins) and NPK fertilizers influence earthworm dynamics inducing a remarkable biological tillage under the emerging grass stand?

Nursery propagation of propagation material on substrates with low doses of fertilizers and growth hormones would induce a more abundant and less expensive production of offshoots and stump fragments. This could favorably interfere on the rhizospheric soil ecology (increased fertility) through a biological tillage operated by the roots and earthworms whose dynamics would be improved as a result.

The global hypothesis of this research is formulated as follows:

The multiplication of *Pennisetum purpureum* propagation materials in a nursery, on a substrate provided with increasing doses of hormones (auxins AIA) and NPK fertilizers would induce, in the same order, an increasing production of stump sprouts/shavings and a more important dynamics of earthworms on degraded soil.

Three specific hypotheses are derived from the above hypothesis:

1. The application of increasing doses of hormones (AIA auxins) and NPK fertilizers in the nursery on degraded soil would induce, in the same order, a higher recovery rate and a higher tillering coefficient;
2. The application in nursery of increasing doses of hormones (auxins AIA) and NPK fertilizers on degraded soil would induce, in the beds, a much higher soil-root interface (total porosity, root density) (biological tillage);
3. The application of increasing doses of hormones (auxin AIA) and NPK fertilizer to degraded soil in the nursery would induce, in the same order, greater earthworm dynamics (biological tillage).

To experimentally measure the rate of cuttings recovery, the tillering index of shoots, the soil-root interface under the emerging grass stand and the dynamics of earthworms in response to the application of increasing microdoses of hormones (auxin AIA) and NPK fertilizers in the nursery.

The following specific objectives are targeted:

1. To experimentally measure the rate of cuttings recovery and the tillering index of P. purpureum transplants in the field, in response to a nursery application of increasing microdoses of hormones (auxins AIA) and NPK fertilizers.
2. Experimentally measure, in nursery beds, the soil-root interface (total porosity, root density) under the emerging grass stand in response to nursery application of increasing microdoses of hormones (auxins AIA) and NPK fertilizers,
3. To experimentally measure, in the nursery beds, the dynamics of earthworms (number of earthworms/m^2/30cm) under the emerging grass stand, in response to a nursery application of increasing microdoses of hormones (auxins AIA) and NPK fertilizers.

Materials and Methods

Environment

Geographic location and experimental period

Our experimental field was established in Kisangani, capital of the province of Tshopo, in the Democratic Republic of Congo, more precisely at PK12 on the Banalia axis, in the annex commune or Collectivity-Secteur of Lubuya-Bera, at the agroforestry station of the Faculty of Management of Renewable Natural Resources (FGRNR) of the University of Kisangani.

The geographical coordinates of the city of Kisangani are as follows Latitude: 0° 11' North; Longitude: 25° 1' East; Altitude: 390 to 410 m with an average of 400 m. The city of Kisangani is located in the central basin straddling the Congo River. Its relief is a set of plateaus interspersed with river beds and streams (Kenga, 2012).

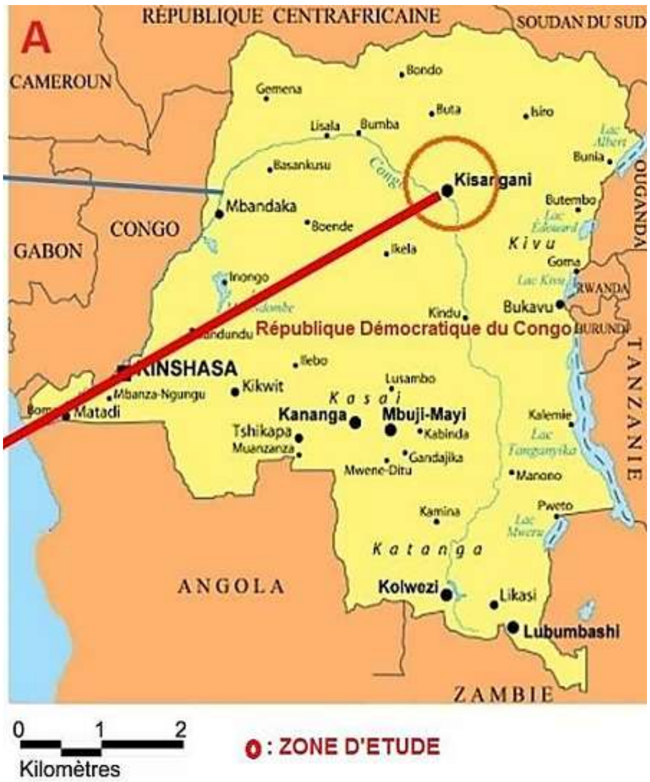

Figure 1: Map of the location of the study area

The city of Kisangani has an equatorial climate, characterized by high heat and humidity. This makes Kisangani an environment where total hydrolysis predominates.

According to the classification of KOPPEN, this climate is of the Af type characterized by a monthly height of precipitations of the driest month superior to 60mm and an annual average temperature superior or equal to 24°C. The average relative humidity is very high all year round and is between 80 and 88%, the annual average being 84%. The insolation is 1972 hours. Annual rainfall is over 1800mm and is divided into two seasons, one very rainy (mid-August to mid-December) and the other rainy month (mid-March to mid-June).

Soil of the experimental site and previous crop

The soil of our experimental site, as with all the soils around Kisangani, is ferralitic (Pyame, 2015). The previous crop was cassava and soybean. We could also observe some grasses such as *Cyperus sp* (Cyperaceae), *Croton hirtus* (Euphorbiaceae), *Comelina diffusa* (Comelinaceae), *Euphorbia heterophilla* (Euphorbiaceae), *Panicum maximum* (Poaceae), *Spermacos latifolia* (Rubiaceae), *Pueraria phaseolides* (Fabaceae), and *Cynodon dactylon* (Poaceae), which was predominant.

Materials

A variety of materials were used in the course of this study. It is about the small current material and the glassware of use to the laboratory of pedology, the aratoire material used during the works of opening and maintenance of the field (nursery), the reagents of laboratory, the hormone of the kind auxin (Indole Acetic Acid) and the material of propagation made of cuttings of *Pennisetum purpureum*.

Biological material

The biological material used was cuttings of *Pennisetum purpureum.*

Figure 2: *Pennisetum purpureum* in the experimental field (© Utshudi, 2022)

Farming and other materials

A long machete for clearing the land, a watering can for occasional watering, 50 cm stakes and string for delimiting the plot, a hoe for minimum plowing and weeding, a decameter for measuring the surface area of the plots, a 5-liter basin for transporting and spreading fertilizer, six plastic buckets for transporting and spreading manure, three nylon ropes for sowing and, finally, one motorcycle for transportation to the research station.

Laboratory equipment

A variety of equipment was used in the laboratory: pressure balance, suspension balance, copecky cylinder, beakers, aluminum trays, porcelain crucibles, oven and desiccator.

Fertilizing equipment

Restoring fertility to degraded soils involves not only the application of crop residues to the surface, but also the return of nutrients exported by crops and the application of humus amendments to replenish humus (CIAT-TSBF, 2009). Thus, we used a raw mulch-compost made of fans of the previous crop, NPK 17/17/17 fertilizer and farmyard manure (pig droppings).

Experimental Setup

We adopted a complete randomized block design, following the provisions pertaining to agroforestry experiments, according to Pyame (2015). The sketch below shows the schematic of this design.

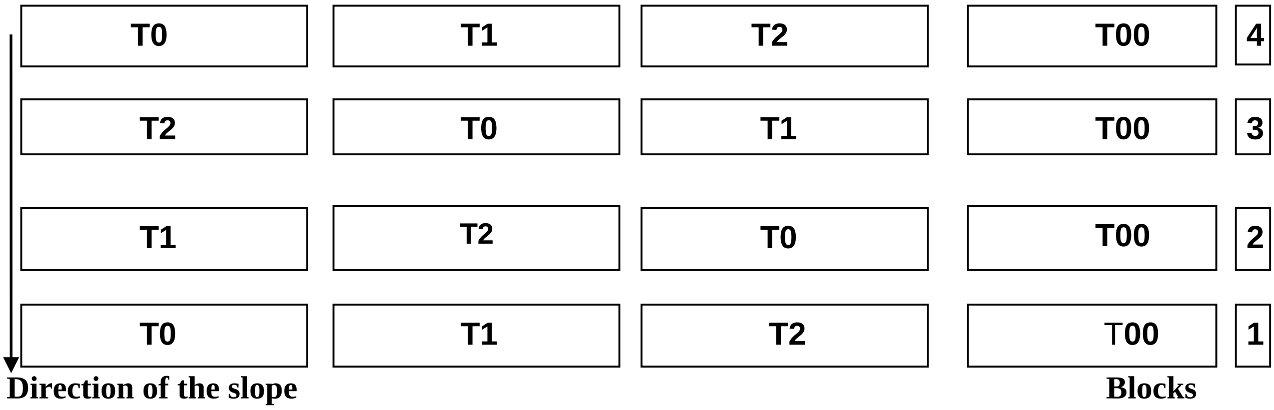

Figure 3: Sketch of the experimental design

Legend: T00= Current planting practice, direct cuttings in the field; T0= production of shoots in the nursery without any additional input; T1= production of shoots in the nursery with a single basal application of auxin (AIA) as a growth hormone and NPK organomineral fertilizer; T2= production of shoots in the nursery with two basal applications of auxin (AIA) as a growth hormone.

Methodological approach of the trial

The methodological approach includes the following operations carried out successively: clearing and cleaning of the land, ploughing, delimitation of the experimental plots, cutting of *Pennisetum purpureum*, planting of the cuttings in the beds, watering of the treatment plots (except for the absolute control), application of the improvement treatments, maintenance work and collection of data according to the experimental parameters selected.

Preparatory work for the trial

This consists of the delimitation of the site, its complete clearing and cleaning, the cutting, transport and cutting of the *Pennisetum purpureum*. Stems full of sap, 3m high, were carefully cut with a machete; cuttings with 3 internodes were then stored in the shade for 24h.

Planting of the cuttings and application of ameliorative inputs

The cuttings of *Pennisetum purpureum* were planted in beds of 6 m x 1,2 m, with 2 cuttings in stakes equidistant of 30 cm in all directions. The hormonal solution used was auxin of the Indole Acetic Acid (IAA) type, at a concentration of 10 micromoles per liter, which was subsequently increased to 1µmole/l and 1l/m^2 was applied to the base of the cuttings in the bed. A second application was made 15 days later for treatment T_2, increasing the dose to 2 µmoles/m^2.

Maintenance work in the nursery

This included 2 weedings and regular watering once every 3 days on the different treatments, except for the absolute control representing the current practice of planting *Pennisetum purpureum* in the area.

Data Collection according to the Selected Parameters

Rate of emergence per cutting planted in the nursery and per bunch of 2 cuttings

The number of emerged cuttings was collected, at the end of 15 days after planting, from 2 circumscribed samples (yield square) on the treatment plot considered, at the level of each block. The result was expressed as an emergence rate based on all planted cuttings and on the basis of the 2-cutting pockets planted.

Number of secondary shoots per plant (tillers)

This index, which provides information on the multiplication coefficient of the propagation material, and thus the regenerative capacity of the grass cuttings, was measured by counting the number of branches on each of the planted cuttings included in the yield square in two plot samples.

Earthworm counts in the crop profile

The field survey and all the organizational work that constituted the beginning of this laborious phase of our study were conducted according to Lavelle (1973). The samples were taken in accordance with the manual sorting method recommended by the Tropical Soil Biology and Fertility (TSBF) program. This method has been used in turn by Lavelle (1983), Lavelle *et al.* (1995) and Mulotwa (2001).

Eight cultivation profiles, two per treatment, were laid out, each with a monolith (soil block) of 25 cm x 25 cm x 30 cm, equivalent to approximately 30 kg of soil, at its center. The monolith was subdivided into four quadrants, the results of which were counted separately. Three main 10 cm soil slices were distinguished on each quadrant to facilitate the subsequent manual sorting.

The monolith was thus explored from top to bottom by passing successively through 3 strata namely (1) 0 to 10 cm, (2) 10 to 20 cm and (3) 20 to 30 cm. The earthworms isolated in each stratum were counted and the respective values carefully recorded, thus giving the spatial structure of colonization of the cultural profile. From this, a total per quadrant, per soil stratum, and then per m^2 of surface area was produced at the end of each operation.

Apparent density and spatial structure of the rooting of the agro system (RLD and RMD)

This investigation, carried out concomitantly with the apparent density of the soil, made it possible to identify the root density profile, i.e., the evolution of root density in the depth of the soil (Pyame, 2015). A Coppécky cylinder of standard volume (100 cc) was used to sample under the grass stand 4 successive soil strata (5 cm) invaded by roots, thus realizing a cultural profile of 20 cm on 2 of the 4 replicate plots, for each treatment.

Immediately brought back to the laboratory, the successive weighing of the samples contained in cylinders of known weight (W) led, at the end of the drying in the oven, to the obtaining of constant weights (CW). Subsequently, the contents of the cylinders are gently pulverized to collect all the roots. These will then be weighed (RMD in g/100 cc) and measured with a batten and the values summed (Root Length Density in cm/cm^3).

Statistical treatment of the data

The data of the study were processed by means of Excel and Statgraphics software, using in turn the analysis of variance and Duncan's tests for the significance of the differences between the evaluated treatments.

Results and Discussion

This chapter presents and interprets the results below, followed by a brief general discussion.

We present alternatively (1) the average recovery rates and the average tillering indices of the shoots in the nursery for the confronted treatments, (2) the parameters defining the soil-root interface, i.e. the total porosity and the root density (Root Mass Density and Root Length

Density) for the confronted treatments, and finally (3) the dynamics of the earthworms under the grassy cover of the beds.

The analysis of variance tables representing the statistical treatments are in the appendix, while the probability index "p" allowing the discrimination of the treatments are placed under the diagrams of the results.

The average recovery rates and the average tillering indices of the shoots in nursery.

These results are presented in Figures 4, 5 and 6 below.

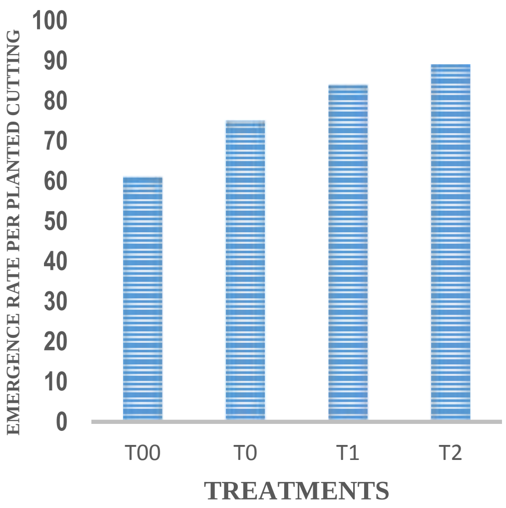

Figure 4: Emergence rate of cuttings per stake per park

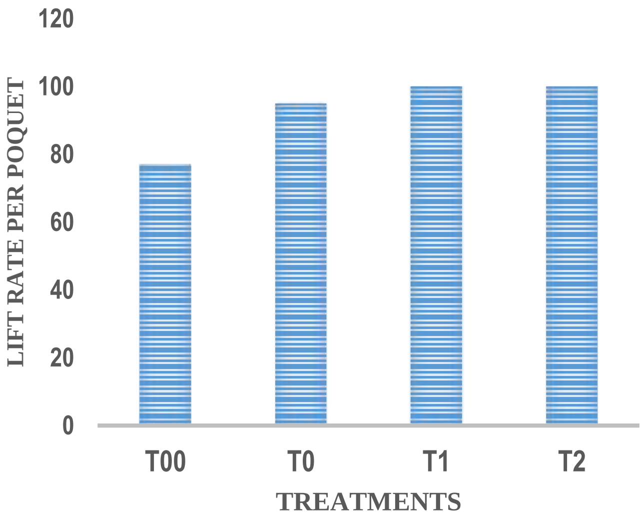

Figure 5: Emergence rate of 2 cuttings per stake

From the results in Figures 4 and 5, it is clear that the regrowth rates of the shoots are much higher in the three treatments using nursery work (T0, T1 and T2) than in the absolute control (T00) using simple cuttings without roots and leaves as propagation material.

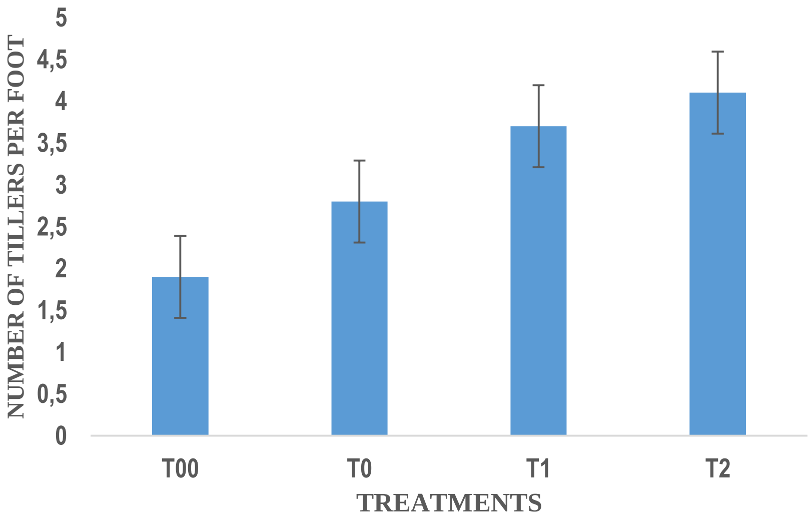

Figure 6: Number of tillers per plant of *Pennisetum purpureum* according to treatments

On examination of the results in Figure 6, it is clear that the tillering indices of the shoots are clearly higher in the three treatments using nursery work (T0, T1 and T2) than in the absolute control (T00) bypassing the latter. Moreover, there is a clear superposition of the curves relating to the organo-mineral fertilization of the rejection beds, the value of the p-index leading to a highly significant difference between these 3 treatments.

It can be said that the hormonal application on the cuttings and the organo-mineral fertilization used on the transplanting beds, under the conditions of our trials, have clearly influenced the rhizogenesis and the metabolic activity of the vegetative apparatus during the stay of the cuttings in the nursery. This suggests, without question, that an effective residual effect related to the nutrient content of the plant material and other growth factors would accompany the field transplantation of well-fed, nursery-activated shoots.

Parameters defining the soil-root interface

Bulk density and porosity

These results are presented in Figures 7 and 8 below.

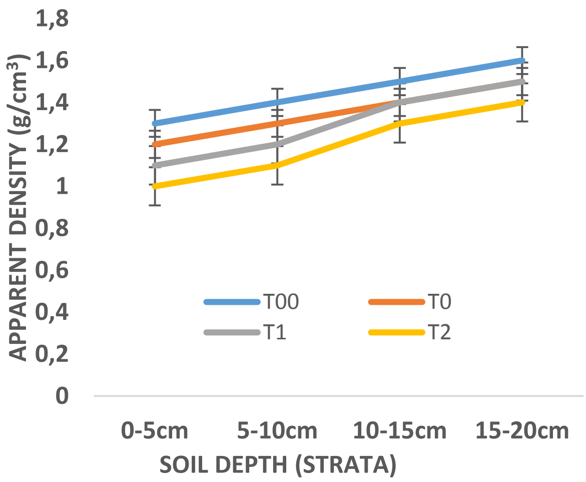

Figure 7: Bulk density in g/cm^3

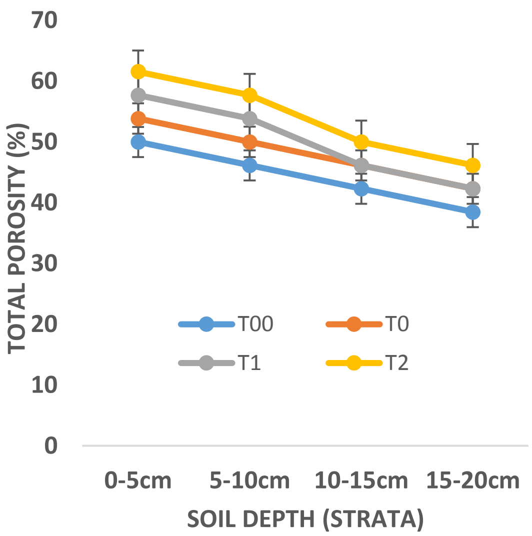

Figure 8: Total porosity (%)

On examination of the results in Figures 7 and 8, it is clear that the diagrams relating to the different treatments are clearly distinct, but supplant each other more or less proportionally to the doses of hormones and NPK fertilizers used in the organo-mineral fertilization, the absolute control referring to the current practice of establishing *Pennisetum purpureum* by direct cuttings being the least performing treatment.

Root density (RMD) and soil-root interface (RLD)

These results are presented in Figures 9 and 10 below.

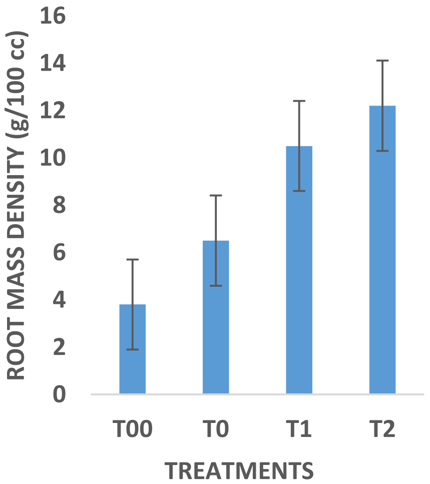

Figure 9: Root density in g/100 cc

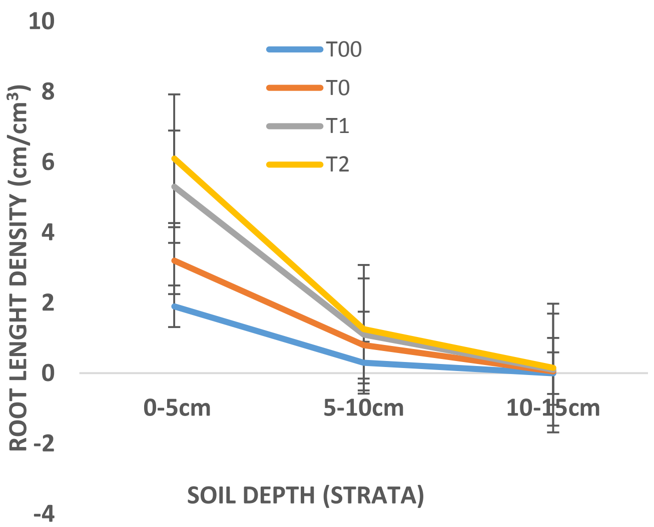

Figure 10: Soil-root interface in cm/cm^3

Examination of the results in Figures 9 and 10 shows that the diagrams for the different treatments developed in the nursery are significantly higher than those for the absolute control, which refers to the current practice of establishing *Pennisetum purpureum* by direct cuttings, and is therefore considered the least effective.

In addition, the plots for the treatments using increasing doses of NPK 17-17-17 fertilizer (0, 50 and 100kg/ha) in combination with a constant dose of pig manure (5t/ha) in the nursery beds stood out.

The value of the discrimination index of the treatments "p", in the analysis of variance led to a significant difference between these treatments. This clearly shows that organomineral fertilization, under the conditions of use defined in our experiments, did not clearly influence the average height of the plants and the average number of leaves per plant.

Dynamics of earthworms under grassy cover in the beds

These results are presented in Figure 11 below.

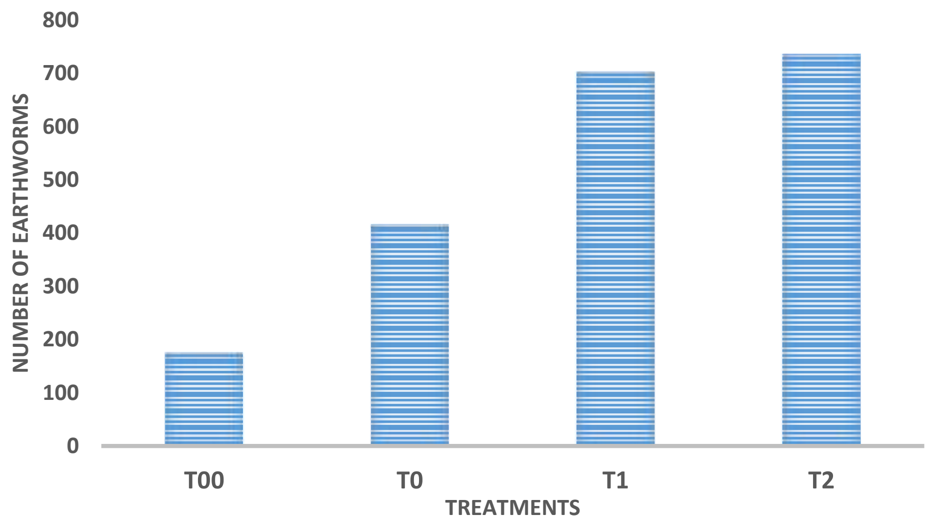

Figure 11: Number of earthworms under grassy cover in flower beds

Examination of the results in Figure 11 shows that the plots for the different nursery treatments are significantly higher than the absolute control, which is the least efficient way of establishing *Pennisetum purpureum* by direct cuttings.

In addition, the plots for the treatments using increasing doses of auxin and NPK 17-17-17 fertilizer (0, 50 and 100kg/ha) applied to the nursery beds, combined with a constant dose of pig manure (5t/ha), stood out from each other.

The value of the discrimination index of the treatments "p", at the analysis of variance, led to a highly significant difference between these treatments. This clearly shows that organo-mineral fertilization, under the conditions of use defined in our experiments, had a clear influence on the average height of the plants and the average number of leaves per plant, in proportion to the doses of fertilizer used.

Discussion

The concern raised at the beginning of this research was to know if the multiplication in nursery of the propagation material, on substrates provided with increasing microdoses of fertilizers and hormones (auxins) could induce a more abundant and less expensive production of shoots. It was also necessary to verify to what extent this could favorably interfere on the rhizospheric soil ecology (increased fertility) through a biological ploughing operated by the roots and the earthworms, whose dynamics are, therefore, improved.

At the end of this work, it should be said that the application, in nursery, of auxins (AIA) and increasing doses of fertilizer NPK 17-17-17 coupled with pig manure has very clearly influenced the rate of resumption of shoots at transplantation compared to the practice of direct cutting in the field (100% against 80%).

Similarly, the tillering index increased with the use of auxin (17,5 and 35 kg/ha) and fertilizer (50 kg and 100 kg with 5 t of manure per hectare), i.e. 1,9%, 2,8%, 3,7% and 4% respectively for direct cuttings in the field without watering, and the non-use of fertilizer + manure in the nursery.

At this level, we can affirm that hypothesis 1 of our research is verified.

Similar research using fertilizer inputs under grass cover has been undertaken by different authors:

1. a high level of microbial activity and assimilable P was found to be directly proportional to the density of the hairy root system and to the amount of inputs regularly accumulated above ground under conservative cultivation (Salinas-Garcia *et al*., 2001; Wright, 2009; Dos Santos *et al*., 2011);
2. perennial grass hedges, especially those of grasses, as well as micro-pools play an effective role in reducing water, ion and soil losses under cultivation and soil humus richness (Comino and Druetta, 2010; Huang *et al*., 2010; Xiao *et al*., 2012).

With respect to parameters defining the soil-root interface (porosity, root density, and earthworm numbers), in general, nursery-developed treatments clearly outperform T00, the local practice of direct field planting of *Pennisetum purpureum* cuttings. There is therefore a clear advantage to pre-production of cuttings in the nursery, with or without chemical inputs.

Furthermore, the plots for the treatments using increasing doses of auxin AIA (0g, 17,5g and 35g/ha) and NPK 17-17-17 fertilizer (0, 50 and 100kg/ha) combined with a constant dose of pig manure (5t /ha) in the nursery beds stood out from each other.

The value of the discrimination index of the treatments "p", in the analysis of variance led to a highly significant difference between these treatments. This clearly shows that auxin AIA and organomineral fertilization, under the conditions of use defined in our experiments, have clearly influenced the soil-root interface (porosity, root density, earthworm population) under the grass cover of the nursery beds.We can, finally, affirm that our second hypothesis is verified.

Finally, we can affirm that our second hypothesis is verified.

Indeed, research undertaken on the modalities of application of increasing doses of NPK fertilizer on the green carpet of *Pennisetum purpureum* associated with leguminous plants has been very successful on the yields of integrated food crops, namely rice, maize and banana (Pyame, 2015; Ntamwira, 2021).

It has also been established that crops and grasslands managed under grass cover develop high root density and higher hydromineral demand creating conditions for high soil exploration (Yang, 2004, 2005; Herold *et al*., 2014), thus proving to be more productive than those in conventional agriculture (Carvalho *et al*., 2012).

Finally, the following performance factors underlying the observed results on growth parameters should be mentioned. These include, but are not limited to:

1. The importance of a living, vigorously rooting, forward-looking canopy as a relay to mineral fertilization or nutrient blotting to enhance the value of input supplies (Rosolem *et al*., 2002; Eekeren *et al*., 2010; Dube *et al*., 2012),
2. Strategic organo-mineral fertilization directed at grass covers, thus forming mycorrhizal enrichment pools (Pande & Tarafdar, 2004),
3. Concerning total porosity and earthworm density under grass cover.

A variable density of earthworms is reported by several authors comparing zero tillage to conventional tillage: 80 vs. 49 (Norgrove *et al*., 2011), 81 vs. 52 (Xu *et al*., 2013), 319 vs.

61 (Errouissi *et al*., 2011), 572 vs. 280 (Schmidt *et al*., 2003). The high densities of earthworms recorded in this experiment are attributed to the favorable climatic conditions, but also and especially to the high production of recyclable inputs and carboydrates by the *Pennisetum purpureum* grass fallow. The balanced application of organic inputs stimulates earthworm activity, influencing aggregate dynamics (Erikson *et al*., 2009; Jouquet *et al*., 2012).

Although many factors can contribute to aggregation (microbial juices, extraradical mycelium of mycorrhizae, root excreta), the action of earthworms remains the most decisive, thus making the contribution of labile substances emanating from metabolism effective (Bohlen *et al*., 2002).

Conclusions and Suggestions

The aim of our research was to experimentally measure the rate of cuttings recovery, the tillering index of shoots, the soil-root interface under the emerging grass stand and the dynamics of earthworms in response to the application in the nursery of increasing microdoses of hormones (auxins AIA) and NPK fertilizers.

The following major findings emerged from this research, confirming all of our hypotheses:

The application in nursery of increasing doses of hormones (auxins AIA) and NPK fertilizers on a degraded soil would induce, in the same order, a rate of recovery and a tallage coefficient more important;

The application in nursery of increasing doses of hormones (auxins AIA) and NPK fertilizers on a degraded soil would induce, on the beds, a soil-root interface (total porosity, root density) much more important (biological tillage);

The application of increasing doses of hormones (auxins AIA) and NPK fertilizers on a degraded soil would induce, in the same order, a more important dynamics of earthworms (biological tillage).

As regards the rate of recovery and the tillering coefficient, it is necessary to say that the hormonal application on the cuttings and the organo-mineral fertilization employed on the beds of rejection, under the conditions of our tests, clearly influenced the rhizogenesis and the metabolic activity of the vegetative apparatus during the stay of the rejections in nursery. This suggests that an effective residual effect related to the nutrient content of the plant material and other growth factors would accompany the transplantation of well-fed and nursery-activated seedlings into the field.

As for the soil-root interface, it should be noted that the diagrams relating to the different treatments are clearly distinct, but supplant each other more or less proportionally to the doses of hormones and NPK fertilizers used in the organo-mineral fertilization, the absolute control referring to the current practice of establishing *Pennisetum purpureum* by direct cuttings being the least performing treatment. The value of the discrimination index of the treatments "p", in the analysis of variance led to a significant difference between these treatments. This clearly shows that the organo-mineral fertilization, under the conditions of use defined during our experiments, did not clearly influence the root density and the soil-root interface.

Concerning the dynamics of earthworms, the results show that the value of the discrimination index of the treatments "p", in the analysis of variance, led to a highly significant difference between these treatments. This clearly shows that organo-mineral fertilization, under the conditions of use defined in our experiments, had a clear influence on the number of earthworms under the grassy cover of the strip-plant, in proportion to the doses of fertilizer and hormones used.

It would be desirable for this study to be continued in longer-term trials with a variety of plant hormones and fertilizers for a wider range of food crop species, cover crops, and fertilizer plants.

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