Scientific Paper

Agroproductive effect of silkworm rearing waste as biofertilizer in two forage species

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Abstract

The objective of the study was to evaluate the agroproductive effect of silkworm rearing waste as biofertilizer, combined with earthworm humus and solid ferment of native microorganisms (NM) on *Brachiaria* hybrid cv. Yacare and *Mucuna pruriens*. Two trials were conducted: in the first one low doses of biofertilizer in *Brachiaria* hybrid cv. Yacare were studied; and, from the results, in the second experiment the biofertilizer doses were increased, silkworm rearing waste alone was included and *M. pruriens* was used. The experimental design was randomized blocks with 15 replicas. The height of cv. Yacare at 30 days tended to be higher in the treatments with biofertilizer. At 60 days all the treatments differed from the control, and silkworm rearing was-te + NM surpassed silkworm rearing waste + humus. The production of seeds and dry biomass in silkworm rearing waste + NM (1,25 and 11,33 g, respectively) was higher than in the other combinations and the control (0,62 and 5,65 g). In *M. pruriens* the emission of branches stood out when applying silkworm rearing waste alone and silkworm rearing waste + humus. The length of the main branch in the control (41,19 cm) differed at 45 days with regards to that of all the fertilized plots (between 82,37 and 92,39 cm). In silkworm rearing waste + NM, with or without humus, the best characteristics of the root system were found at 75 days. It is concluded that the use of biofertilizers obtained from silkworm rearing waste and their combination with NM and humus constitute efficacious practices to improve the agroproductive indicators of *B.* hybrid cv. Yacare and *M. pruriens*.

Keywords: *Brachiaria* hybrid cv. Yacare, silkworm, humus, *Mucuna pruriens*

Introduction

The management of organic wastes, as resource for soil amelioration and the increase of productivity through recycling and regular applications, constitutes a priority to guarantee the food security of the population and environmental resilience in fields and cities.

The actions carried out in Cuba in that sense are insufficient, when there are organic and biological sources, technologies and generated products, legal support for that objective and documented information about the use of biofertilizers and fertilizers from peat, sugarcane filter cake, manures, compost and vermicompost (García, 2014).

It is known that, at local scale, organic fertilizers can substitute mineral fertilizers, because due to the law of return part of the nutrient exports cannot be returned to their original status in the soil. In addition, the nutritional efficiency of chemical fertilizers is often higher than that of organic fertilizers, and this means a gap in yield between conventional and organic systems (De Ponti *et al*., 2012).

For such reason, to maintain long-term and large-scale sustainable organic fertilization, the carbon and nutrient cycle, should be included, in an optimum way, along the whole productive chain, utilizing and combining all the harvest residues and animal wastes; and the efficiency of the transformation of the organic matter into biofertilizer can also be improved.

Brachiaria hybrid cv. Yacare and *Mucuna pruriens* could be protagonists in the tests that are conducted on the effect of recycling on agricultural production; because they are promising crops in the production of animal feed, green manure and cover crop. The former is a grass characterized by its high degree of persistence in pasturelands, even higher than that of *Brachiaria brizantha* cv. Marandú. In an experimental test, the new hybrid, after four years of being planted, showed a cover of 83 % of the area; while Marandú barely covered 53 %. Yacare can reach a forage production equivalent to 15 t DM ha^{-1}, comparable to that of Marandú (13 t DM ha^{-1}); responds linearly to nitrogen fertilization; shows high nutritional value and good *in vitro* organic matter digestibility; tolerates bad drainage or waterlogging for long periods; and is capable of reaching a mean production of 1 066 adventitious roots per plant, comparable to *Brachiaria humidicola*.

On the other hand, the species *M. pruriens* is widely used as green manure and plant cover in different countries of the tropical region, allows to preserve the soil moisture in zones where the water resource is scarce, reduces soil losses due to erosion, and improves aggregate stability (Sanclemente -Reyes and Patiño-Torres, 2015).

The objective of the study was to evaluate the agroproductive effect of silkworm rearing waste as biofertilizer, combined with earthworm humus and with solid ferment of native microorganisms (NM), on *B.* hybrid cv. Yacare and *M. pruriens*.

Materials and Methods

Two experiments were conducted under semi-controlled conditions, in the Pastures and Forages Research Station Indio Hatuey, located in the Perico municipality –Matanzas province, Cuba–, at 22º 48' 7'' North latitude and 81º 2' West longitude, at 19 m.a.s.l.; on a Ferralitic Red soil characteristic of the zone (Hernández-Jiménez *et al.*, 2015).

In the first trial the work was done with *B.* hybrid cv. Yacare and relatively low doses of biofertilizers. From the obtained results, in the second experiment the biofertilizer doses were increased and the evaluation with silkworm rearing waste alone was included. The species *M. pruriens* was used with the purpose of comprising two families of forage plants (grass and legume).

Design and treatments. In both experiments a randomized block experimental design was used with 15 replicas. In Yacare, the evaluated treatments consisted in:

- Control without fertilization, soil (100 % of the substrate).
- Soil (99,5 %) + silkworm rearing waste (0,25 %) + earthworm humus (0,25 %)
- Soil (99,5 %) + silkworm rearing waste (0,25 %) + solid ferment of native microorganisms, NM (0,25 %)
- Soil (99,25 %) + silkworm rearing waste (0,25 %) + earthworm humus (0,25 %) + NM (0,25)

The evaluated indicators were:

- Plant height (cm) at 30 and 60 days
- Number of emitted stems (u) at 60 days
- Number of shoots per tiller (u) at 60 days
- Number of rachises (u) at 60 days
- Distance of the rachis (cm) at 60 days
- Seed production per plant (g) at 120 days
- Production of dry biomass per plant (g) at 120 days
- DM concentration (%) at 120 days

In *M. pruriens*, the treatments consisted in:

- Control without fertilization, soil (100 % of the substrate)
- Soil (95,0 %) + silkworm rearing waste (5,0 %)
- Soil (95,0 %) + silkworm rearing waste (3,0 %) + earthworm humus (2,0 %)
- Soil (95,0 %) + silkworm rearing waste (4,0 %) + NM (1,0 %)
- Soil (95,0 %) + silkworm rearing waste (1,5 %) + earthworm humus (3,0) + NM (0,5 %)

The following indicators were evaluated:

- Seed emergence (u) at 15 and 30 days
- Emission of leaves and branches (u) at 15, 30 and 45 days
- Length of the main branch and lateral branches (cm) at 15, 30 and 45 days
- Plant survival (u) at 75 days
- Number of roots (u) at 75 days
- Dry weight of the roots per plant (g) at 75 days
- Root length (cm) at 75 days

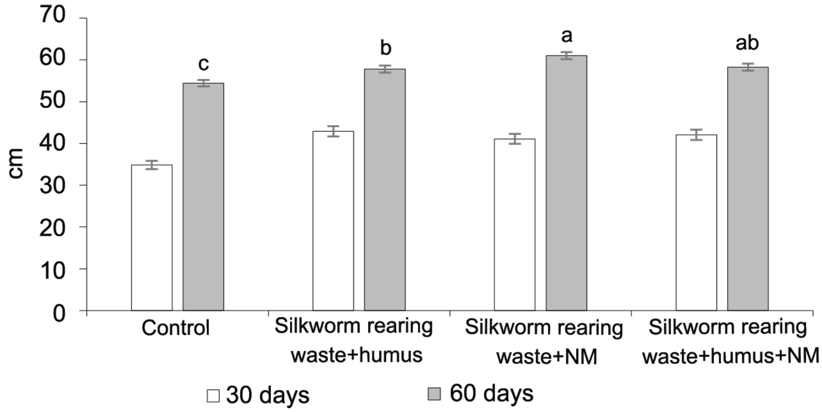
The silkworm rearing waste as well as the solid NM ferment was maintained 60 days in maturation process, under environmental conditions. Afterwards the mixtures were prepared according to the treatment, and the biofertilizers were placed on the soil surface. The cultivation was carried out in perforated plastic buckets, of 30 cm diameter x 40 cm depth. The sowing was done with sexual seed, and irrigation took place daily.

Statistical analysis. Descriptive analysis was made through the arithmetic mean to the indicators emergence and number of live plants, and in the other variables ANOVA was used; the means were compared through Duncan's test (1955) at $p \leq 0,05$. For such purpose the statistical program Infostat 2008 (Di Rienzo *et al.*, 2008) was used.

Results and Discussion

The growth in height of *B.* hybrid cv. Yacare at 30 days tended to be higher in the treatments with biofertilizer. At 60 days all the treatments differed from the control, and silkworm rearing waste + solid NM ferment differed from silkworm rearing waste + humus (fig. 1).

The seed and dry biomass production in the treatment with silkworm rearing waste + NM was statistically higher than that of the other combinations and the control without fertilization (table 1). This suggests that in this formulation there were significant variation factors derived from high contributions of N, P, Ca and K; in addition, it is inferred that there was high compatibility among the



Different letters indicate significant differences at $p \leq 0,05$ (Duncan, 1955)

Figure 1. Plant height of *B. hybrid* cv. Yacare

Table 1. Agroproductive effect of the biofertilizers on the grass *B.* hybrid cv. Yacare.

Treatment	No. stems emitted 60 days (u)	No. shoots per tiller 60 days (u)	No. spikes 60 days (u)	No. rachises (u)	Distance of the rachis (cm)	Seed production (g)	DM 120 days (%)	Dry biomass per plant, 120 days (g)
Control	11[b]	5[b]	5	9	6,07	0,62[b]	21,27[b]	5,65c
Silkworm rearing waste + humus	15[a]	7[a]	6	11	5,84	0,84[b]	25,91[a]	10,11[ab]
Silkworm rearing waste + NM	17[a]	7[a]	6	12	6,40	1,25[a]	26,78[a]	11,33[a]
Silkworm rearing waste + humus + NM	14[ab]	8[a]	7	8	6,29	0,58[b]	25,02[a]	9,20[b]
Sign.	0,002	0,002	0,62	0,12	0,08	0,05	0,002	<0,0001
SE ±	0,61	0,28	0,27	0,71	0,08	0,04	0,57	0,41

Different letters indicate significant differences at $p \leq 0,05$ (Duncan, 1955).

microorganisms present in the silkworm rearing waste and in the NM ferment, which participate in the decomposition and mineralization of the organic matter and in the processes related to the absorption of nutrients by plants.

In that sense, it is known that the excreta of herbivore species has, as average, 50 % of N, 80 % of P and 95 % of K contained in the feedstuffs consumed by the animals (Nicolás Medina, personal communication), besides a high protein percentage (15,3 %) contributed by the mulberry foliage (*Morus alba* cv. Yu 62) in the daily diet of the silkworm, part of which is not digested and forms the rearing bed (Prieto, 2015).

The efficient NM present in the solid ferment which was combined with the silkworm rearing waste constitutes a mixture of photosynthetic or phototrophic bacteria (*Rhodopseudomonas* sp.), lactic acid bacteria (*Lactobacillus* sp.) and yeasts (*Saccharomyces* sp.), in concentrations higher than 10^5 colony forming units per milliliter. The phototrophic bacteria synthesize useful substances from the secretions of organic matter and harmful gases, using sunlight and heat as energy sources; the synthesized substances comprise aminoacids, nucleic acids, bioactive elements and sugars; and lactobacilli favor the presence of metabolites in the substrate (Suárez and Martín, 2012).

Among the treatments with higher influence the combination silkworm rearing waste + earthworm humus also stood out, particularly on the number of emitted stems at 60 days and on the production of dry biomass at 120 days; as well as rearing waste + earthworm humus + NM on the number of shoots emitted per tiller at 60 days and on the dry matter concentration in the biomass 120 days after planting (table 1).

These results corroborate the multiple benefits that are ascribed to earthworm humus, related to its influence on the formation of stable aggregates in the soil and on the increase of the content of assimilable nutrients (N, P, K, Ca, Mg) and of biologically active substances through secondary metabolism, such as free aminoacids, vitamins (provitamin D, vitamin B), phytohormones, growth regulators, gibberellins, cytokinins and auxins (Martínez *et al*., 2013). In this sense, López-García *et al*. (2017) obtained in *B. brizantha* higher values of green biomass production, dry matter yield (3 383 kg ha^{-1}), length of primary roots and leaf/stem ratio when applying earthworm humus; while in the presence of bokashi they found the highest values of DM digestibility (62 %), crude protein (9,8 %) and ash (9,5 %).

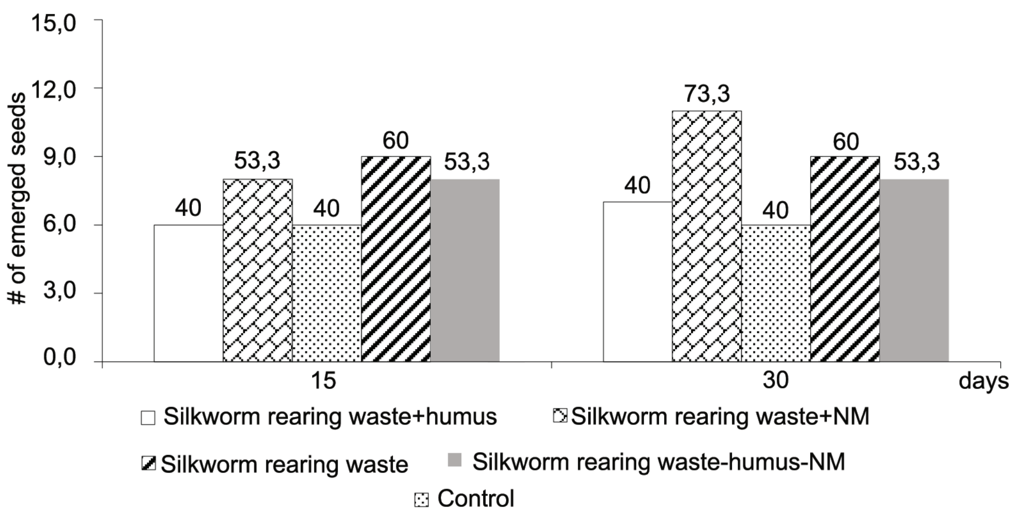
Laulate-Lavinto (2017) stated that, with a higher dose of enriched liquid humus applied to the grass *B. brizantha*, the quantity of nutrients and microorganisms contributed to the soil will be higher.

It must be emphasized that there are many reports in scientific literature about the effect of earthworm humus, compost and NM in different crops and application forms. In this sense, Fleitas *et al*. (2013), when comparing the production of radish in nursery stage, obtained that the agronomic yield was significantly higher with the humus leachate, followed by manure dissolved in water. Luna-Murillo *et al*. (2016) reported that the application of earthworm humus plus humic acid, followed by bokashi plus humic acid, increased the agricultural production; and suggested that this could have been related to the effect of the present phytohormones, mainly auxins and humic substances of low molar mass, to which properties are ascribed of stimulation in synthesis of metabolites, such as aminoacids and proteins. Another possibility lies on the fact that the organic fertilizers could be favoring the absorption of N-NO_3 and the activity of H+ATPase, essential for growth. The results obtained by Moya *et al*. (2017), who observed a positive effect of efficient microorganisms on the growth and yield of rice (*Oryza sativa* L.), can also be used for comparison.

The results in *M. pruriens* during the first 30 days showed higher emergence of the seeds (fig. 2) in the presence of silkworm rearing waste + NM; which is an indicator of better qualities of the enriched substrate regarding its physical structure, microbiological activity and sufficiency of nutrients and phytohormones.

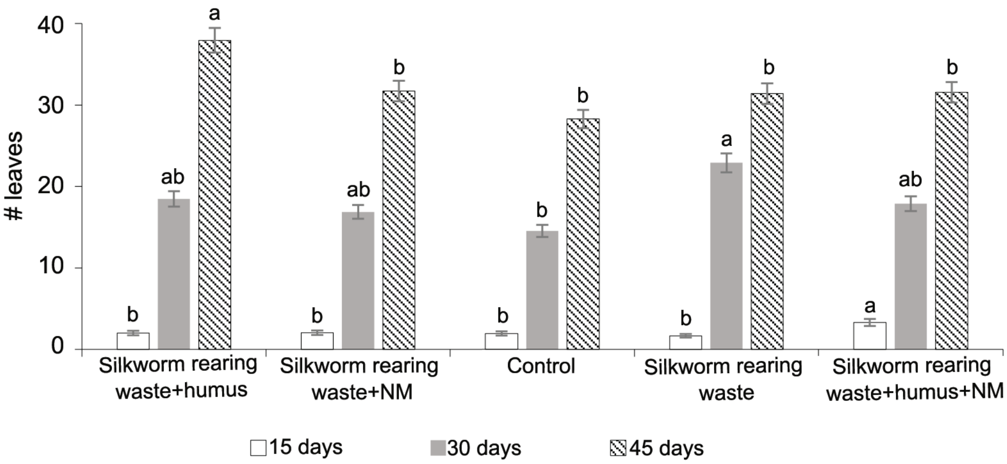
Leaf emission was more influenced during the first 30 days by silkworm rearing waste alone and silkworm rearing waste + humus + NM, and at 45 days by silkworm rearing waste + humus (fig. 3). The emission of branches stood out in general in the treatments with silkworm rearing waste alone and silkworm rearing waste + humus (fig. 4).

Regarding the main branch length (fig. 5), the superiority of the fertilized plants was significant compared with the control, which differed at 15 days from the treatments with silkworm rearing



The values on the bars indicate the percentage value represented by the number of emerged seeds.

Figure 2. Seed emergence of *M. pruriens* during the initial growth stage.



Different letters indicate significant differences at $p \leq 0{,}05$ (Duncan, 1955).

Figure 3. Emission of leaves during the initial growth stage.

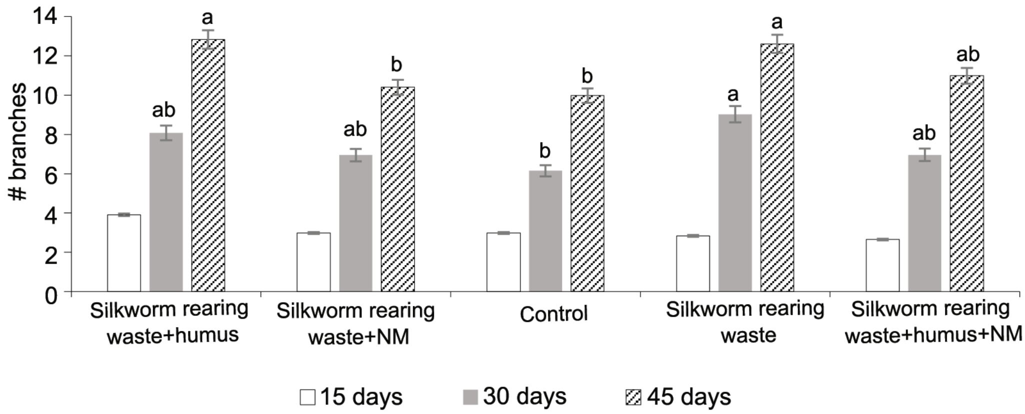


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Figure 4. Emission of branches during the initial growth stage.

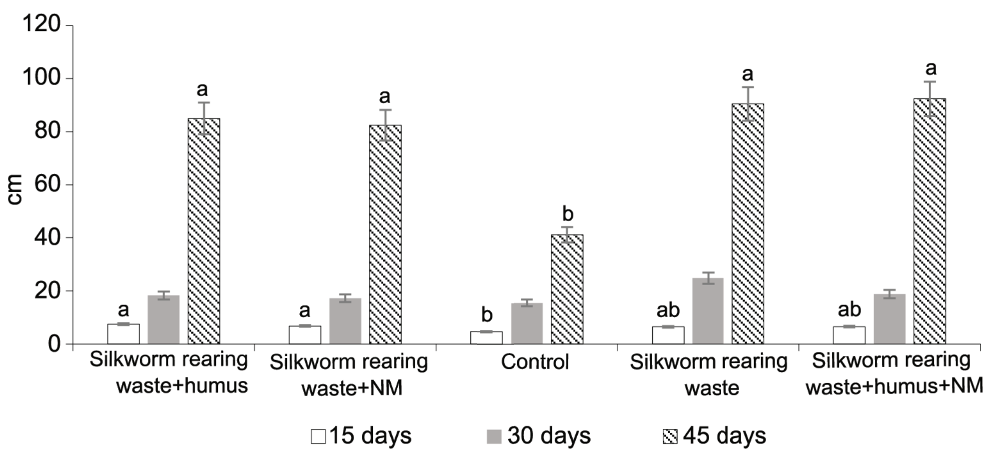

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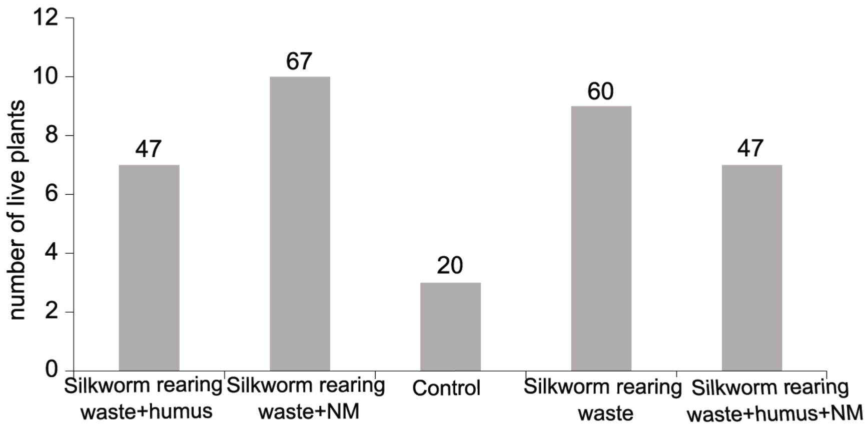
Figure 5. Length of the main branch during the initial growth stage.

waste + humus and silkworm rearing waste + NM, and at 45 days, from all the fertilized plots. The length of the branches corroborated the advantages of silkworm rearing waste + humus, and this treatment in turn did not differ from silkworm rearing waste alone and from the combination silkworm rearing waste + humus + NM. The plants fertilized with silkworm rearing waste + NM and silkworm rearing waste alone showed higher survival at 75 days (fig. 6).

The results corroborated the advantages of the use of silkworm rearing waste as fertilizer, combined or not with NM ferments and earthworm humus. This is explained, to some extent, by the high contents of P, Ca and K present in the excreta of silkworm (Patiño-Ospina, 2008); and, additionally, the high nutrient levels concentrated in the mulberry foliage, which, according to Prieto (2015), reach 24,1 % of DM; 20,1 % of protein; 15,33 % of CF; 0,58 % of P; 4,18 % of K and 2,17 % of Ca, allowing to generate high-quality biofertilizer.

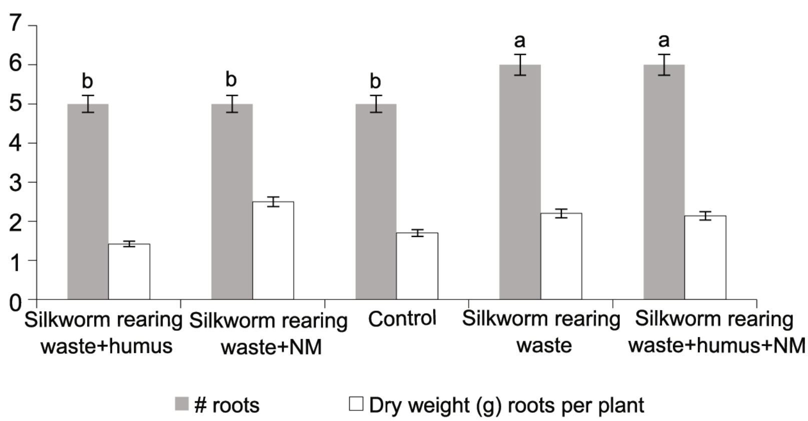
Martínez *et al*. (2013) stated, about the availability of nutrients in the organic fertilizers of animal origin, that nitrogen is best held and higher utilization coefficients by the plants are reached than with mineral fertilizers. According to González-Cañizares (2014), potassium is found in large amount and it is easily available; while phosphorus, mainly present in the solid excreta and the rearing beds, reaches higher coefficient of utilization by plants (35 % higher than the report for mineral fertilizers).

The treatments with biofertilizers from silkworm rearing waste + NM, with or without earthworm humus, showed the best characteristics of the root system 75 days after sowing (figs. 7 and 8). In this regard, Gutiérrez *et al*. (2012) reported that effi-



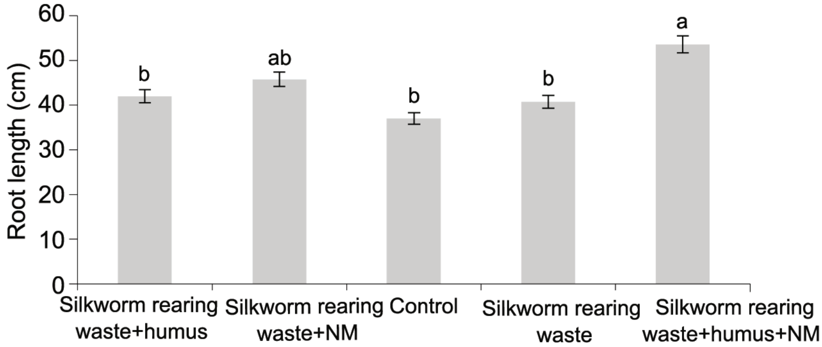
The values on the bars indicate the percentage value represented by the number of live plants.

Figure 6. Plant survival 75 days after sowing



Different letters indicate significant differences at $p \leq 0,05$ (Duncan, 1955)

Figure 7. Root number and weight 75 days after sowing.



Different letters indicate significant differences at $p \leq 0,05$ (Duncan, 1955).

Figure 8. Root length 75 days after planting

cient microorganisms optimize the effects of organic fertilizer, and this favors directly plant growth.

It is concluded that the use of biofertilizers obtained from silkworm rearing waste and its combination with solid ferment of native microorganisms and earthworm humus constitute efficacious practices to improve the agroproductive indicators of *B.* hybrid cv. Yacare and *M. pruriens*.

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