Effect of a *Pseudomonas fluorescens*-based Biofertilizer on Sweet Potato Yield Components

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ABSTRACT— *A field experiment was conducted to study the effect of a Pseudomonas fluorescens-based biofertilizer on sweet potato (Ipomoea batatas (L) Lam) yield. The application was by immersion of cuttings before sowing for 0, 5, 10 and 15 minutes with combination doses of 0, 50 and 100% of NPK mineral fertilizer in a randomized block design with three replications (12 treatments-combinations). During the harvest (130 days after planting), some measurements related to yield components were recorded on ten randomly selected plants from each plot. All treatments with Pseudomonas' immersion showed a positive productive response. With 100% NPK and the immersion in the biofertilizer for 15 min showed the highest yield (56.09 tha^{-1}), followed by the other treatments with 100% NPK and without statistical differences among them. The treatment with 50% NPK and the immersion in Pseudomonas for 15 min (49.58 tha^{-1}) had no statistical differences with the control variant (100% NPK, 51.60 tha^{-1}). Based on the results, it can be concluded that this biofertilizer could be an appropriate alternative to increase the sweet potato yield, saving the 50% of the current quantity of the recommended mineral fertilizer, through a more friendly environmental techniques to promote a sustainable, efficient and productive agriculture.*

Keywords— biofertilizer, plant nutrition, sustainable agriculture, sweet potato crop

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1. INTRODUCTION

The world needs an alternative agricultural development paradigm, one that encourages more ecologically sound, biodiverse, resilient, sustainable and socially just forms of agriculture. The agricultural productivity and hence, food and nutrition security are being affected by the climate change with a negative impact on crop productivity [1]. Consequently, it is necessary to move towards a more sustainable and resilient production, in which the agroecological approach is the way forward to produce the necessary food. It requires the implementation of environmentally friendly crops and strategies, as well as timely training of producers for its use.

Sweet potato (*Ipomoea batatas* (L) Lam) (batata, boniato, camote) is a species of American origin, an important food

crop, but considered as an 'orphan crop' [2]. Globally, 112 835 316 t of this tuberous root are produced in 9 202 777 ha with a yield of 12.26 t ha^{-1}; in Cuba, sweet potato yields on smallholder farms stand at an average of 10.87 t ha^{-1} [3]. It is a typical food for food safety since it can be harvested in just 4-6 months, an easily propagated crop able to provide carbohydrates, minerals and β-carotenes (pro-vitamin A). Its ability to face adverse climatic conditions, effective response to meteorological phenomena, its versatility and vegetative reproduction, places it above other crops of higher production [2].

As in other crops, correct and timely fertilization are very important to get high yields. Sweet potato productivity is constrained by poor fertility, especially low potassium (K), phosphorus (P), nitrogen (N), sulfur (S), and some micronutrients [4]. Phosphorus requirements are quantitatively lower than K and N doses, but it affects increasing the average weight and the number of roots [5], and then, crop yields. Due to its slow diffusion and high degree of fixation, phosphorus is generally less available in the soil solution but, its uptake and utilization are essential on the final yield of agricultural crops [6].

On the other hand, the application of chemicals in agriculture is often the cause of soil erosion and environmental deterioration, mainly when used indiscriminately. Fortunately, relationships between plant and some microorganisms improve the assimilation of nutrients and, therefore, that allows to obtain better yields. Among the most commonly used biofertilizers in agricultural crops are mycorrhizae, azotobacter or phosphorin [7; 8]. Other bioproducts, such as: Fitomas® [9], VIUSID agro® [10] that stimulate vegetative development are also used successfully. Many studies have been carried out on sweet potato growth and productivity, including the effect of organic and inorganic fertilizers applications ([11]; [12].

Among the most important beneficial microorganisms, different bacterial species of the genus *Pseudomonas* have been described; they act in a double way on crops: they promote plant growth and suppress pathogenic microorganisms. It has also been suggested that they stimulate the establishment of other beneficial microorganisms associated with roots, such as mycorrhizae [13]. *Pseudomonas* produces an increase in the availability of phosphorus and nitrogen in an assimilable way for the plant, due to the production of phytohormones that stimulate a vegetative activity, as well as the degradation of ethylene precursors [14]. *Pseudomonas fluorescens* Migula is a Gram-negative, aerobic bacilliform bacterium that has several polar flagella. They are known for their ability to stimulate the growth of plants that live in contact with them [15].

Recently, researchers from the Cuban Business Group LABIOFAM developed a biofertilizer based on a *P. fluorescens* strain phosphate solubilizer [16]; the new product is under the technical validation process for its future use in agriculture.

In the case of sweet potato, the effect of this biofertilizer or the most effective way to apply it to achieve sustainable productions is unknown. It is not also known if with the combined use of this bioproduct and doses of mineral fertilizer, the current doses of the chemical, expensive and environmental pollutants, could be reduced. Therefore, the objective of this work was to determine the effect of a *P. fluorescens*-based biofertilizer on the development and yield of sweet potato (*Ipomoea batatas* (L.) Lam) cv. INIVIT B 240-2006.

2. MATERIALS AND METHODS

2.1 Plant Material and experimental design

The experiments were conducted on a neighboring experimental field site located at the Farm "La Dora" (Cienfuegos city, Cuba), between December/2018 – April/2019 (low rainy period). The soil is brown without carbonates [17] with 3.2 % of organic matter and a pH= 6.8.

The commercial sweet potato cultivar ′INIVIT B 240-2006′, obtained by the breeding program from the Research Institute of Tropical Roots and Tuber Crops (INIVIT) was used. It is an early cultivar (four months with potential yields greater than 55 t.ha^{-1}) that has many appreciated characteristics by the producers: a good culinary quality of its elongated tuberous roots, the flesh or pulp is white, sweet and without fibers, and the skin is light red and smooth.

The effect of a *P. fluorescens*-based biofertilizer was evaluated at the dose recommended by the manufacturer (Labiofam) of 20 Lha^{-1}. The application method was by immersion of cuttings before sowing for 0, 5, 10 and 15 minutes and the studies included the combination of the bioproduct with doses of 0, 50 and 100 % of the NPK mineral fertilizer. In field, plants from all tested groups were exposed to the same agricultural conditions according to the Technical Instructive [18].

On-farm field experiments were conducted with 12 treatments:

T1. Absolute Control (a control group without the application of any mineral fertilizer or *Pseudomonas*)

T2. Cuttings with immersion in *P. fluorescens* for 5 min

T3. Cuttings with immersion in *P. fluorescens* for 10 min

T4. Cuttings with immersion in *P. fluorescens* for 15 min

T5. Cuttings with 100% of NPK mineral fertilizer (a control group according to the Technical guide [18]

T6. Cuttings with 100% NPK fertilization plus immersion in *P. fluorescens* for 5 min

T7. Cuttings with 100% NPK fertilization plus immersion in *P. fluorescens* for 10 min

T8. Cuttings with 100% NPK fertilization plus immersion in *P. fluorescens* for 15 min.

T9. Cuttings with 50% NPK fertilization

T10. Cuttings with 50% NPK fertilization plus immersion in *P. fluorescens* for 5 min

T11. Cuttings with 50% NPK fertilization plus immersion in *P. fluorescens* for 10 min

T12. Cuttings with 50% NPK fertilization plus immersion in *P. fluorescens* for 15 min.

All treatments with the previous immersion of cuttings in a *P. fluorescens* biofertilizer received one extra application of the biofertilizer 21 days after planting with the objective to improve the soil's bacterial concentration. In all cases, the application was by means of direct aspersion on the soil surface using a manual backpack and the same dose recommended by the manufacturer. The 100 % NPK fertilizer was applied as 90 kg N ha^{-1}, 75 kg P$_2$O$_5$ ha^{-1} and 150 kg K$_2$O ha^{-1}, according to the technical recommendations [18].

The vine cutting, 20-30 cm in length, from the apical part of matrix plants from the INIVIT experimental field (certificated seed) was used as planting material. A unique planting distance of 0.90 m X 0.30 m was used. Plants were grown in field conditions and experiments were conducted in a completely randomized blocks design with 12 treatments in three replicates (plots of 10.80 m^2).

The plantation was manually, on the ridge and with the soil properly humid, and in independent plots. Irrigation was carried out according to crop needs and the routine agronomic package of practices and plant protection measures recommended for this crop were applied to raise a good crop [18]. Each treatment was represented in three replicates with 50 plants.

2.2 Evaluation and statistical analysis

The measurements were recorded on ten randomly selected plants from the central ridges of each plot and they consisted in different qualitative and quantitative characters (Table 1).

Table 1. Morphological descriptors for the characterization of the sweet potato cultivar INIVIT B-240-2006 during the evaluation of the effect of a *P. fluorescens*-based biofertilizer

No.	Variable	Code	Reference
1	Color of mature leaf	CML	[19]
2	Color of immature leaf	CImL	[19]
3	Tuberous root form	TRF	[19]
4	Predominant color of root skin	PCRS	[19]
5	Predominant color of roots pulp	PCRP	[19]
6	Inter-knots length (cm)	IKL	[19]
7	Diameter of inter-knots (cm)	DIK	[19]
8	Petiole length (cm)	PeL	[19]
9	Length of the main stem (cm)	LoMS	[20]

Special value was given to those variables related with the yield: number of total tuberous roots per plant (NTTR), number of commercial (NCTR) and non commercial (NNCTR) tuberous roots per plant, fresh weight of commercial (FWCTR, g) and non commercial (FWNCTR, g) tuberous roots per plant, and commercial yield per hectare (CY, t ha^{-1}). The collected data were averaged to get mean values of these characters that have been affected by the studied treatments.

After 130 days of growth, the plots were harvested and data for the mentioned characters, especially the number and weight of marketable tubers per plant were recorded. Root and vine characteristics were described previously after 90 days of growth, according to the sweet potato classification system defined by [19] and [20].

The collected data were subjected to the analysis of variance (ANOVA) appropriate to the design of completely randomized blocks with factorial arrangement (3 x 4), where the factors were: A- four immersion times in a *P.*

fluorescens-based biofertilizer (0, 5, 10 y 15 min), and B- three doses of NPK mineral fertilizer (0, 50 y 100 %). All statistical procedure was according to the experimental design and using the tools from the SPSS/PC+ statistical package version 15.0 for Windows® [21]. Whenever differences existed among means values, the comparison of them was carried out with the Tukey's honest significant difference (HSD) for P≤ 0.05.

3. RESULTS

Observations of the sprouting percentage 20 days after planting always behaved above 95 % (absolute control treatment without any fertilizer) for a general average of 98.06 %.

The effect of different treatments on the growth parameters of sweet potato was observed. However, the morphological characteristics did not change, especially those related with the color of immature (lightly purple) and mature leaf (green), the tuberous root form (lengthened), and the predominant light red skin color and the white tuberous roots pulp. All plants, regardless of the treatment received, maintained the characteristics of the cultivar INIVIT B-240-2006, among them: vigorous foliage with green stems and green nodes. The leaves were heart-shaped, slightly dentate of medium size and green color, with green ribs slightly pigmented on the underside with the purple limbo-petiole insertion point.

All morphological measured variables expressed an important effect when the immersion in the biofertilizer was applied during 15 minutes together with the 100 % of the mineral fertilizer (Figure 1).
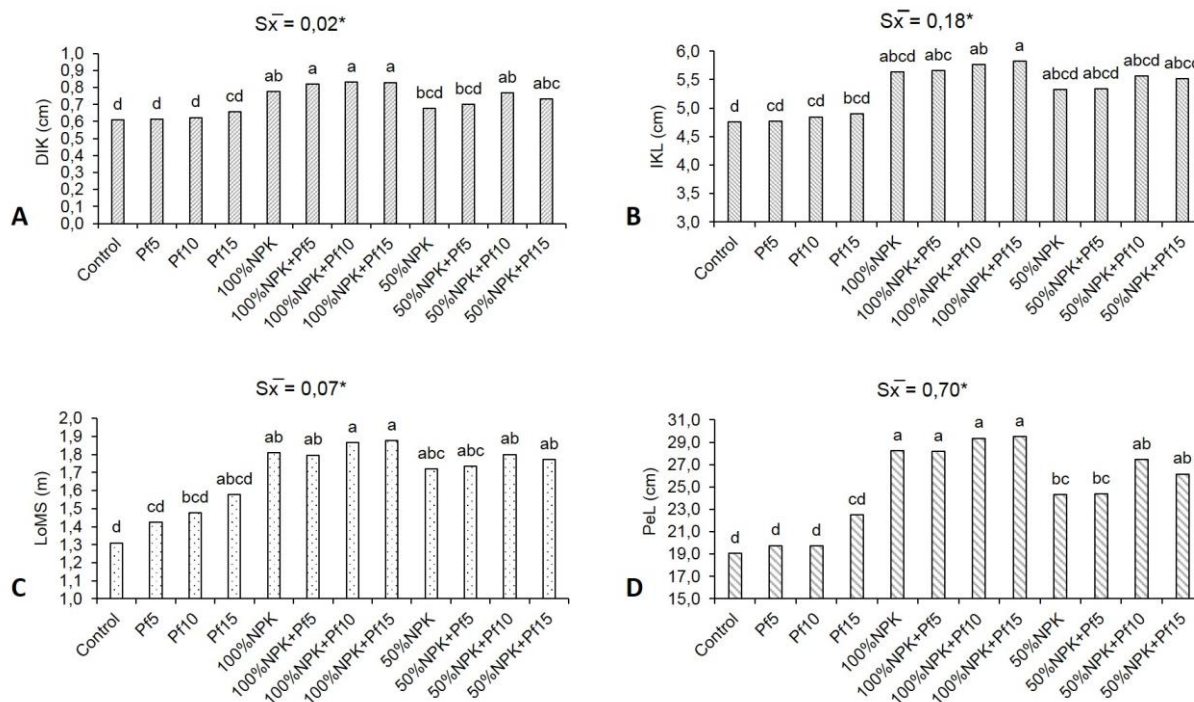
Legend: (A) DIK- Diameter of inter-knots (cm); (B) IKL - Inter-knots length (cm); (C) LoMS - length of the main stem (cm); (D) PeL - Petiole length (cm)

Figure 1. Effect of a *Pseudomonas*-based biofertilizer on some morphological variables of the sweet potato cultivar INIVIT B 240-2006.

The effect was especially evident in two variables: the diameter of inter-knots (DIK) and the petiole length (PeL) with significant differences from the control treatment. The thickest inter-knots were observed when the 100% of the mineral fertilizer (0.78 cm) was combined with the immersion in Pseudomonas during 15, 10 or 5 minutes (0.83, 0.83, 0.82 cm, respectively), or in those variants with the 50% of fertilizer and 10 or 15 minutes immersion (0.77, 0.73 cm), without statistical differences between all of them.

The longest petioles measured an average of 29.51 cm and it was observed in plants from the treatment with 100% of mineral fertilizer and immersion in the *Pseudomonas*-based fertilizer during 15 min, without statistical differences with the other three combinations 100% NPK-*Pseudomonas* (29.36, 28.16, 28.24 cm) neither with treatments that include 50% of mineral fertilizer and immersions in the biofertilizer for 10 (27.46 cm) or 15 min (26.16 cm). All they differ significantly from the control treatment (without applications) (only 19.06 cm).

On the other hand, a positive response was observed on the evaluated agronomic variables in all treatments where the

immersion of cuttings in the solution of *P. fluorescens* (20 t ha^{-1}) was carried out (Table 1); when the biofertilizer was combined with 100 % of NPK fertilization, the results were significantly higher over the rest of treatments.

Table 1. Effect of a *P. fluorescens*-based biofertilizer on the yield components on the sweet potato cultivar INIVIT B 240-2006 at La Dora farm (Abreus, Cienfuegos).

TREATMENTS	NTTR	NCTR	FWCTR (g)	NNCTR	FWNCTR (g)	CY (tha^{-1})
Absolute control	2.11	1.45 b	546.11 d	0.67	54.11	19.84 d
P. fluorescens 5 min	2.22	1.67 ab	552.78 d	0.55	39.11	20.08 d
P. fluorescens 10 min	2.33	1.67 ab	570.00 d	0.67	48.67	20.70 d
P. fluorescens 15 min	2.34	1.78 ab	653.33 d	0.56	47.00	23.73 d
100 % NPK (control)	3.33	2.89 a	1420.55 ab	0.55	33.33	51.60 ab
100 % NPK + Pf 5 min	3.44	2.89 a	1437.78 ab	0.44	38.56	52.23 ab
100 % NPK + Pf 10 min	3.44	2.89 a	1498.89 ab	0.44	40.11	54.45 ab
100 % NPK + Pf 15 min	3.45	3.00 a	1543.89 a	0.44	44.33	56.09 a
50 % NPK	2.55	2.11 ab	1093.89 c	0.45	27.33	39.74 c
50 % NPK + Pf 5 min	2.78	2.22 ab	1139.44 c	0.56	40.00	41.39 c
50 % NPK + Pf 10 min	2.78	2.22 ab	1181.11 c	0.56	35.00	42.91 c
50 % NPK + Pf 15 min	3.22	2.89 a	1365.00 b	0.78	36.11	49.58 b
$S_{\bar{x}} =$	ns	0.21*	22.41*	ns	ns	0.66*

* Means followed by the same letter in a same column are not significantly different according to Tukey's HSD test for P≤ 0.05.

Legend: NPK-mineral fertilizer (complete formula 9-13-17), Pf- *P. fluorescens*, NTTR-number of total tuberous roots per plant, NCTR-number of commercial tuberous roots per plant, FWCTR (g)-fresh weight of commercial tuberous roots per plant, NNCTR-number of non commercial tuberous roots per plant, FWNCTR- fresh weight of non commercial tuberous roots per plant (g), CY-commercial yield (t ha^{-1}).

No statistical differences were shown among the treatments for the number of total tuberous roots (NTTR), the number of non commercial tuberous roots (NNCTR) and its fresh weight (FWNCTR). However, significant differences appeared for other variables, especially for those related with the yield.

The worst treatment for all variables was the absolute control (without any fertilizer) with significant statistical differences with the rest of combinations. The control treatment with 100% of the mineral fertilizer (NPK) and its combinations with 5, 10 and 15 min immersion in the biofertilizer expressed statistical differences regarding to the rest of treatments. The mixture of 50% NPK and the immersion during 15 min in the biofertilizer don't differ statistically with the control with 100% NPK. That's a good finding because it shows the possibility to save mineral fertilizer to increase new areas when immersions for 15 min in the *P. fluorescens*-based biofertilizer could be made to improve sweet potato production significantly.

About the effect of the bioproduct on the yield, the combination with 100 % NPK and the immersion in *P. fluorescens* for 15 min showed the highest yield (56.09 tha^{-1}), followed by the other treatments with 100 % NPK and without statistical differences among them. It was interesting that the treatment with 50 % NPK and the immersion in *P. fluorescens* for 15 min (49.58 tha^{-1}) had no statistical differences with the control variant (100 % NPK, 51.60 tha^{-1}). In general, the effect of this biofertilizer and its ability to improve plant growth and productivity confirms an option to minimize the agricultural chemical footprint on sweet potato.

4. DISCUSSION

The positive sprouting percentage observed in all treatments, was a good result and it coincides with the one expected by this commercial cultivar and it was higher to the 90 %, technically demanded as a permissible value on the sweet potato crop production [18].

The vigor and the affirmative response observed in all treatments is firstly related with the good quality of cuttings (tips) that came from areas of categorized seed (original seed) of the cultivar at the Research Institute of Tropical Roots and Tuber Crops (INIVIT). In addition, cuttings with 30 cm are the best option to get a better development of this crop; a recent study made by [22] confirmed that 30 cm-long sweet potato vine cuttings produced the greatest growth and yield. Therefore, the planting material used had enough moisture and nutrients to achieve an optimum sprouting, after the agrotechnical work to the soil was carried out properly before, during and after sowing, especially the irrigation.

The qualitative characteristics observed in all treatments for the foliage and roots of this cultivar are representative of this variety [18]. This demonstrated that the biofertilizer improved the general development and yield of the crop but did not produce genetic variability or other significant changes.

A better development of morphological characteristics can result in a big plant with more leaves and better conditions to take the nutriments from the soil, and all that can improve physiological processes, especially the photosynthesis, resulting in an excellent productive response of the crop.

The genera *Pseudomonas* possesses the property to produce different substances whose main advantages are: to stimulate the germination of seeds, to accelerate the growth of the plants especially in their first stadiums, to induce the initiation radicular and to increase the formation of roots, according to [23]. Jorquera et al. [24] affirmed this bacteria increases the growth, yield and plants stress tolerance. They have too the quality to improve seeds germination [25] and the seedling vigor [26]. These statements agree with the sprouting vigor and the better development observed during this investigation for the evaluated morphological characters.

The observed results had points of coincidence with se same effect view by [27] when they studied the response of a triple superphosphate fertilizer and phosphate bio-fertilizer application (seed inoculated with phosphate bio-fertilizer containing *Pseudomonas fluorescens*). They concluded that pod number per plant and pod yield increased by 16 % and 15 %, respectively, when phosphate bio-fertilizer was applied.

Pseudomonas is denominated as a Plant Growth Promoting Rhizobacteria (PGPR) because it is able to colonize the rizosphera of plants (bacterias have to colonize the plant endosphere after colonizing the rhizosphere to confer some benefits to the plant [28]) and to present antagonistic activity toward diverse pathogens [29]. Recently, [12] recognized that beneficial *Pseudomonas* strains are frequently found associated with plants where they act as PGPB and the biofertilizer liberates growth promoting substances and vitamins that helps in maintaining the soil fertility; these authors confirmed that they acts as antagonists and suppress the incidence of soil borne plant pathogens. On the other hand, [30] emphasized that microorganisms, as a general concept, are one of the most important organisms that can develop beneficial associations with plants.

They are one of the most important organic sources, containing beneficial viable-organisms which have ability to mobilize nutritionally important elements from non-usable to usable form through biological processes [31]. In case of this experiment, evaluations showed the best development and yield in those treatments when the biofertilizer was applied by immersion during 15 minutes together with the 100 % of mineral fertilizer, without statistical differences with the treatment that combines 50 % of NPK fertilizer with the immersion in the *Pseudomonas*-based biofertilizer during 15 minutes. In absence of other factor, the biofertilizer was the cause of the observed yield increment. There is another reason, it is common for *Pseudomonas* populations to outshine other diazotrophic genera, due to their short latency period, rapid growth rate, and metabolic versatility [32].

These results coincided with previous findings published by [33]. They studied the effect of the commercial product named Gluticid® on the tomato crop (*Solanum licopersicum* Mill) and they found a good bioproduct effectiveness to obtain vigorous plants and acceptable yields when they used the seeds submersion during 30 minutes and one foliar aspersion 10 days after planting. The Gluticid® bioproduct was obtained in Cuba starting from active metabolites of *Pseudomona aeruginosa*, and its effect has been evaluated in the growth and development of different agricultural crops.

On the other hand, there are many examples where the combination of inorganic fertilizer with biofertilizers produce better productive results than when they were applied individually; [34] reported an improved root quality and productivity in sweet potato when combining arbuscular mycorrhizal fungi (*G. mosseae*) inoculum with the recommended P level (100 % P_2O_5) (superphosphate fertilizer).

Usually, chemical fertilizers make the difference necessary to guarantee the increase of yields [35], but, the excessive mineral fertilization to one side have adverse financial effects and also represents an environmental burden [36]. The vision to reduce the dependency on synthetic fertilizers requires effective biological-based alternatives. In this sense, *P. fluorescens* is a new input for sweet potato crop that can reduce the application rates of chemical fertilizers, offering an alternative to traditional agricultural practices like in other crops [37; 38]. This kind of scientific results are important because they help to develop the sweet potato, one orphan crop but vital in the developing world [39].

Due to the worldwide current multifactorial crisis (ecological, economic and social), the increase of the world population and the necessity of foods for millions of people, where climate change represents a threat to agriculture and food security, agroecological methods offer comprehensive solutions for food systems. Because of that, bioproduct use has gradually increased in the agriculture of countries promoting change towards an insecticide model more in harmony with the environment.

Farmers have to fight with all sorts of problems, including the high prices of fertilizers, the soil degradation, the reduction of crops productivity, the increment of plagues and droughts, among others. In this context, the application of environment-friendly farming practices is not an alternative, is the urgent response to guarantee the food sovereignty.

Definitely, one of the alternatives to the derived problem of the fertilization is the application of PGPR [40], and many researchers have been demonstrated their efficiency or advantages in front of the use of chemical fertilizers and they favor the environment preservation and the development of a sustainable agriculture in many crops [41; 42 and 43], including some studies in sweet potato [8; 44].

In consequence, although it would be necessary to know the productive response during the rainy period (in execution), the results of this research are the first scientific report about the use of this new *P. fluorescens*-based biofertilizer to improve the sweet potato yield significantly. This is a very important alternative for more sustainable agricultural practices without affecting the growth and productivity of this important crop.

5. CONCLUSION

The study concludes that the *P. fluorescens*-based biofertilizer stimulated the commercial yield of sweet potato (*Ipomoea batatas* (L.) Lam) cv. INIVIT B 240-2006; the combination of 50 % NPK fertilization and the immersion of vine cuttings in *P. fluorescens* during 15 min before planting, can equal the productive results reached with 100% of the mineral fertilizer. This research demonstrated that the use of biofertilizers based on Plant Growth Promoting Rhizobacteria (PGPR), as *P. fluorescens*, is a viable alternative to decrease the load of mineral fertilizer applied in agriculture.

6. ACKNOWLEDGEMENT

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