Effect of phosphate - solubilizing bacteria and compost on the nutritional characteristics of the oil palm crop (*Elaeis guineensis* Jacq.) in Casanare, Colombia

Efecto de la adición de bacterias solubilizadoras de fósforo, y compost sobre características nutricionales del cultivo de palma de aceite (*Elaeis guineensis* Jacq.) en Casanare, Colombia

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ABSTRACT

In accordance with interest to include biological practices in fertilization programs for commercially important crops, the effect of a bioinoculant application based on phosphate solubilizing bacteria along with compost was evaluated on oil palm cultivation in the nursery stage and in a definitive area. The five treatments that were evaluated included: (C) compost, (CQ) compost and chemical fertilizers 50/50, (IC) compost and inoculant, (IQ) chemical fertilizers and inoculant and (ICQ) inoculant, compost and chemical fertilizers 50/50; as a positive control it was used a plant group fertilized with traditional chemical compounds. Organic matter was added at 2% (w/w) at nursery stage and 15 kg/plant in the definitive area. Response variables includedagronomic variables were evaluated (total height, height to bifurcation, bulb diameter and number of leaves) and soil physicochemical variables (pH, oxidizable organic carbon (OOC), extractable phosphorus and total boron), measured during 8 months in the nursery area and 6 months in the definitive area. The results showed that the evaluated compost constitutes an alternative for palm fertilization in the definitive area, as source of nutrients that meet crop demand at this stage of the crop, matching the nutritional levels of the control plants ($P \geq 0.005$). Meanwhile, in the nursery area, chemical fertilization is essential to ensure the quality of the plants during the first stage of growth, since, at this stage, plants require high amount of N, which is not supplied by the compost. Finally, it was not possible to demonstrate the promoting effect of the microbial inoculant on plant growth, so it is necessary to complement this research in regard to this product.

Key words: bio-stimulation, organic fertilizers, chemical fertilizers, agronomic variables.

RESUMEN

Frente al interés por incluir prácticas biológicas en los programas de fertilización en cultivos de importancia comercial, se propuso evaluar el efecto de la aplicación de un bioinoculante a base de bacterias fosfato solubilizadoras junto con la adición de compost, en un cultivo de palma de aceite en etapa de vivero y en zona definitiva. Se evaluaron cinco tratamientos que comprendieron: (C) compost únicamente, (CQ) compost con fertilizantes químicos 50/50, (IC) compost con inoculante, (IQ) fertilizantes químicos con inoculante e (ICQ) inoculante, compost y fertilizantes químicos 50/50; como control positivo se usó un grupo de plantas con la fertilización química tradicional. La materia orgánica se adicionó a razón de 2% (p/p) en vivero y 15 kg/planta en zona definitiva. Las variables de respuesta incluyeron variables agronómicas (altura total, altura hasta bifurcación, diámetro del bulbo y número de hojas) y fisicoquímicas del suelo (pH, carbono orgánico oxidable (OOC), fósforo extractable y boro total), medidas durante 8 meses en zona de vivero y 6 meses en zona definitiva. Los resultados obtenidos demostraron que el compost evaluado constituye una alternativa para la fertilización de palma en zona definitiva, ya que fue una fuente de nutrientes que logró suplir la demanda en esta fase del cultivo, igualando los niveles nutricionales de las plantas del control ($P \geq 0,005$). Por su parte, en zona de vivero resulta indispensable la fertilización con productos químicos para asegurar la calidad de las plantas desde la primera etapa de crecimiento, dado que en esta fase las plantas demandan una alta cantidad de N, que no pudo abastecer el compost. Finalmente, no fue posible evidenciar el efecto estimulador del crecimiento vegetal del inoculante microbiano, por lo que se hace necesario complementar la investigación en relación a éste producto.

Palabras clave: bio-estimulación, fertilización orgánica, fertilizantes químicos, variables agronómicas.

Introduction

Recently, the Colombian oil palm sector has seen a steady increase in cultivated areas, amounting to 360,000 ha (Fedepalma, 2012), although the gap in competition with other countries such as Malaysia or Indonesia is notable, due to, among other factors, the fact that production costs in Colombia are about 190% higher than in Malaysia;

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attributed to inadequate use of inputs which results in nutritional imbalances that affect the growth and yield of oil palm, associated with an inefficient use of the nutrients (Weng, 2003; Acuña, 2005).

Palm cultivation includes three phases: pre-nursery, nursery and renewal or definitive area, the site the plants occupy for 20-25 years which can be in new areas (usually with a past use for cattle) or old plantation areas that are renewed with young plants. In the nursery stage, seedlings are grown for 8-10 months, monitoring the conditions of irrigation and nutrition to ensure phenotypic quality, which are then planted in two different areas: new soil called definitive zone or planting and old soil called renewal zone. During the first three years, the demands of N, P, K, B and Mg increase and are of great importance for the development of more vigorous and productive plants (Corley and Tinker, 2003). For this reason, in the nursery stage, monthly applications of chemical fertilizers are carried out along with an initial application of organic matter, while the renewal zone is fertilized with 60% of the amount applied in the nursery, given the lower nutritional requirements of adult plants (Corley and Tinker, 2003). Given this, the use of organic and biological inputs produced from renewable resources could be an alternative for nutritional management in growing oil palm. Heriansyah (2010) demonstrated that the addition of organic fertilizers in oil palm cultivation at the nursery stage generated a 15% increase in total dry matter and a 35% increase in yield of mature plants. This response is attributed to the microbiological content of the manure and its effects on nutrient availability, mineralization and PGPR activity (Plant Growth Promoting Rhizobacteria), as well as production of phytohormones, enzymes, siderophores, stimulation of biological nitrogen fixation, production of antibiotics and solubilizing of P (Hargreaves *et al*., 2008). However, despite the limitations of phosphorus (P) in acid soils, in Colombia, there are no known studies on the effect of beneficial microorganisms such as P solubilizing bacteria with organic material in oil palm plantations. Amir *et al.* (2005), in Malaysia, found that the application of growth promoting microorganisms in greenhouse oil palm favors the uptake of N and P.

The application of bioinoculants based on phosphate solubilizing microorganisms in Colombia has been studied in various crops such as the mangrove and *Citrullus vulgaris* plants (Galindo *et al*., 2006), demonstrating a beneficial effect on plant growth; just as Faccini *et al.* (2007), and Martinez and Martinez (2007) showed, that the use of these microorganisms in Criolla potato (*Solanum phureja*) and sugar cane (*Saccharum officinarus*) generated an increase in plant height, dry matter of tuber roots and leaves, and a greater availability of P and N at the ground level. However, the use of microbial inoculants has not yet been widely adopted as an agricultural practice in different production systems in the country, so it is important to innovate and promote the technology in the Colombian agricultural sector, in this case the oil palm sector.

The present study aimed to evaluate the addition of a bioinoculant based on phosphate solubilizing bacteria to oil palm (*Elaeis guineensis* Jacq.) along with the addition of compost in the nursery stage and renewal area for eight and six months respectively, taking into account physicochemical and agronomic variables.

Materials and methods

The study was conducted on the plantations of Palmar de Oriente, located in Villanueva (Casanare, Colombia), whose geographical coordinates are X = -166831.06529 and Y = 442736.2526 (Zone 43, UTM: WGS84), at an altitude of 300 m, during October-December 2007 and January-June 2008, during which time there was an average maximum temperature of 38°C, a minimum average of 20°C and a relative humidity of 78%.

Compost organic matter

The organic matter evaluated was a class A mature compost according with NTC 5167/2011, produced under controlled piles, (Rodriguez *et al*., 2007), using vegetal waste from horticultural industry. Chemical characteristics included: 36% humidity, oxidizable organic carbon (%CO)10.9%, C/N ratio 10, 30.5 cmol kg^{-1} CEC, total N 1.08% and 0.99% total P; in addition, it was verified the absence of phytopathogens that could affect the oil palm crop: *Fusarium oxysporum*, *Helminthosporium* spp. and *Pestalotia* spp. using the plate count technique (IICA, 2006).

Phosphate solubilizing bacteria inoculant (PSB)

The inoculant applied was a product developed by Angulo *et al*. (2012), which consisted of a mixed inoculant based on five bacterial strains capable of solubilizing inorganic P (IS=1.33) and phosphatase activity (UP=12.9), with a concentration of $6.0x10^{11}$ ufc/mL.

Experimental design

The test was carried out during dry season (November to March) and rainy season (April to June), in two areas of evaluation: nursery and definitive area; using oil palm plants (*Elaeis guineensis* Jacq.) tenera variety; 3 months old in the nursery area and a year old in the definitive area.

The experiment was established using a balanced design, taking as factors: the application of microbial inoculant, compost and chemical fertilizer, the latter two with two levels (100 and 50%); which were tested with 5 treatments and 1 control (Tab. 1), which corresponded to the conventional nutritional plan implemented in the plantation (100% level). In the experiment, 100 plants were used per treatment both in the nursery and in the field.

TABLE 1. Description of treatments performed both in the nursery and field.

Treatment	Description
Control	100% fertilization: monthly application of 5.5 g/palm of ammonium phosphate di basic (18-46-00), 26 g/palm of N-P-K compound 06-18-17, 1.25 g/palm sodium borate , 5.5 g/palm magnesium sulfate and 0.12 and 0.4 mL/palm of gibberellic acid and P-K 0-28-6, applied three times during the 8 months of evaluation, in the renewal area, consisted of the semiannual addition of sodium borate in an average concentration of 110 g/palm and a N-P-K-Mg mixture with an average ratio of 13-11-24-4.
C	Compost 100% nursery 2% (w/w) manually applied on top of the bag. Renewal area: 15 kg/palm applied superficially with coater.
CQ	Chemical fertilizer 50% and compost 50% (1% (w/w) for nursery and 7.5 kg/plant.
IQ	100% chemical fertilization and the addition of the PSB inoculant at 500mL/plant with a 1:10 dilution applied with a sprayer.
IC	100% Compost with the addition of the PSB inoculant 500 mL/plant.
ICQ	Chemical fertilizer 50% and compost 50% and the addition of the PSB inoculant 500 mL/plant.

Every month, for 8 months in the nursery and 6 months in the renewal area, agronomic variables were evaluated such as: total length (cm), number of leaves, length to bifurcation or stem (cm) and the presence or absence of significant nutritional deficiencies in the oil palm according to Corley and Tinker (2003) with manifestation of signs. In the nursery phase, further assessment included differentiation of leaflets, bulb diameter (cm) and percentage of seedlings suitable for transplant (Corley and Tinker, 2003). Also, in both zones, foliar analysis examined P (%) and B (mg kg^{-1}) concentration in each treatment, at month 0 and 2 in the renewal area and at month 2 and 4 in the nursery stage, in order to verify the nutritional status of the plants; in addition soil analysis determined pH and oxidizable organic C (%) with the wet method (Arrieche and Pacheco, 1998).

Statistical analysis

The results were analyzed using descriptive statistics with Microsoft Excel® software and inferential statistics with JMP software for Windows, using the Shapiro-Wilk test to verify the normality of the data, Wilcoxon Kruskal-Wallis test to show differences between treatments and Tukey-Kramer to show which treatments were significantly better.

Results and discussion

Analysis of agronomic variables

The behavior of the total height and height to bifurcation of the treatments evaluated in the nursery area showed significant differences only in the last sampling time (240 days after the start of the trial); here it was seen that at the end of the study there were significant differences between the control and the other treatments ($P \leq 0.001$), with the control being significantly higher than the treatments (Fig. 1), indicating that the supply of chemical fertilizer provides a higher quantity of nutrients with respect to the other treatments. On the other hand, treatment C had the lowest values for both variables, showing that the concentration of total nitrogen (N) (1.08%) in the compost did not provide the available N needed to meet the demand of the plants at this stage of development. However, it was evident that treatment C was similar to CQ and different from ICQ (Fig. 1), which confirms the synergistic effect of the three assessed factors. This behavior is due to the presence of organic matter which improved physicochemical and microbiological properties of the soil (Heargreaves *et al.*, 2008), soil promotes substances (Goenadi, 1998; Joo *et al.*, 2005), in combination with chemical fertilizers which provide the

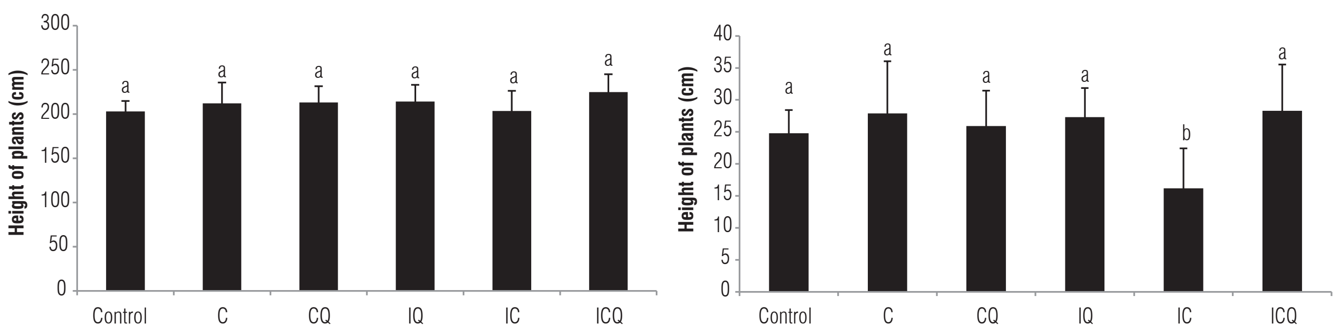

FIGURE 1. Height of plants in the nursery area after eight months of evaluation. A. Total height. B. Height to bifurcation. Treatments with the same letters indicate no significant differences, Tukey-Kramer test ($P \leq 0.05$).

available nutrients needed for plant development (Havlin *et al*., 2005). In this integral nutrition system, it is possible to optimize the agricultural productivity. The ICQ treatment did not exceed the control because it contained only 50% of the nutrient dose required for optimum growth.

Moreover, unlike evidenced in the nursery, treatments in the renewal area did not differ significantly (P=0.1547), showing a behavior equal to the control for total height over the 6 month evaluation in all treatments (Fig. 2A), similar to results reported by Rochels (2010), who found that the application of compost does not generate a difference in height in oil palm.

For the monthly average number of leaves of plants, both in the nursery area and the renewal area, there were no statistically significant differences between treatments (P=0.105). However, in both areas, was evident that CQ and ICQ treatments had on average of one leaf more than the control (Tab. 2), indicating that the compost mixed with half the dose of chemical fertilizer promotes leaf biomass synthesis, which directly affects photosynthetic capacity and thus future yield of the plant (Munévar, 1999). Compared to technical reports from Central America, using palm plants at the end of the nursery stage, those plants have 6 to 8 leaves on average (IICA, 2006); the present study achieved a high number of leaves, up to 12 leaves per plant in the IC and ICQ treatments. The results showed plants with more leaves, that mean a higher photosynthetic rate, and therefore a greater possibility of synthesizing biomass (Gómez *et al*., 2006).

The bulb is a sustaining and plant reserve organ whose development is directly related to nutrition, root development and the presence of new leaves, and constitutes a reservoir of nutrients useful for the synthesis of new tissues (Munévar, 1999). This parameter in the nursery area showed that the IQ treatment and control had values significantly higher ($P\leq$=0.0001) (9.78 and 9.73 cm on average, respectively), compared to the other treatments: C (7.63 cm), CQ (7.9 cm), IC (6.9 cm) and ICQ (8.12 cm) (Fig. 3). This result demonstrates that the addition of adequate doses rates of chemical fertilizers supplies the needs of the plant and could promote root development by the bioinoculant action, either by increased P availability and/or phytohormone production. This synergistic effect contributes to the proliferation and elongation of meristematic cells, improving nutrient absorption (Joo *et al*., 2005) as reflected in the diameter of the bulb. With the results, it was possible to conclude that the addition of compost only at this stage of the crop, or mixed with chemicals at a half dose, does not provide nutrients for adequate plant development because the compost does not offer elevated quantities of macro elements such as P and N that are important to the development of roots and this organ (Corley and Tinker, 2003), but it is important to maintained biological and physical conditions of cropping soils.

The selection of plants in nurseries, in order for planting in the definitive of renewal zone is one of the most important agronomical practice because the suitable establishment and subsequent productivity of the definitive zone depend on the vigor characteristics of these plants such as: height,

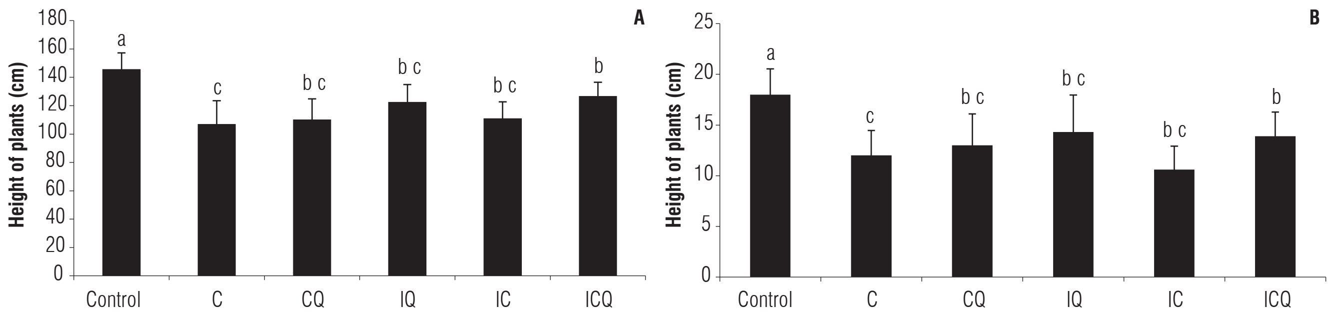

FIGURE 2. Height of plants in the renewal area after six months of evaluation. A. Total height. B. Height to bifurcation. Treatments with the same letters indicate no significant differences, Tukey-Kramer test ($P\leq$0.05).

TABLE 2. Number of leaves at the end of the test in both areas $P\leq$0.05).

Zone \ Treatment	Control	C	CQ	IQ	IC	ICQ
Field	10±1	11±3	11±2	10±1	9±1	11±2
Nursery	9±1	8±1	9±1	10±1	9±2	10±1

Conventions: C: Compost complete dose; CQ: compost and chemical fertilizers at a half dose. IQ: Chemical fertilizers complete dose and microbial inoculant. IC: Compost complete dose and microbial inoculant. ICQ: Compost and chemical fertilizers at a half dose with microbial inoculant.

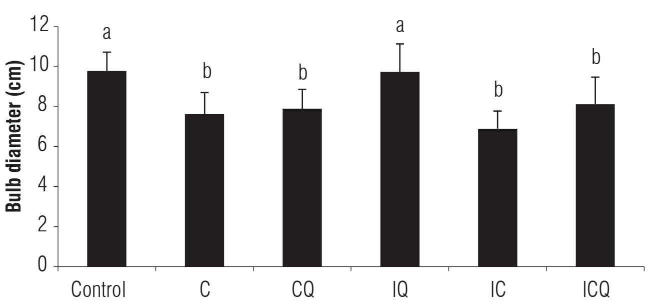

FIGURE 3. Bulb diameter of plants in the nursery during the last month of the evaluation for the different treatments. Treatments with the same letters indicate no significant differences, Tukey-Kramer test ($P \leq 0.05$).

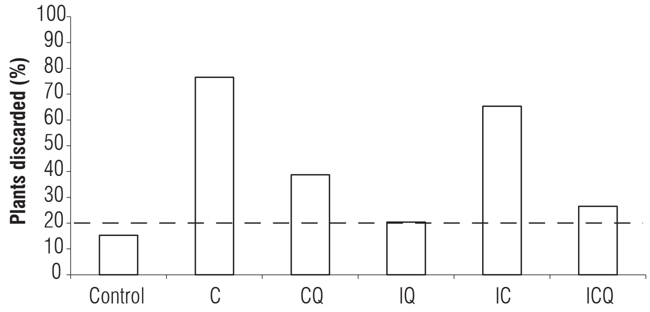

FIGURE 4. Percentage of plants in the nursery area discarded after six months of evaluation.The maximum limit set at 20% is indicated by the dotted line (Corley and Tinker, 2003).

color, number of leaves, leaf differentiation and diameter. It has been established that in nursery a minimum of 80% of the plants must be acceptable, so, a maximum of 20% of the plants have tocould be discarded (Corley and Tinker, 2003). The percentages of discarded plants in the studied nursery showed higher values in treatments C and IC, passing 20%; only treatments with added fertilizer of 100% (Control 15% and IQ 20%) maintained the ideal percentage for discarded plants (Fig. 4). However, considering that it was observed that the ICQ treatment had a tendency to improve the agronomic parameters of total height and leaf number as compared to other, it would be highly useful to verify whether said mixture with doses of 100% provides benefits that exceed control standards.

Soil and foliar analysis

When organic residues are incorporated into the soil, the microorganisms usedmineralized organic compounds, so and total content of OMorganic matter (and also OOC) slowly decreases over time since after metabolism. A fraction is lost as CO_2 and the rest becomes as part of the microbial biomass or simple organic and inorganic molecules. Therefore the organic matter is eventually exhausted and periodic supply system becomes necessary (Hachicha *et al*., 2008). This behavior are sthe results reported was observed in the present study; it was evident that the tested treatments showed a decrease in the soil COOC at over time, achieve levels similar to those of control treatment for both evaluated areas (Fig. 5). When this process of mineralization occurs in very short time periods, as in the case of the present study (2 months), it becomes a negative factor on the compost fertilizer capacity and is related to the low organic C content of the product (Hachicha *et al.*, 2008). It should be noted that the mineralization of organic matter was favored in both areas by high temperatures on the plantation (about 38°C on average), and the controlled humidity conditions of the nursery, which also stimulate mineralization process (Sylvia *et al*., 2005). Meanwhile, atypical behavior presented in the renewal area, as was to be expected, the treatments with the addition of compost had a higher concentration of organic C, at least at the beginning of the trial, which did not happen in all cases (Fig. 5 B). This could be due to the heterogeneous organic matter content of the evaluation area, where independent on soils and not for the evaluated treatments evaluated, there where vegetal residues from old palms and kudzu cover crop (*Pueraria lobata*); therefore there was not possible to demonstrate the effect of compost on the soil

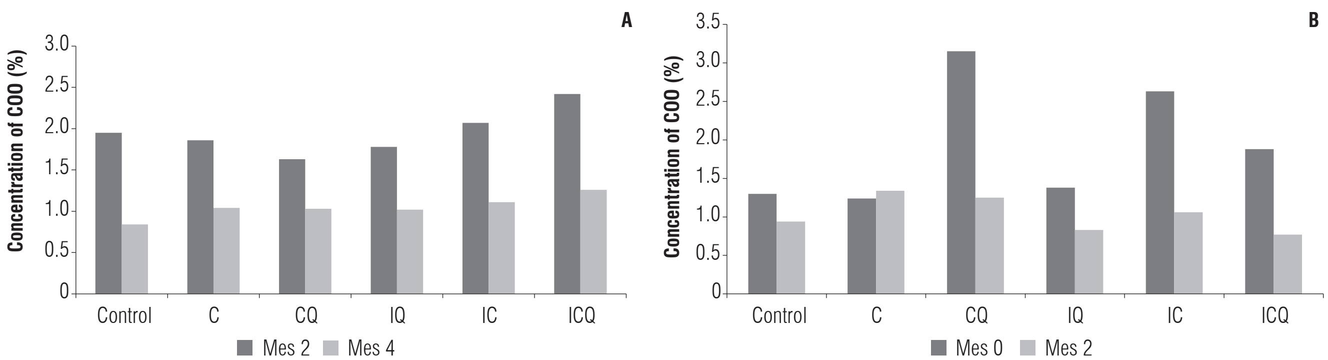

FIGURE 5. Concentration of OOC in soils of the tested treatments. A. Nursery area, 2 and 4 months of the evaluation. B. Renewal zone 0 and 2 months of the evaluation.

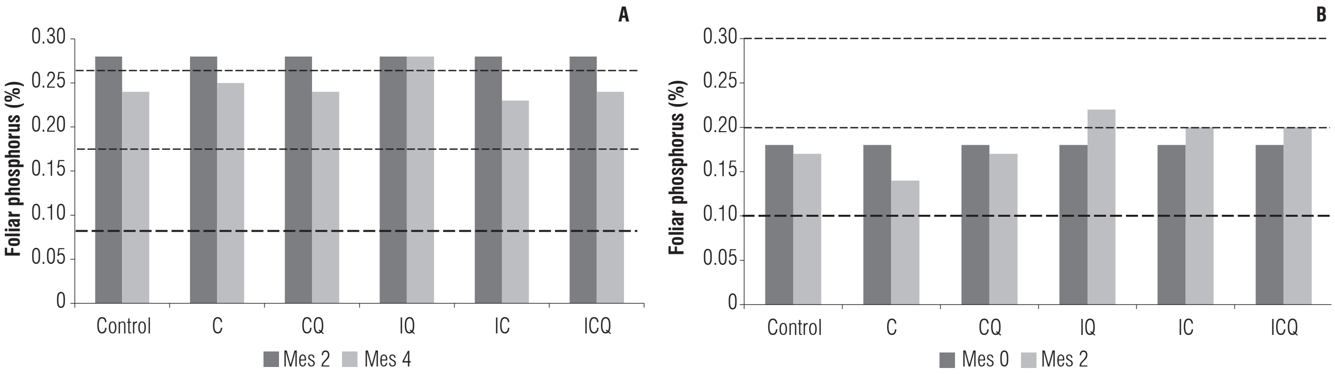

FIGURE 6. Percentage oftotal foliar phosphorusin the different treatments. A. Nursery area. B. Renewal area. Dotted lines represent high, medium and low from top to bottom ofthe y axis.

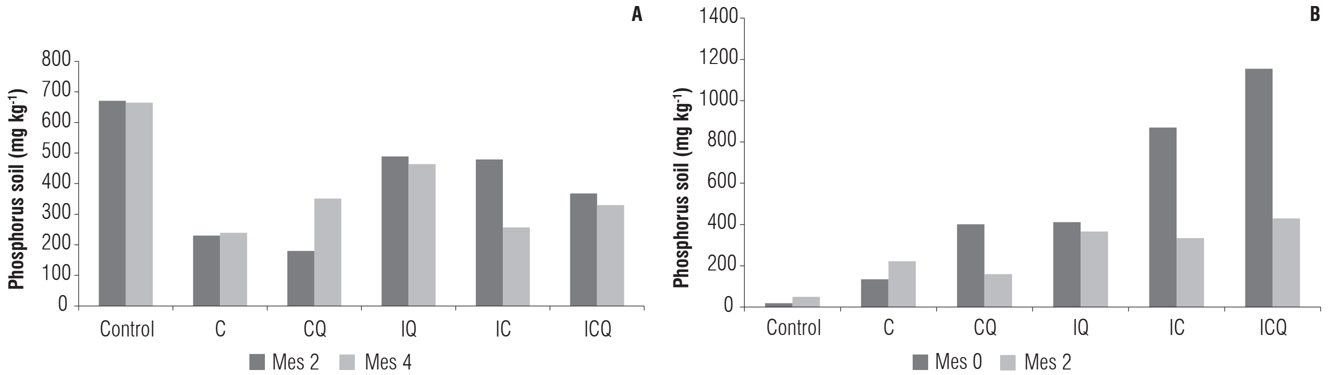

FIGURE 7. Extractable phosphorus soil concentration in the treatments. A. Nursery area. B. Renewal area.

organic carbon. because the plant residue as kudzu and palms leaved in crops could affect the carbon content in soil and was difficult to demonstrate the behavior of organic C incorporated in compost treatment.

Moreover in palm cultivation, phosphorus is an essential element for plant growth, and it is necessary to establish a correct fertilization program to maintained crop sustainability (Leon, 2004). The application of organic material, in good mineralization conditions, increases total phosphorous and stimulates microbial and enzymatic activity (Sylvia *et al*., 2005). This behavior was evident in the nursery area and the renewal zone, since in all treatments reach increase the P demand by plants, and was reflected in an optimal foliar P concentration, higher than 0.16% (Fig. 6) (Corley and Tinker, 2003), with medium-high P levels in the nursery and medium P levels in the renewal area (Fig. 6).

On the other hand, boron (B) deficiencies are associated with low organic matter content (<1%), pH and soil texture (Fujiwara and Tanaka, 2007). In oil palm plantations, B is an element of great importance since it intervenes in the synthesis of sugars and carbohydrates and the metabolism of nucleic acids and proteins. B is also essential to meristematic activity, therefore boron deficiency affects the tips of the leaflets of the leaves, resulting in deformation of the leaves and "fishbone leaf" (Espinosa, 2000). Organic matter is the largest available source of boron, which is released and becomes available to the plant through the continuous decomposition process; since B does not move through the phloem, it must be available to the root system, whereby the addition of organic matter is necessary (Yamada, 2000). Thus, environmental conditions such as humidity and aeration define the presence or absence of this micro element (Yamada, 2000). Therefore, the treatments containing compost in both areas had appropriate results for leaf tissue analysis regarding this element (Fig. 8). Compost applications in the nursery area provided a medium-high boron level, and although the chemical fertilization presents a high level, it should be noted that the CQ treatment provided the greatest amount of boron to the plants, suggesting that chemical fertilization of this element could be decreased or substituted by adding compost, since it is able to meet the B demands of the plants.

Meanwhile, in the renewal zone, the C and CQ treatment presented differences in boron nutrition with respect to the control, because basically the compost improved a higher

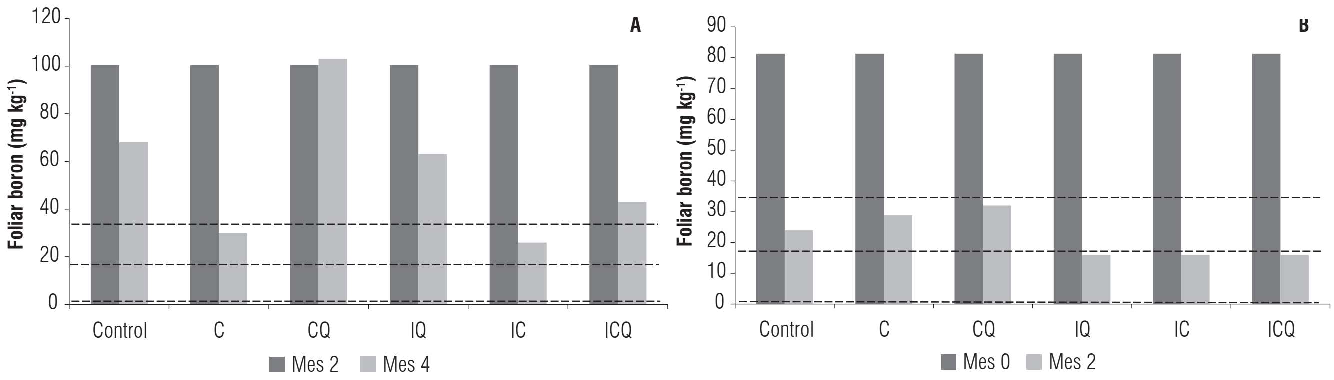

FIGURE 8. Total foliar boron concentration in the different treatments. A. Nursery area. B. Renewal area. Dotted lines represent high, medium and low from top to bottom of the y axis.

amount of boron during the mineralization process (month 2), considering that fertilization with borate sodium in the renewal zone is a less frequent than in the nursery. Likewise, it was evidenced the impact of the addition of the inoculant on a decrease in foliar boron due to consumption of the same on the ground for sustaining bacteria. Although boron metabolic functions in these organisms is not well studied, it was established that in some bacteria it is involved in the synthesis of secondary metabolites such as bromomycine, tartrolon A and B, phenyl boronic acid and orthoboric acid, which are antimicrobials (Ahmed *et al*., 2007) and therefore, it is possible that the added microorganisms in the inoculant could cause a decline in the acquisition of this element, which was evident in both areas (Fig. 8).

Conclusions

Under the study conditions, the use of compost as the sole source of fertilizer in the nursery area was not a viable alternative because it cannot supply the N demand of the plants, a key element for vegetative growth at this stage; however, organic fertilization acts as good source of P and micronutrients such as B, and therefore can be used as part of an integrated fertilizer plan involving organic and inorganic sources of nutrients. On the other hand, in the renewal area, compost was a source of nutrients equivalent to the control, indicating that at this stage of the crop, compost is an organic source that gradually delivers nutrients in amounts that meet the demands of the palms in this developmental stage. However, it is important to determine the effect of organic nutrition in the renewal area on crop yield. Finally, although a tendency to improve some agronomic parameters was seen with the application of microbial inoculants, it was not possible to determine a direct effect on the crop, so an evaluation of an integrated nutrition plan involving suitable doses of organic matter and chemical fertilizers is recommended, to directly view the effect on root synthesis in the nursery stage.

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