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Rice straw effect on phosphate rock from Morocco (PRM) phosphate solubilizing bacterium (PSB) combination on acid soil in Man (Ivory Coast, West Africa).

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Key words: Rice straw, phosphate rock from Morocco, PSB, Acid soil, Man.

Submitted 16/12/2023, Published online on 29/02/2024 in the *Journal of Animal and Plant Sciences* (J. Anim. Plant Sci.) ISSN 2071 – 7024

1 ABSTRACT

The study examines the problem of phosphorus (P) limited availability to plants. The experiment evaluates impact of rice straw, combined with Phosphate Solubilising Bacteria (PSB) and Phosphate Rock from Morocco (PRM) amendments, on P availability and soil chemical parameters. The results demonstrate the importance of the straw: it significantly lowers pH, to 4.80 in sterilised soil and 4.88 in non-sterilised soil. It encourages proliferation of PSB, increasing from 105 to 107 bacteria g⁻¹ of dry sterile soil to 105 and 106 bacteria g⁻¹ of dry non-sterile soil. The presence of straw also increased the level of dissolved phosphorus, reaching 9.77 mg.kg⁻¹ and 13.68 mg.kg⁻¹ of dry non-sterile soil compared with 5.05 mg.kg⁻¹ and 9.55 mg.kg⁻¹ of dry sterile soil. Furthermore, the introduction of straw exceeded the critical threshold for Dissolved Organic Carbon (DOC), exceeding 0.3 mg.kg⁻¹ of dry soil. Without rice straw, whatever the soil type, DOC remained below this threshold, while pH increased and phosphorus levels remained lower than those observed with the presence of straw. The mineralisation of rice straw acidifies the solution in the medium, increases P, encourages the proliferation of bacterial micro fauna and increases the medium's DOC. The addition of straw had a significant effect on the parameters of the growing medium.

2 INTRODUCTION

Phosphorus (P) is a crucial element in the growth and productivity of cereal crops such as rice and wheat; it is involved at different stages of development proper to maintain physiological, physiognomic and genetic functioning of plants. However, P deficiency in tropical soils leads to lower productivity and yields of plants in general and rice in particular (Kotchi et al., 2010; Rehman and Qayyum, 2020). The percentage of plant-available best phosphorus in soil solution is ranges between 2 and 5 p.c. This deficit of P directly assimilable by the plant limits the activity of microorganisms specialized in the mineralization of soil organic matter, whose content is also particularly low (Rabeharisoa, 2004; Randriamanantsoa *et al.*, 2013). To alleviate this problem, soluble chemical fertilizers, at prohibitive cost for farmers, are applied to soils to achieve appreciable yields. However, poorly applied phosphate fertilizers can reduce soil fertility, through the mobilization of organic colloids (Siemens *et al.*, 2004; Ilg *et al.*, 2005); and excessive use of mineral fertilizers leads to a reduction in nutrient availability, particularly phosphorus and nitrogen, with adverse effects

on the atmosphere (Aulakh and Adhya, 2005). Also, according to Taktek (2015), only 0.1 p.c of total P exists in the soluble inorganic form easily assimilated by plants. For Khan et al, (2007) and Servín, (2014), the majority (70-90 p.c) of phosphate fertilizers used in agriculture precipitate after application. They form metal complexes, thus limiting their effectiveness, which depends on edaphic conditions (Li and Stanforth, 2000; Riggle and Von Wandruszka, 2005; Servín, 2014). Due to its richness in nitrogen, phosphorus, potassium, sulphur and other micronutrients, Organic Matter (OM) helps to increase crop yields by improving soil fertility (Lompo et al., 2009). Although OM improves the physical, chemical and biological properties of soils, the question of its availability in sufficient quantities remains problematic. Thus, one low-cost alternative is the use of phosphate rock (PR), which is a true source of phosphorus (Kotchi et al., 2010). But one of the main obstacles to the direct application of phosphate rocks to soils is the insufficient release of P to support plant nutrition due to their low solubility in soil depending on soil characteristics (Smalberger et al., 2010; Servín, 2014). PSBs enhance the amount of P solubilized in the soil and from Phosphate

2 MATERIALS AND METHODS

2.1 Study site: The study site is located between latitudes 07°18' and 07°36' North and longitudes 07°27' and 7°53' West, at the National Center of Agronomic Research (CNRA®) of Man, in western Côte d'Ivoire (West Africa). The climate is humid tropical and the rainfall regime is unimodal, with a rainy season from March to October followed by a dry season from November to February. Our site has a ferralitic soil highly desaturated in bases with a strongly acid pH.

2.2 Chemical and natural materials: Two types were used of inputs: chemical inputs such

Amendments (PAs) to make it available to plants (Khan et al., 2007). Also, organic P contained in organic matter can only be made available to plants after decomposition and mineralization by microorganisms (Smith et al., 2003; Pereda Campos, 2008). Making P from PR available to the plant has led researchers to partially acidify PRs (Kpomblekou and Tabatabai, 2003), to manufacture phospho-composts based on organic residues and PR (Saleem et al., 2013), to combine natural phosphates with water-soluble phosphate fertilizers (Mashori et al., 2013) and to solubilize phosphate rocks with microorganisms (Servín, 2014; Taktek et al., 2015). However, phosphate fertilization methods need to be tailored not only to plant requirements for this element, but also to the mechanisms by which it becomes available and used by plants to increase yields. This study was undertaken in order to phosphate introduce amendments (PAs), composed of different proportions of PR and TSP, to decrease soil pH and improve phosphate nutrition in plants. The aim of this study is to effect of rice straw on evaluate the mineralization process of phosphate rock from Morocco (PRM) by phosphate solubilizing bacteria (PSB) and on the physico-chemical parameters of the environment.

as TSP (Triple Superphosphate), a granulated fertilizer with a water pH between 1 and 3, a minimum solubility of 90% in water and containing 30 p.c P₂O₅, and two natural inputs such as phosphate rock from Morocco (PRM), also containing 30 p.c P₂O₅ with a solubility of 3 p.c in water, and rice straw collected from farmers' plots. PRM and TSP were supplied by OCP-Africa (Office Chérifien des Phosphates). The chemical composition of the PRM is given in Table 1, and the chemical composition of the straw in Table 2.

Table 1: Chemical composition of phosphate rock from Morocco

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Chemical components	Concentration (%)
P_2O_5	30
CO_2	6.44
SO_3	1.29
SiO ₂	6.64
CaO	49.54
MgO	1.16
Fe ₂ O ₃	0.2
Al ₂ O ₃	0.41
F	2.21
H ₂ O	2.13

Table 2: Concentration of N, P, K, Ca, Mg and C in rice straw

Nutriment	Concentration (g.kg ⁻¹)
Ν	140
р	1.6
Κ	26.2
Ca	1.7
Mg	2.8
С	448.2

2.3 Biological material: The biological material consisted of a bacterial isolate from the soil at the study site. It was selected following isolation tests based on the characteristics described by Sharma and al. in 2011. The results of the isolation tests are reported in Table 3.

2.4 Conducting the tests

2.4.1 Soil sampling: In the field, soil samples were taken along the diagonals of the plot at different points at a depth of 0-20 cm and mixed to obtain a composite sample representative of the study site. The composite sample was divided into three parts. The first part was kept in a freezer for culturing and counting the microorganisms, and then for isolating the phosphate-solubilising bacterial strains. The second part was air-dried and used for the physico-chemical characterisation of the plot before setting up the experiment. The third part was used for batch testing.

2.4.2 Isolation of strains: Isolation was carried out in three stages: pre-culture, selection and purification of bacterial strains on solid Pikovskaya medium (PVK).

Pre-culture consisted of inoculating the wells of microplates containing liquid PVK medium with soil suspensions for 7 days of incubation. The optical densities (OD) of each well were then determined using a spectrophotometer. The results were processed by a statistical programme to determine the Most Probable Number (MPN) of bacteria present in the sample, expressed as CFU (Number of Colony Forming Units) per gram of dry soil. The selection consisted of using the positive wells for inoculation and incubation for 7 days of petri dishes on solid PVK medium according to the morphological diversity of the strains. Morphological distinction of colonies was based on criteria described by Sharma et al (2011). These include shape, colony colour, appearance, colony diameter and solubilisation halos, followed by the solubilisation index. Purification consisted of isolating bacterial strains according to their shape, colony colour, appearance, colony diameter and solubilisation halos. We used isolates with an IS >2 to reseed petri dishes containing solid PVK medium (Figure 1). This procedure was repeated five (05) times until pure bacterial strains were obtained. The strain with the highest solubilisation index was used to set up our batch experiment.

The diameter of the colony and the surrounding halo were measured to determine the

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solubilisation index (SI) according to the following formula:



Figure 1: Uninoculated control microplate (A) and inoculated microplate showing a halo of solubilisation around a colony (B) (Source: Kpan, 2019).

2.5 Batch experiments: To complete the study on the capacity of the selected bacterial strain to effectively solubilise phosphate, a batch experiment was carried out in the laboratory in which the soil sample was divided in two and then one part was sterilised by heat treatment (autoclave) at a temperature of 120° C for 15 minutes on three (03) occasions at one (01) day intervals. In each pot of 400 cm² surface area, 30g of sterile or non-sterile tray soil was introduced. Then 1.2 g (300 kg.ha⁻¹) of phosphate amendments composed of different

proportions of phosphate rock (PR) and/or triple super phosphate (TSP) to give 0.144g P /pot or 90 kg P_2O_5 .ha⁻¹ and finally 300 ml of sterilised distilled water to give a moisture content of 80 p.c were added, then homogenised. The treatments received 250 µl of bacterial isolate, corresponding to 108 bacteria per pot. The pots were shaken and incubated for sixty (60) days in a randomised complete block design with 4 blocks. The treatments applied are shown in Table 3.

Type of soil	Inputs (kg.ha ⁻¹)	Quantity of straw	Inoculum	
	PRM+TSP	P_2O_5	supplied (t.na ⁻¹)	(I)	
SterileSoil	300	90	0	+ I	
SterileSoil	300	90	0.15	+ I	
Non-Sterile Soil	300	90	0	+ I	
Non-Sterile Soil	300	90	0.15	+ I	

2.6 Data collection: After 0, 10, 20, 30, 40, 50 and 60 days of incubation, 5 ml of the different solutions were taken from each pot and centrifuged at 4,000 rpm for 15 minutes. The

supernatant obtained was then used to determine changes in the parameters (pH, P, dissolved organic carbon (DOC) and number of bacteria) of the culture medium. The pH was determined electrometrically using a pH meter with a glass electrode (744 pH metermetrohm). Total organic carbon was determined by the Walkley-Black method using oxidation in a mixture of potassium dichromate and sulphuric acid. This method assumes that the quantity of oxygen consumed is proportional to that of the carbon to be determined. Phosphorus in solution was determined spectrophotometrically using the Spectroquant phosphate kit, a method



Figure 2: Standard curve for phosphorus

From the measurement of the optical density (OD) of dilutions (100 to 108) of bacterial cultures, a calibration curve of bacterial growth as a function of OD was established (Figure 3). The number of bacteria in the lowest dilution was determined using the Thomas cell, giving based on the determination of orthophosphate ions. In a sulphuric solution, orthophosphate ions form phosphomolybdic acid with molybdate ions. This is reduced by ascorbic acid to blue phosphomolybdenum (BPM), which was measured photometrically and the absorbance was measured at 790 nm and then converted to mg.kg⁻¹ using the established standard range (Figure 2).

the straight line y = ax + b (where y = number of bacteria and x = OD). Once the ODs of the culture media have been determined, the values are used to determine the number of bacteria using the pre-established standard curve (Figure 3).





Figure 3: Standard curve for the isolate (strain 1) used in the pot experiment.

2.7 Statistical analysis: Using SAS 9.0 software, analyses of variance (ANOVA) were performed using the Student Newman_Keuls (SNK) test to determine the significance of the different treatments on the mean levels of

3 RESULTS

3.1 Physico-chemical characteristics of the soil: The soil has a sandy – silty – clay loam texture, moderately acid with a pH water of 5.2 and a pHkcl = 3.6. The variation in pH (Δ pH= pHwater - pHkcl) is Δ pH >1. The soil contains a small amount of nitrogen (less than 100 mg.kg⁻¹) and its carbon and phosphorus contents are 143 mg.kg⁻¹ and 5 mg.kg⁻¹ respectively. However, although it's organic matter content is high (246

solubilised P, dissolved organic carbon (DOC), the number of bacteria and pH, using the least significant difference (LSD) method at the 5 p.c significance level.

mg.kg⁻¹), there is little decomposition of organic matter, resulting in a high C/N ratio (C/N>10) (Table 4). The CEC is low (1.54 cmol⁺.kg⁻¹), and the saturation rate (18.23 p.c) shows that the soil is desaturated in exchangeable cations such as calcium, magnesium, potassium and sodium, with respective values of 1.00 cmol⁺.kg⁻¹; 0.32 cmol⁺.kg⁻¹; 0.11 cmol⁺.kg⁻¹; 0.11 cmol⁺.kg⁻¹ (Table 4).

Table 4: Physico-chemical	characteristics o	f the soil in	the 0 - 20 cm	horizon	before ex	perimentat	ion
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Soil characteristic	Value
Argile (%)	28.65
Limon (%)	16.35
Sable (%)	55
pHeau	5.2
pH _{KCl}	3.6
Passi (mg.kg ⁻¹)	5
C organique (mg.kg ⁻¹)	143
N total (mg.kg ⁻¹)	13
MO (mg.kg ⁻¹)	246
C/N (mg.kg ⁻¹)	11
K ⁺ (cmol ⁺ .kg ⁻¹)	0.11
Na+ (cmol+.kg-1)	0.11

Ca++ (cmol+.kg-1)	1.00
Mg++ (cmol+.kg-1)	0.32
CEC (cmol ⁺ .kg ⁻¹)	1.54
S/T (%)	18.23

3.2 Macroscopic characteristics of isolated strains: Overall, four (04) bacterial strains were identified on solid PVK medium from the Plateau soil sample. The macroscopic study of the bacterial strains showed that the diameters of the halo zone varied from (0.2 to 1cm) and the solubilisation indices (SI) (from 0.6 to 3.6) (Table 4). The colour of the strains identified is green-yellow (S1), brown (S2), brown-green (S3) and green (S4) and they have a rounded (S3 and S4) and subangular (S1 and S2) shape but they all have a cottony appearance. Furthermore, only strain S1 obtained a solubilisation index IS>2, which is equal to 3.6 (Table 4).

Table 4: Macroscopic characteristic	s of the BSPs found	on the Man p	lateau soil.
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Isolates	Morphological characteristics	Halo diameter (cm)	IS
S1	Cotton aspect, green-yellow colour, subangular shape	1.03±0.15	3.6±1.2
S2	Cotton aspect, brown colour, subangular shape	0.2 ± 0.01	0.6 ± 0.04
S 3	Cotton aspect, brown-green colour, rounded shape	0.7 ± 0.02	1.2 ± 0.3
S 4	Cotton aspect, green colour, rounded shape	0.4 ± 0.03	1.5 ± 0.5
SI = Solubilisation index			

3.3 Changes in culture medium parameters in Batch studies

3.3.1 **Changes in pH:** The evolution of the parameters medium during the batch experiments shows that on sterilised soil, the initial pH values (day 0) vary according to the presence or absence of straw. The pH on the first day in the absence of straw was 5.7 compared with 6.57 in the presence of straw. From the 1st to the 10th day after the start of the experiment, the pH in the presence of straw in the medium fell rapidly and reached 5.53, while the pH in the absence of straw remained constant. From the 10th to the 60th day of the experiment, the pH in the presence of straw fell progressively to reach a value of 4.8, while the pH in the absence of straw remained constant until the 50th day before increasing to reach 6 on the 60th day (Figure 4). On non-sterile soil, at the start of the experiment, the pH in the presence of straw (pH = 5.6) and in the absence of straw (pH = 5.72) were low compared with the pH under sterile soil. The pH trend is identical to that of the sterile soil. However, variations from the 1st to the 50th day of the experiment were significantly lower than in the sterile soil. Between days 50 and 60, the pH in the presence of straw fell sharply to 4.88, while in the absence of straw it rose rapidly to 6.02 (Figure 4).



Figure 4: pH evolution during incubation times in the presence or absence or rice straw in sterilized and none sterilized soil

3.3.2 Changes in P content: Generally speaking, P levels increased in both types of soil (sterile and non-sterile), whether or not straw was present in the medium from day 1 to day 60 of the experiment. However, the P content of the non-sterile soil was higher, fluctuating between 9.77 mg.kg⁻¹ and 13.68 mg.kg⁻¹ of dry soil in the absence of straw, compared with 11.18 mg.kg⁻¹ and 17.2 mg.kg⁻¹ of dry soil in the presence of rice straw. The P content of sterile

soil in the presence of rice straw varied between 5.05 mg.kg⁻¹ and 9.55 mg.kg⁻¹ of dry soil without straw, compared with 6.24 mg.kg⁻¹ and 10.27 mg.kg⁻¹ of dry soil in the presence of rice straw (Figure 5). Despite this general gradual increase, the P content of the sterile soil that had received the straw increased rapidly from day 1 to day 10 and from day 40 to day 50 of the experiment (Figure 5).



Figure 5: P content evolution during incubation times in the presence or absence of rice straw in sterilized and none sterilized soil

3.3.3 Changes in the number of PSB and DOC: The number of bacteria on the nonsterile soil was higher than the number of bacteria on the sterile soil from the beginning to the end of the experiment. However, on the sterile soils the number of bacteria increased significantly over the course of the experiment (from 105 to 107 bacteria g⁻¹ of dry soil in the presence of rice straw, whereas in the absence of straw the number fluctuated between 105 and 106 bacteria g⁻¹ of dry soil). On non-sterile soils, the number of bacteria increased progressively in the presence or absence of rice straw up to day 20. But from day 20 to day 60, compared with

the medium with rice straw, the number of bacteria in the absence of rice straw was higher, with a peak of 4.06.108 bacteria g⁻¹ of dry soil on day 30. After 60 days, the non-sterile soil with straw contained an average of 3.65.108 bacteria in the non-sterile soil without straw (Figure 6). In the absence of rice straw in the growing medium, dissolved organic carbon (DOC) was below the threshold (<0.3 mg.kg⁻¹) for all soil types. But in the presence of rice straw in the medium, on infertile soils we obtained 1.39 mg.kg⁻¹ DOC compared with 1.14 mg.kg⁻¹ DOC on infertile soils (Table 5).



Figure 6: Bacteria number evolution during incubation times in the presence or absence of rice straw in sterilized and none sterilized soil

The ANOVAs carried out on the pH values, phosphorus content, number of bacteria and dissolved organic carbon indicate a drop in soil pH (4.81 and 4.86) highly significant at P = 0.0001 after 60 days of incubation in the presence of straw and an increase in pH (5.74 and 6.13) highly significant at P = 0.0001 after 60 days of incubation in the absence of straw (Table 5), regardless of the type of soil (sterile or non-sterile). There was a highly significant increase in phosphorus content and DOC at

P<0.05 after 60 days of incubation in the presence of straw compared with the treatment without straw (Table 5) on both sterile and nonsterile soil. The number of bacteria was significantly greater at P<0.01 in the presence of straw than in the absence of straw on the sterile soil. But on the non-sterile soil, bacterial proliferation under the straw treatment was not significantly different from that without straw (Table 5).



	Non sterile			Sterile				
Treatments	pН	Р	PSB	DOC	pН	Р	PSB	DOC
		(mg.kg-1)		(mg.kg-1)		(mg.kg-1)		(mg.kg ⁻¹)
Rice straw	4.8b	17.40a	16.10 ⁸ a	1.39a	4.86b	13.80b	4.3.10 ⁵ a	1.14a
Without Rice straw	6.13a	9.72b	15.10 ⁸ b	<0.3b	5.74a	9.58a	3.7.10 ⁵ a	<0.3b
CV (%)	3.43	7.87	42.92	19.79	3.768	12.204	11.45	8.95
Pr>F	0.001*	0.0009**	0.01*	0.0002**	0.006**	0.022*	0.17 ^{ns}	< 0.0001***

Table 5: Statistical test for changes in environmental parameters with or without rice straw

The natures in the table are the averages of the parameters measured during the follow-up of the experiment. Data in the same column followed by the same letter are not significantly different according to the Newman-Keuls test p<0.05. (0.3 is the COD detection threshold) *** very bighty significant at the p<0.05 threshold; ** very significant at the p<0.05 threshold;

4 **DISCUSSION**

4.1 Effect of rice straw on the pH, bacterial count and DOC of the medium: The analysis showed that the addition of rice straw to the growing medium led to a gradual drop in pH from the beginning to the end of the experiment compared with the medium without rice straw, while the number of bacteria in the medium increased from the beginning to the end of the experiment. These two phenomena are thought to be linked by the fact that the rice straw added to the medium was a source of nutrients for soil-dwelling organisms (Lompo et al., 2009). In fact, the processes of decomposition and mineralisation of rice straw by soil microorganisms lead to acidification of the environment through the release of acids such as oxalic, citric and tartaric acids and organic carbon (DOC) into the environment (Caribou, 2013; Plassard et al., 2015). This rice straw decomposition activity allows soil microorganisms to proliferate. These results are in line with those of Bongoua et al., 2012, who observed a drop in soil pH and an increase in the number of bacteria after application of organic amendment in rice field soils in Thailand, affected by salinity problems, probably due to the reducing Ferri activities of bacteria. The increase in pH in the absence of rice straw is thought to be due to the mineralisation of the P contained in the treatments applied during the experiment. RPM contains 49.54 p.c Ca, whose release into the environment would create a liming effect that reduces the H⁺ concentration of the soil solution and increases the pH (Traoré, 2009; Hartemink and Barrow, 2023).

4.2 P content and number of bacteria: The initial pH of the soil used for the experiment

predisposed it to better solubilisation of the RPM contained in the treatments applied at the start of the experiment, which helped to increase the quantity of P in the medium. This result is in line with those of Plassard et al. 2015, whose work on the involvement of plants and biological processes in the bioavailability of phosphorus revealed that an acid soil favours the solubilisation of Ca phosphates, while reducing the solubility of iron and aluminium phosphates. The low CV values (<15 p.c) in the ANOVAs indicate that environmental P has another source. This increase in environmental P would also be induced by mineralisation of soil OM and rice straw by soil microorganisms. The degradation of rice straw, which has a high phosphorus content (1.6 mg.kg⁻¹), or of soil OM in the absence of rice straw, will promote the mineralisation of soil phosphorus and consequently increase the soluble P content of the medium (Koulibaly et al., 2010). For Plassard et al. (2015), the addition of organic matter as a source of carbon in the medium stimulated the mineralisation of P in the soil and in the straw. In 2011, a study by Oburger et al. showed that in tropical soils with acid pH, most of the Pi is bound to clay and Fe and Al oxy (hydr) oxides. This quantity of Pi could be released as a result of variations (increase or decrease) in the pH of the environment acting on the sites of P fixation by the OM or clay in the soil (Devau et al., 2009). Our results also show that a variation in pH can favour the availability of P in the environment, as demonstrated by the work of Devau et al, (2011).

5 CONCLUSION

The addition of straw to the growing medium affects the pH of the medium, mineralised P and DOC. The presence of straw in the medium favoured the proliferation of PSBs, which in turn favoured the solubilisation of PAs present in the growing medium. The mineralisation of rice straw acidifies the solution in the medium, increases P, encourages the proliferation of bacterial microfauna and increases the medium's

Conflicts of interest: The authors declare that there are no conflicts of interest.

Authors' contributions : Guety Thierry Philippe, Affi Jeanne BONGOUA-DEVISME and Wondouet Hippolyte KPAN took part in

6 ACKNOWLEDGEMENTS

We would like to sincerely thank OCP-Africa for supplying the fertilizers that were used, and the National Centre for Agronomic Research

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DOC. The addition of straw had a significant effect on the parameters of the growing medium. The combination of rice straw, PSB and PRM is therefore very promising as an alternative to chemical fertilisers for improving soil properties and increasing the efficiency of PR use. However, field trials are needed to confirm the agronomic and economic profitability of applying this combination.

setting up and conducting the laboratory tests, in the design, then in the writing and formatting of the article; Konan-Kan Hippolyte KOUADIO and Franck Michael Lemounou BAHAN were the supervisors at all stages of this work.

(CNRA) of Man for approval to use samples from their plot in this study.

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