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Integrated management of the fertilization for *Tithonia diversifolia* forage production

Manejo integrado de la fertilización para la producción de forraje de *Tithonia diversifolia*

P. J. González-Cañizares[1]*, S. Méndez-Bonet[2], R. Reyes-Rouseaux[3], R. Rivera-Espinosa[1], A. Hernández-Jiménez[1]

[1]*Instituto Nacional de Ciencias Agrícolas, Gaveta Postal No. 1. San José de las Lajas, Mayabeque. Cuba.*
[2]*Universidad Agraria de la Habana Fructuoso Rodríguez Pérez, Carrera Tapaste y Autopista Nacional km 23 ½. San José de las Lajas, Mayabeque. Cuba.*
[3]*Instituto de Investigaciones de Pastos y Forrajes, Avenida Independencia km 8 ½. Boyeros, La Habana. Cuba.*

* *Email: pgonzalez@inca.edu.cu*

The effect of the integrated management of the fertilization with biofertilizer, organic manure and nitrogen fertilizer on *Tithonia diversifolia* forage production was evaluated. A total of nine treatments (application of 0, 70 and 100 kg N ha^{-1}; 0, 70 and 100 kg N ha^{-1} plus the coinoculation with *Azospirillum brasilense* and *Rhizoglomus irregulare*, and 0, 70 and 100 kg N ha^{-1} combine with the coinoculation with both biofertilizer plus the application of 25 t ha^{-1} cattle manure) were studied in a random block design with four repetitions. The inoculum with *R. irregulare* was applied using the *Canavalia ensiformis* as precedent crop and way for the reproduction of mycorrhizal propagules in the soil for Tithonia inoculation, and the inoculum with *A. brasilense*, at 15 d after Tithonia planting, at a rate of 20 L ha^{-1}.The manure increased ($p<0.01$) the contents of OM, assimilable P and k interchangeable of the soil. Their application, combine with the coinoculation with both biofertilizer, caused the higher levels of mycorrhizal colonization, concentrations and extractions of N, P and K in the aerial biomass and yields higher ($p<0.01$) to those reached with the nitrogen fertilization, alone or with the biofertilizer (24 t DM ha^{-1}). It is concluded that the integrated management of the fertilization by the application of cattle manure and the coinoculation with *A. brasilense* and *R. irregulare* is effective to increase the yield of Tithonia forage, reduce the use of nitrogen fertilizer and improve the soil fertility.

Key words: *arbusculars mycorrhizas, forage yield, nutritional state, rhizobacteria*

Se evaluó el efecto del manejo integrado de la fertilización con biofertilizantes, abono orgánico y fertilizante nitrogenado en la producción de forraje de *Tithonia diversifolia.* Se estudiaron nueve tratamientos (aplicaciones de 0, 70 y 100 kg N ha^{-1}; 0, 70 y 100 kg N ha^{-1} más la coinoculación con *Azospirillum brasilense* y *Rhizoglomus irregulare*, y 0, 70 y 100 kg N ha^{-1} combinadas con la coinoculación con ambos biofertilizantes más la aplicación de 25 t ha^{-1} de estiércol vacuno) en un diseño de bloques al azar con cuatro réplicas. El inóculo con *R. irregulare* se aplicó mediante el uso de *Canavalia ensiformis* como cultivo precedente y vía para la reproducción de propágulos micorrízicos en el suelo para la inoculación de Tithonia, y el inóculo con *A. brasilense,* a los 15 d después de la plantación de Tithonia, a razón de 20 L ha^{-1}. El estiércol aumentó ($p<0.01$) los contenidos de MO, P asimilable y K intercambiable del suelo. Su aplicación, combinada con la coinoculación con ambos biofertilizantes, produjo los mayores valores de colonización micorrízica, concentraciones y extracciones de N, P y K en la biomasa aérea y rendimientos superiores ($p<0.01$) a los alcanzados con la fertilización nitrogenada, sola o acompañada de los biofertilizantes (24 t MS ha^{-1}). Se concluye que un manejo integrado de la fertilización mediante la aplicación de estiércol vacuno y la coinoculación con *A. brasilense* y *R. irregulare* resulta efectivo para incrementar el rendimiento de forraje de Tithonia, reducir el uso de fertilizante nitrogenado y mejorar la fertilidad del suelo.

Palabras clave: *estado nutricional, micorrizas arbusculares, rendimiento de forraje, rizobacterias*

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Introduction

The interest of using the *Tithonia diversifolia* as forage resource has been increase in the last years, which respond to their high biomass production, fast recuperation after cut and high nutritional value that exceeds to those from other tropical forages species (Ramírez 2018 and Villegas *et al.* 2020). However, due to their capacity to extract important amount of nutrients, when it is submitted to frequent cuts for forage production, *Tithonia diversifolia* needs an adequate fertilization to maintain their productivity and keep the soil fertility (Botero Londoño *et al.* 2019).

The high prices of mineral fertilizers and the need to promote a friendly livestock with the environment suggest the search of fertilization strategies that guarantee the adequate nutrition of forages crops, decrease the use of external inputs and, in turn, guarantee the protection of natural resources. The cattle manure is the organic waste most abundant in the livestock agroecosystems, and their use for the fertilization of these crops constitutes an economic and ecologically viable alternative because it contributes to the recycle of the extracted nutrients with the intake biomass by the cattle, as well as to the reduction of mineral fertilizer demand and to the improvement of the soil fertility (Jiang *et al.* 2021).

Recently, it has been also paying attention to the inclusion of biofertilizer in the fertilization systems of forage plants due to its direct effect on the improvement of the biological properties of soils, on the productivity increase and on the biomass nutritive value, as well as on the reduction of the use of synthetic fertilizer (Guimarães *et al.* 2022). Among the microorganisms used as biofertilizer is the associative bacterium *Azospirillum brasilense*, able to fix atmospheric nitrogen and improve the productivity and quality of harvests, in addition to provide other benefits such as the phytohormones production, phosphates solubilization and the plants protection against abiotics stresses (Leite *et al.* 2019).

Likewise, the biofertilization with arbuscular mycorrhizal forming fungus (AMF), whose structures increase the soil volume that the roots explore and makes easy the nutrients and water absorption, among other benefits, has showed their effectiveness in different agricultural crops (Chandrasekaran 2020). In fact, in the Tithonia roots it has been proved the presence of a big group of AMF species and their contribution to the absorption of non available ways of phosphorous in the soil (Scrase *et al.* 2019).

Although it's known the benefits of the organic fertilization (Reis *et al.* 2018) and the favorable effect of the double inoculation with *A. brasilense* and AMF on the increase of Tithonia productivity and nutritive value (Méndez *et al.* 2022), the studies aimed to design fertilization strategies based on the integrated management of organic manure, mineral fertilizer and both microorganisms are limited, despite their possible advantages to improve yields, reduce the external inputs and to keep the soil fertility.

When considering these premises, this study was performed to evaluated the effects of the integrated management of fertilization, based on biofertilizer, cattle manure and nitrogen fertilizer, on soil fertility indicators and *T. diversifolia* forage production.

Materials and Methods

Experimental conditions. The experiment was performed in the typical dairy 23 from Unidad Básica de Producción Cooperativa (UBPC) "Juan Oramas", located in Guanabacoa municipality.

La Habana province, Cuba, at 23°08' north latitude and 23°08' west longitude, on a calcic cambisol soil, according to the Base Referencial Mundial del Recurso Suelo (IUSS 2015). Their main chemical characteristics are showed in table 1.

The soil of the experimental area has high contents of assimilable P, pH alkaline, average content of OM, high basis exchange capacity (BEC) and higher contents of interchangeable K (Paneque and Calaña 2001).

During the experimental period (May 2019- June 2020) the total rainfall was 1705 mm, 83% occur during the months corresponding to the rainy season (May- October, 2019 and May - June, 2020), as is show in figure 1.

Treatments and experimental design. A total of nine treatments were evaluated, constituted by the applications of 0. 70 and 100 kg N ha^{-1}; 0, 70 and 100 kg N ha^{-1} plus the coinoculation with *Azospirillum brasilense* and *Rhizoglomus irregulare*, and 0, 70 and 100 kg N ha^{-1} plus the coinoculation with *A. brasilense* and *R. irregular* and the application of 25 t ha^{-1} of cattle manure in a random block design with four replications. The plots constitute the experimental unit and had total surface of 24 m^2 and calculation area of 16 m^2.

Table 1. Chemical characteristics of the soil of experiments (deep: 0-20 cm)

pH H_2O	OM, %	P,	Ca^{2+}	Mg^{2+}	Na^+,	K^+	CIB
		mg kg^{-1}	cmol$_c$ kg^{-1}				
7.7	4.50	70	49.5	4.8	0.25	0.57	54.9
(7.5-7.8)	(4.25-4.76)	(65-76)	(47.3-51.7)	(4.1-5.2)	(0.22-0.28)	(0.51-0.63)	(53.7-56.4)

OM: organic matter, CIB: basis exchange capacity, Values in parenthesis show confidence intervals of means (α=0.05)

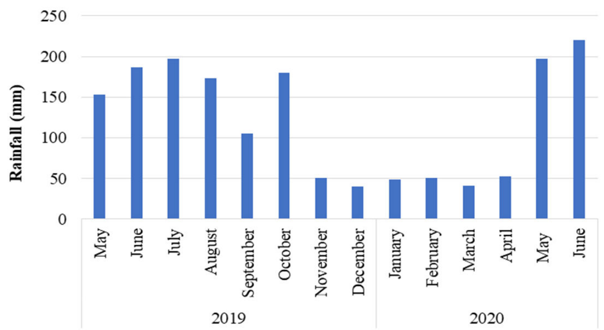

Figure 1. Distribution of the rainfalls during the execution period of the experiment (data taking in the experimental area)

Inoculants used. For the biofertilization with AMF, inoculant that has the INCAM-11 strain from the *R. irregulare* species was used (Sieverding *et al.* 2014). The inoculants came from the Instituto Nacional de Ciencias Agrícolas (INCA) and it was previously multiply in a clayey substrate sterilized in autoclave at 120 °C for an hour during three days, with the use of *Urochloa decumbens* cv. Basilisk as host plant. It has 50 spores per gram of solid inoculant, as well as many fragments of rootlet of the host plant. For the biofertilization with *A. brasilense,* the commercial product Nitrofix®, from Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA) was used, which has the strain 8I, with concentrations of 10^9 UFC mL^{-1}.

Soil preparation, sowing, inoculants application and fertilization. The soil was prepared by labors of plowing (plow), harrow, cross (plow) and harrow, at approximate intervals of 25 d between each one. The cattle manure was applied on the plots surface and it was incorporated to the soil with the second plowing work. This came from the stabling areas of the dairy and has a time of three deposition months in the dunghill. Their chemical characteristics and humidity content at the moment of their application are showed in table 2.

In May, 2019, all the treatments were sowing with canavalia (*Canavalia ensiformis*) as way to reproduce in the soil in those that led the inoculation with *R. irregulare*, sufficient amount of mycorrhizal propagules for the subsequent inoculation of Tithonia. The method of mycorrhizal inoculants application was fallowed for this crop, used by Méndez *et al.* (2022).

The canavalia was sowing at distances of 50 cm between rows and 25 cm between plants, with density of 100 kg ha^{-1} of seeds. In the treatments to inoculate with *R. irregulare*, the seeds were cover with an amount of mycorrhizal inoculants equivalent to 10 % of their mass. For the cover, the seeds were submerging in a fluid paste, prepared through the mixture of the solid mycorrhizal inoculants and water, in 1:0.6 m/v proportion. Later, they were dried in shade and were sowing.

The canavalia was cut at 60 d after sowing and their aerial biomass was removed of the field for the animals feeding. Table 3 show the number of spores of AMF that were in the soil after the canavalia cut and at the moment of Tithonia planting.

After the canavalia cut, it was plow through again and the tithonia was planted using vegetative propagules of approximately 30 cm in length. They were taking from the superior and medium part of the stems of a cultivated field

Table 2. Chemical characteristics (dry base) and humidity content of the cattle manure as organic fertilizer in the experiment

OM, %	N, %	C/N ratio	P, %	K, %	Ca, %	Mg, %	pH	Humidity, %
61.7	2.25	16.6	0.65	1.83	2.92	0.71	7.2	52.7
(61.2-62.2)	(2.22-2.28)	(14.7-18.5)	(0.63-0.67)	(1.80-1.86)	(2.87-2.97)	0.69-0.73)	(7.0-7.4)	(52.1-53.3)

Average of ten samples taking at the moment of manure application. Values in parenthesis show confidence intervals of means (α=0.05)

Table 3. Number of spores of AMF that were in the soil in each treatment after the canavalia cut

No	N, kg ha^{-1}	Cattle manure, t ha^{-1}	Biofertilization	Number of spores/50g
1	0	0	NB	133 (118-148)
2	70	0	NB	145 (128-162)
3	100	0	NB	138 (125-151)
4	0	0	B	520 (501-539)
5	70	0	B	492 (459-525)
6	100	0	B	518 (483-553)
7	0	25	B	526 (494-558)
8	0	25	B	499 (472-523)
9	70	25	B	519 (486-552)

NB: not biofertilized, B: biofertilized with *Azospirillum brasilense* and *Rhizoglumus irregulare.*

Values in parenthesis show confidence intervals (α=0.05) with this species, very close to the experimental area. The propagules were planting at 1.0 m between furrows and 0.5 m between plants, for a density of 20000 propagules ha^{-1}.

The inoculation with *A. brasilense* was performed at 15d after the tithonia sprouting. For this a mixture of liquid inoculum and water (1:10 v/v) was prepared and applied to the soil very close to the furrows with a manual knapsack sprayer at a rate of 20 L ha^{-1} of commercial product.

The nitrogen fertilizer was used according to the doses described in the treatments, fractioned in two moments: 50 % at 30 d after planting and the rest, after the third cut, both coincide with rainy seasons. For the application, there were made small furrows with a hoe of 10 cm deep, at 10 cm of the plants rows and were covered after fertilizer application. As carrier of the nitrogen fertilizer the urea was used. There were not applied potassium and phosphoric fertilizer because it was considered that the high contents of both nutrients in the soil (table 1) were sufficient for the crop. The experiment was conducted under dryland farming.

Samplings and evaluations. A total of four cuts were made. The first one at 120 d after plantation (November, 2019), and the rest in February, May and July, 2020 at a height of 30 cm of soil surface. In each cut the fresh mass of the aerial part corresponding to the calculation area of plots was weighed. From each treatment a total of three subsamples of the fresh mass of the aerial part were taking and they were homogenized to form a sample of 200 g. The subsamples were taking to an air circulation oven at 70 ºC during 72 h to determine the dry matter (DM) percentage and to estimate the DM yield. In the second and third cut, the concentrations (g kg^{-1} of DM) of N, P and K in the biomass of the aerial part were determined, according to the manual of analytical techniques for the analysis of soil, leaf, organic manure and chemical fertilizers (Paneque *et al*. 2011).

The extraction of N, P and K in the biomass of the aerial part was calculated. For that the average values of the concentrations of these nutrients in the moment of the second and fourth cut and the accumulated yield of the aerial biomass (sum of the four cuts) were taking. The fallowing formula was used:

Extraction of N, P and K (kg ha^{-1}) = [accumulated yield of DM, kg ha^{-1} x concentration of the element in the DM of the aerial part, g kg^{-1}] / 1000

In the moment of the second and fourth cut, in the rainy and dry season, respectively, from each plot a total of three subsamples of roots and soil of the rhizosphere were taking, at a deep of 0–20 cm using a metallic cylinder of 5 cm diameter and 20 cm height. The sampling points were equidistant and separate distributed at 10cm from the rows. The subsamples were homogenized to form a sample compound by plot, and 1 g of rootlets was extracted for their dying and clarification (Rodríguez *et al*. 2015). The frequency of mycorrhizal colonization was evaluated by the intercept method (Giovanetti and Mosse 1980), the visual intensity or colonization intensity, in accordance with Trouvelot *et al*. (1986) and the number of spores in the rhizosphere from the sieve and decanted by humid way of these structures and their observation in microscope (Herrera *et al*. 1995).

In the area where the experiment was performed, previous to the canavalia sowing, a total of 10 samples of soil were taking with a drill by the zigzag method, at 0-20 cm deep for their chemical characterization. After the second cut, a total of three subsamples of soil at a deep of 0-20 cm were taking to form a compound sample and determine their contents of OM, P assimilable and K interchangeable (Paneque *et al*. 2011).

Statistical analysis. The data, once the normality and homogeneity of variances were proved, they were processed by the analysis of variance and Tukey test ($P<0.05$). In the variables corresponding to the initial chemical characterization of the soil and to the AMF spores counting

(number of spores/50g) that leave in the soil after the canavalia cut, the confidence interval ($\alpha=0.05$) was used as dispersion statistics (Payton *et al*. 2000). In all cases the statistical program SPSS 25 (2017) was used.

Results and Discussion

There was a significant effect of the addition of cattle manure on the contents of OM, P assimilable and K interchangeable of the soil (table 4), which was in correspondence with the amounts of this elements provide by the organic fertilizer. According to their composition, with each ton of manure (in humid base) there were incorporated to the soil 290 kg of OM, 3 kg of P and 9 kg of K, and this has effect, on the increase of their contents in the soil. The manure did not have effects on the pH, probably due to the alkaline reaction of the soil and to the neutral pH of the manure.

Although the benefits of the applications of cattle manure in the soil fertility are known, their inclusion in the fertilization systems of forages crops continues being an acceptable strategy by the many environmental services that generates in the cattle agroecosystems by their contribution to the recycle of nutrients which are extracted from the soil with the biomass intake by the animals, as well as by their direct impact in the reduction of the pollutant effect that their incorrect use cause (Sileshi *et al*. 2019).

Table 5 show the effect of treatments on the fungi variables in the moment of the second and fourth cut. In the second one, the join application of both biofertilizers, alone or with N, increased the frequency and intensity of the colonization, as well as the number of spores in the rhizosphere with relation to the non biofertlizers treatments. However, these variables reached the highest values with the application of the biofertilizers combine with the cattle manure, except where the higher dose of N was added.

Table 4. Effect of the nitrogen fertilization, biofertilization and the application of cattle manure on the contents of OM, pH, P assimilable and K interchangeable of the soil.

Treatments			OM, %	pH, H_2O	P, mg kg^{-1}	K, $cmol_c$ kg^{-1}
N, kg ha^{-1} year	Cattle manure, t ha^{-1}	Biofertilization				
0	0	NB	4.40^b	7.5	62^b	0.57^b
70	0	NB	4.50^b	7.7	65^b	0.62^b
100	0	NB	4.36^b	7.5	61^b	0.52^b
0	0	B	4.62^b	7.6	70^b	0.58^b
70	0	B	4.35^b	7.4	63^b	0.61^b
100	0	B	4.39^b	7.6	67^b	0.53^b
0	25	B	5.46^a	7.7	116^a	1.28^a
70	25	B	5.54^a	7.8	121^a	1.31^a
100	25	B	5.49^a	7.6	118^a	1.26^a
	SE±		0.21	0.2	6	0.15
	P		0.0001	0.332	0.0001	0.0002

NB: non biofertilized. B: biofertilized with *Azospirillum brasilense* + *Rhizoglomus irregulare*. Averages with common letters in the same row did not significantly differ, according to Tukey (P<0.05) test

Table 5. Effect of the nitrogen fertilization, biofertilization and the application of cattle manure on the fungi structures of Tithonia in the moment of the second and fourth cut

Treatments			Second cut, February 2019			Fourth cut, July 2020		
N, kg ha^{-1} year	Cattle manure, t ha^{-1}	Bio-fertilization	Colonization frequency, %	Colonization intensity, %	Spores/ 50 g	Colonization frequency, %	Colonization intensity , %	Spores/ 50 g
0	0	NB	23.9^c	3.15^c	87^c	33.7^d	3.27^d	125^d
70	0	NB	22.8^c	2.89^c	92^c	34.3^d	2.93^d	132^d
100	0	NB	21.3^c	3.19^c	89^c	33.9^d	3.19^d	129^d
0	0	B	34.7^b	4.63^b	223^b	41.4^c	4.53^c	313^c
70	0	B	33.9^b	4.61^b	225^b	52.7^b	5.55^b	473^b
100	0	B	33.5^b	4.57^b	237^b	51.9^b	5.38^b	451^b
0	25	B	38.3^a	5.91^a	393^a	64.2^a	6.75^a	613^a
70	25	B	39.5^a	6.22^a	418^a	63.8^a	6.97^a	587^a
100	25	B	34.9^b	4.59^b	242^b	40.5^c	4.39^c	309^c
	SE±		1.6	0.28	23.5	1.8	0.26	32
	P		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

NB: non biofertilized. B: biofertilized with *Azospirillum brasilense* + *Rhizoglomus irregulare*. Averages with common letters in the same row did not significantly differ, according to Tukey (P<0.05) test

In the moment of the fourth cut, different to the second, among the biofertlized treatments without the addition of manure, the frequency and intensity of the colonization and the number of spores in the rhizosphere were significantly higher with the application of 70 and 100 kg ha^{-1} per year of N, than with the dose of 0 kg ha^{-1} of N. Therefore, the highest values of these variables were also reached in the treatments where the biofertilization and the addition of cattle manure was combined, alone or with nitrogen fertilization, except where the higher dose of N was applied.

It has to highlight that, during this cycle, the plants growth in the rainy season and, therefore, the nitrogen fertilizer was applied, which did not happen during the second cut, whose plats growth in the dry season and it was not possible the application of N. These could explain the direct effect of the nitrogen fertilization, combine with the application of the biofertilizers on the increase of the fungi structures.

It has been showed that the availability of nutrients in the soil control the growth of intra and extraradical hyphae of the AMF. In this way, when the plants are inoculated with effective AMF strains, the fungi structures can increase in presence of moderate amounts of nutrients and reduce with the application of dose that exceed the needs of the inoculated crops, since the delivery of recourses from the soil to the host plant through the AMF loss importance (Liu *et al.* 2019). This could explain the increase of the frequency, intensity of colonization and the number of spores in the rhizosphere that was observed in both cuts with the application of biofertilizers and manure, as well as their decrease in the treatment where the biofertilizers and organic fertilizer were combined with the highest dose of nitrogen.

The results also prove the effectiveness of the use of canavalia as proceeding crop and way for the production of mycorrhizal propagules in the soil for the inoculation of successor crops (Rivera *et al*. 2020, Simó *et al*. 2020 and Méndez *et al*. 2022) and the effectiveness of the inoculation with *R. irregulare* and *A brasilense* in the increase of mycorrhizal structures of tithonia (Méndez *et al*. 2022).

In the moment of the second as in the fourth cut there was significant effect of the treatments in the concentrations of N on the biomass of the aerial part (table 6). In the second one, among the non inoculated treatments there were not differences between them and showed the lowest values in relation to the rest. With the biofertilized were reached higher concentrations of N, but the highest values were obtained when they were combined with the application of manure and the doses of 0 and 70 kg ha^{-1} of N.

In the moment of the fourth cut, different to the previous, between the non biofertilized treatments there was increase of the N concentrations in the biomass of the aerial part with the increase of the nutrient dose, as direct consequence of the nitrogen fertilization applied during this cycle. However, with the biofertilization and the applications of 70 and 100 kg ha^{-1} of N highest values were reached. Therefore, the higher concentrations of N were obtained with the application of manure and the biofertilizers, except where the highest dose of this nutrient was applied.

The treatments did not have effects on the concentrations of P and K of the aerial biomass, which proved that the high contents of both elements in the soil at beginning of the experiment were sufficient for the crop. In fact, this criterion was taking into account for not introduce phosphoric and potassium fertilizers in the fertilization plan.

The effect of the nitrogen fertilization on the increase of the N concentrations in the biomass of the aerial part of Tithonia has being mentioned by Botero Londoño *et al*. (2019) and Astúa *et al*. (2020); whose attribute it to the high demand of this nutrient, which cannot generally be cover from their contents in the soil, when it is expect to reach a high forage production.

However, the results of this study showed that with the biofertilization with *R. irregulare* and *A. brasilense* and the cattle manure, without additional applications of N, the plants are better supplied of this element than with the application of the nitrogen fertilizer. In fact, the addition of 100 kg ha^{-1} of N, combined with the biofertilizers and the addition of manure, dressed the N concentrations. This could be consequence of the decrease of the effectiveness of biofertilization, as it was showed when evaluating the effect of treatments on the fungi variables. That is, to the positive effect of the biofertilizers on the nitrogen nutrition, was added the improvement of their effectiveness by the addition of manure and the N contribution of the organic fertilizer. This guarantee that the plants were better supplied with this nutrient than with applied doses of nitrogen fertilizer.

The treatments had a significant effect on the forage production (table 7). In the cuts made on November, 2019 and July, 2020, whose plants growth in the rainy season and received the direct effects of the nitrogen fertilization, among the treatment that only received N it was recorded increase of the DM yields with the increase of the doses, until reaching the highest values with the annual application of 100 kg ha^{-1} of N during the year (50 kg ha^{-1} in one and other cut).

Table 6. Effect of the nitrogen fertilization, biofertilization and cattle manure on the concentrations of N, P and K in the biomass of Tithonia aerial part

Treatments			Second cut, February 2019			Fourth cut, July 2020		
N, kg ha^{-1}	Cattle manure, t ha^{-1}	Biofertilization	N	P	K	N	P	K
			g kg^{-1}					
0	0	NB	23.1^{c}	2.6	21.5	22.1^{e}	2.1	17.9
70	0	NB	22.8^{c}	2.4	22.1	24.5^{d}	2.3	18.3
100	0	NB	23.5^{c}	2.6	21.7	26.9^{c}	2.1	17.5
0	0	B	27.8^{b}	2.5	22.3	26.7^{c}	2.0	18.1
70	0	B	28.2^{b}	2.4	22.8	30.3^{b}	2.3	16.9
100	0	B	29.3^{b}	2.6	21.9	29.5^{b}	2.1	18.1
0	25	B	31.2^{a}	2.4	22.7	33.3^{a}	2.3	17.9
70	25	B	31.3^{a}	2.5	22.3	32.9^{a}	2.0	16.3
100	25	B	27.5^{b}	2.6	21.8	30.1^{b}	2.3	18.7
	SE±		0.4	0.2	0.3	0.4	0.2	0.3
	P		0.0001	0.335	0.418	0.0001	0.382	0.402

NB: non biofertilized. B: biofertilized with *Azospirillum brasilense* + *Rhizoglomus irregulare*. Averages with common letters in the same row did not significantly differ, according to Tukey (P<0.05) test

Table 7. Effect of the nitrogen fertilization, biofertilization and cattle manure on the yield (t ha^{-1} DM) of Tithonia forage per cut and the accumulated during the year

Treatments			t DM/ha				
N, kg ha^{-1} year	Cattle manure, t ha^{-1}	Biofertilization	First cut, November 2019	Second cut, February 2020	Third cut, May 2020	Fourth cut, July 2020	Accumulated, Nov 2019-July 2020
0	0	NB	3.89^{e}	3.25^{c}	2.97^{c}	4.13^{e}	14.24^{e}
70	0	NB	4.64^{d}	3.33^{c}	3.11^{c}	4.83^{d}	15.91^{d}
100	0	NB	5.38^{c}	3.41^{c}	3.07^{c}	5.67^{c}	17.53^{c}
0	0	B	4.55^{d}	4.15^{b}	3.93^{b}	4.92^{d}	17.61^{c}
70	0	B	6.52^{b}	4.31^{b}	3.89^{b}	6.45^{b}	21.22^{b}
100	0	B	6.41^{b}	4.23^{b}	3.78^{b}	6.51^{b}	20.93^{b}
0	25	B	7.13^{a}	4.89^{a}	4.51^{a}	7.63^{a}	23.83^{a}
70	25	B	7.21^{a}	4.83^{a}	4.63^{a}	7.23^{a}	23.90^{a}
100	25	B	6.37^{b}	4.1^{b}	3.46^{b}	6.31^{b}	20.24^{b}
SE±			0.202	0.181	0.194	0.221	0.415
P			0.0001	0.0001	0.0001	0.0001	0.0000

NB: non biofertilized. B: biofertilized with *Azospirillum brasilense* + *Rhizoglomus irregulare*. Averages with common letters in the same row did not significantly differ, according to Tukey ($P<0.05$) test

In both cuts, in the treatments which were biofertilized with *A. brasilense* and *R. irregular* without the addition of manure, was only necessary to apply 70 kg ha^{-1} of N per year to reach biomass yields, even higher to those obtained with the higher dose of N without biofertilizers. Therefore, in the rest of cuts, the yields reached with the jointly application of biofertlizers were higher with respect to the treatments that only receive N. But when the biofertilizers were jointly applied with the manure, the N applications were not only unnecessary, so they reached yields higher to those recorded with the biofertlizers, alone or with the nitrogen fertilization.

A more clear perception of the effect of treatments on the biomass production was observed in the accumulated yield, where it can state that when it was only applied nitrogen fertilization, the highest response was obtained with 100 kg ha^{-1} of N. While, with the biofertilization, without the N addition, the biomass production reached values similar to those that were obtained with the higher dose of this nutrient. However, in presence of biofertilizers, the highest yields were reached with the applications of 70 and 100 ha^{-1} of N. When combining the biofertilizers with manure were obtained the maximum forage yields without need to apply N, it was observed decrease with the addition of 100 kg ha^{-1} of this nutrient.

The effect of fertilization on the forage production of Tithonia has being mentioned by several researchers. All coincide when explain that the highest yields were reached with a supply of high doses of nutrients (Ramírez 2018 and Dos Santos *et al*. 2021). Regarding the biofertilization, different authors has recorded increase in the forage production. Hakim *et al*. (2014) reported them with the combine application of solubilized bacteria of phosphorous and *Azospirillum*. Murillo *et al*. (2020) reported with the inoculation of AMF strains *Claroideoglomus etunicatun* and *Scutellospora calospora*.

It was interest that with the combine application of biofertilizers and cattle manure has been reached the highest forage yields, without applying nitrogen fertilizer. Several aspects seem to explain this performance. The first one can be related with the increase of the biofertilization effectiveness in presence of cattle manure. It has being showed that the biological fixation of nitrogen through the simple inoculation with A. *brasilense* can contribute up to 54 kg de N ha^{-1} year^{-1} (Hungria *et al*. 2016 and Aguirre *et al*. 2020). Also, the biological fixation of N as the effectiveness of the inoculation with AMF, can be potentiate through the contribution of C and other nutrients and the increase of the microbial activity in the soil, that cause the addition of organic fertilizers (Ren *et al*. 2021). In fact, Aguirre *et al*. (2020) find in perl sorghum increase of the mycorrhizal colonization and decrease of 50 % of the cattle manure dose, when their application was combine with the inoculation with *A. brasilense* and *G. mosseae*.

It is also to highlight the positive effect that the AMF exert in cooperation with other microorganisms of the soil, in terms to the OM mineralization and consequently, the absorption of N and other nutrients derived from this process (Jiang *et al*. 2021). If it is added the contribution of the manure nutrients and their possible effects on the improvement of the physical properties of the soil (Sisouvanh *et al*. 2021) it can ensure the high effect of the join addition of the organic fertilizer and the biofertilizers on the Tithonia yields with respect to the biofertilization, as well as the in the reduction of applying complementary doses of nitrogen fertilizer, at least during the first year of their cultivation. The decrease of yields obtained with the application of 100 kg ha^{-1} of N in presence of the biofertilizers and manure and the reduction of the fungi variables observed in this treatments reaffirms the decrease of the biofertilization activity face a N dose that, in this case, was excessive for the Tithonia biofertlized and fertilized with manure during the period in which the experiment was carried out.

When evaluating the effect of the treatments on the extractions of the primary macronutrients (N, P and K) with biomass of the aerial part during the period in which the experiment was performed (table 8), it was observed increase of the three elements with the increase of the nitrogen fertilization without biofertilizers and manure. In the biofertilized treatments, the extractions were higher, above all when they were combined with the doses of 70 and 100 kg ha^{-1} of N. But the highest values were obtained with the combine application of bifertilizers and manure, except when N at a rate of 100 kg ha^{-1}was added.

Quantitatively, the extractions fallow the order N>K>P and coincide with those find by Botero Londoño *et al*. (2019) and Astúa *et al*. (2020). The first authors also find extraction levels up to 800, 140 and 462 kg ha^{-1} of N, P and K with plantation densities of 1 m^2 per plant and applications of 25, 12 and 11 kg per plant of N, P_2O_5 and K_2O.

Although the exportation levels of nutrients with the Tithonia biomass depends on the soil fertility, fertilizers application, plantation distance and varieties, among other factors. The obtained results by the mentioned authors and also those from this study reaffirm that the crop is highly nutrients extractor from the soil. In addition, despite their rusticity and few edaphic requirements, the fertilization is vital, not only to make higher and to support the forage yields in the time, so to maintain the soil fertility. This involve, especially when it is submitted to frequently cuts for forage production, the need of monitoring the nutrients dynamic in the soil and the exportations of these ones with the biomass and yields, with the purpose of making the appropriate corrections to maintain the yields and to avoid the soil exhaustion.

Conclusions

The integrated management of the fertilization base on cattle manure and the coinoculation with A. *brasilense* and *R. irregular* is effective to increase the yield of Tithonia forage, reduce the use nitrogen fertilizer and to improve the soil fertility.

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Table 8. Effects of the nitrogen fertilization, biofertilization and the application of cattle manure on the extractions of N, P and K with the biomass of Tithonia aerial part

Treatments					
N, kg ha^{-1}	Cattle manure, t ha^{-1}	Biofertilization	N, kg ha^{-1}	P, kg ha^{-1}	K, kg ha^{-1}
0	0	NB	321.8^f	32.8^d	280.5^e
70	0	NB	376.3^e	36.6^c	321.4^d
100	0	NB	441.8^d	40.3^b	343.7^c
0	0	B	479.6^c	38.7^c	355.5^c
70	0	B	619.0^b	48.8^b	420.7^b
100	0	B	615.3^b	47.4^b	418.6^b
0	25	B	768.5^a	54.8^a	483.7^a
70	25	B	767.9^a	52.6^a	471.3^a
100	25	B	579.1^b	50.0^{ab}	421.8^b
	SE±		6.9	1.2	5.7
	P		0.0001	0.0001	0.0001

NB: non biofertilized. B: biofertilized with *Azospirillum brasilense* + *Rhizoglomus irregulare*. Averages with common letters in the same row did not significantly differ, according to Tukey (P<0.05) test

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