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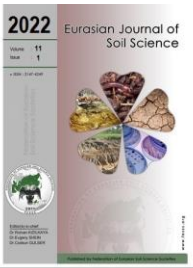

Biomass yield, soil cover and minerals accumulation by two green manures species grown in soils of Chiapas Mexico

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Abstract

The aim of the current study was to assess the performance of *Canavalia ensiformis* and *Mucuna deeringiana* (Leguminosae) as a green manure in the agricultural soil of the Frailesca region of Chiapas, México, in terms of aboveground biomass accumulation, plant height, number of leaves, canopy coverage, and the accumulation of nitrogen (N), phosphorus (P), and potassium (K). Each species was sowed at two population densities under a randomized complete block design with three replications. Every 30 days after sowing (DAS), the following variables were quantified: plant length, number of leaves, canopy coverage, biomass yield, and N, P, and K content. A variance analysis and mean comparison test (Tukey 0.05) were performed for each variable. The biomass yield in *M. deeringiana* fluctuated from 9150 to 33,160 kg ha^{-1} on a fresh basis and from 4490 to 15,890 kg ha^{-1} on a dry basis, whereas the yield in *C. ensiformis* varied from 9343 to 26,390 kg ha^{-1} and from 4513 to 13,150 kg ha^{-1}, respectively. The longest recorded plant length was 513.00 cm in *M. deeringiana* and 155 cm in *C. ensiformis*, with a total of 353 and 322 leaves, respectively. The accumulation of N, P, and K was 463.99 kg ha^{-1}, 84.22 kg ha^{-1}, and 49.26 kg ha^{-1} in *M. deeringiana* and 341.90 kg ha^{-1}, 43.40 kg ha^{-1}, and 36.82 kg ha^{-1} in *C. ensiformis*, respectively. Both *C. ensiformis* and *M. deeringiana* have potential as green manure for the Frailesca region of Chiapas in terms of biomass production and N accumulation.

Keywords: *Canavalia ensiformis*, Canopy coverage, dry matter, ecotechnologies, legumes, *Mucuna deeringiana*.

Introduction

Incorrect management and overexploitation of soil resources has caused continuous degradation (Renté-Martí et al., 2018), as reflected in environmental pollution, erosion, and low fertility, which is mainly due to not considering agroecological practices for soil conservation (Serrano and Cano, 2007). Soil degradation in agriculture is appreciable in the long-term, as the excessive use of agrochemicals and other external technologies maintains crop yield levels; however, the cost of production increases each time and the negative effects to the soil increase (Amézquita et al., 2013). Therefore, one of the alternatives for soil improvement is the incorporation of green manures, mainly species of the Leguminosae family, due to their ability to fix atmospheric N (N_2) in association with bacteria of the genus *Rhizobium* (Mangaravite et al., 2014) and their different benefits to the physical, chemical and biological properties of the soil. Among these benefits is an increase in organic matter (Cruz et al., 2014) and the release of nutrients, mainly N, which is the most limiting nutrient for many of the basic crops with annual growth (Pereira et al., 2016). *C. ensiformis* and *M. deeringiana* are the most important species as green manures and cover crops in the tropics and subtropics, adapting to tropical and subtropical climates (Buckles and Triomphe, 1999), where they present optimal development in

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both clay and sandy soils under conditions of low fertility (Calegari et al., 1993). In some studies, it has been reported that *C. ensiformis* and *M. deeringiana* obtains large amounts of N through biological fixation (Bunch 2016; Sant´Anna et al., 2018). On the other hand in Cuba Renté-Martí et al. (2018) reported high biomass yields and a positive effect on physical properties of soil when the green manure was incorporate into the soil.

This ability to accumulate fresh or dry matter in the organs of a plant is known as crop yield (Barrientos-Llanos et al., 2015), which can be expressed as fresh or dry biomass, height, number of leaves, and stem diameter, among others, through the quantitative process of growth (Werner and Leihner, 2005). Therefore, knowledge of biomass yield and nutrient content throughout the growth cycle of plants is fundamental to establishing the potential for utilization in soil rehabilitation programs (Zapata et al., 2019). However, *C. ensiformis* and *M. deeringiana* have been little used to improve or preserve the fertility of agricultural soils in the tropical and subtropical regions of Mexico due to the competition that it can exert with the main crop, in addition to little knowledge of its use as forage in animal feeding (Eilittä and Carsky, 2003). Knowledge about the development and biomass production of *C. ensiformis* and *M. deeringiana*, as well as their roles in the improvement and rehabilitation processes of agricultural soils, is scarce in tropical regions of south-southeast Mexico. On the other hand, no investigations have indicated the best population densities for cultivation and dates of incorporation into the soil as green manures. In this context, it is important to for studies to focus on the vegetative characterization and biomass production in species that represent sustainable alternatives for improving and restoring soils in tropical and subtropical regions. Therefore, the objective of the present study was to characterize the vegetative development of *C. ensiformis* and *M. deeringiana* at different population densities in terms of biomass accumulation, plant height, number of leaves, canopy coverage, and the accumulation of N, P, and K for their potential use as green manures in agricultural soils of the Frailesca region of Chiapas, Mexico.

Material and Methods

Description of the experimental site

The evaluated species (*C. ensiformis* and *M. deeringiana* were cultivated at the University Center for Technology Transfer, CUTT San Ramón, of the Faculty of Agronomic Sciences, Campus V, of the Autonomous University of Chiapas, located in the municipality of Villaflores, Chiapas, Mexico. The geographical coordinates are 16°15'N and 93°14'W, with an altitude of 610 m.a.s.l., average annual temperature of 22°C, and annual precipitation of 1200 mm (Aguilar et al., 2019). Figure 1 shows the average temperature and precipitation during the experiment.

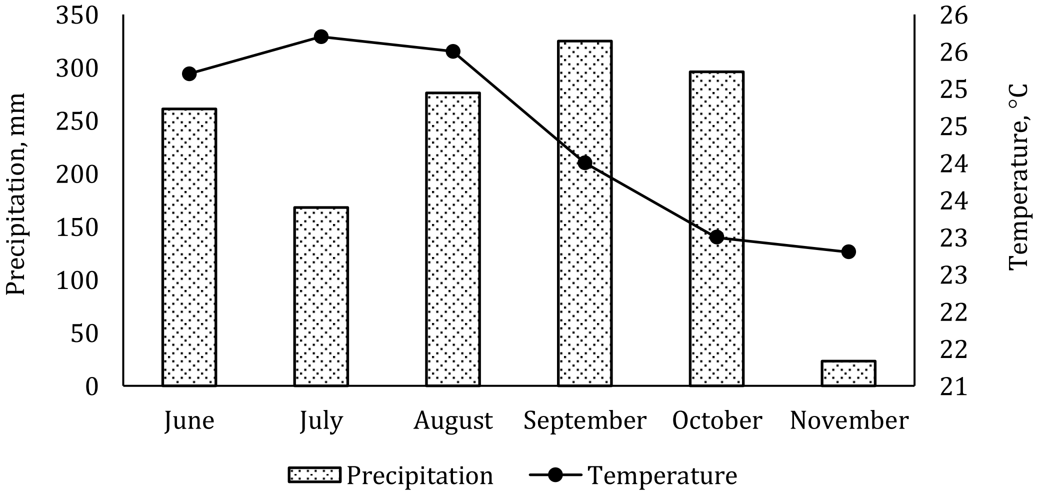

Figure 1. Average precipitation and temperature of the study area during the experiment.

Plant material and experimental design

The *C. ensiformis* and *M. deeringiana* seeds used in this experiment were obtained from the harvest of the previous agricultural cycle, spring/summer 2018, in the same experimental field. The seeds were sowed by hand June 1, 2019, at two different population densities using a randomized block design with four treatments and three replications (Table 1).

Table 1. Treatments, planting density, and population density of *Canavalia ensiformis* and *Mucuna deeringiana.*

Treatment	Planting distance between rows and plants (m)	Planting density (kg ha^{-1})	Population density (plants ha^{-1})
T1: *C. ensiformis*	0.5 × 0.5	95.2	80,000
T2: *C. ensiformis*	1 × 0.5	47.6	40,000
T3: *M. deeringiana*	1 × 1.5	10.66	13,333
T4: *M. deeringiana*	1 × 1	16	20,000

Each experimental unit consisted of 10×8 m plots, with a separation of 1 m between experimental plots. No scarification or inoculation treatment was applied to the seeds. In all four treatments, two seeds were placed per sowing point. The soil type used was a *Chromic Luvisol* (WRB, 2014), soil samples were collected and analysed according to methods described by Carter and Gregorich, (2007) and its initial chemical and physical characteristics are provided in Table 2. No agrochemicals were applied during the evaluation and weed control was carried out by hand.

Table 2. Physical and chemical characteristics of the soil used.

Characteristic	Method	Result
pH	$CaCO_2$	4.02
Organic matter (%)	Walkley and Black	1.41
N total (%)	Kjeldahl	0.07
P (mg kg^{-1})	Olsen	11
K (meq$_c$ 100 g)	Atomic emission	0.3
Ca (meq$_c$ 100 g)	Atomic absorption	2.1
Mg (meq$_c$ 100 g)	Atomic absorption	0.5
Na (meq$_c$ 100 g)	Atomic emission	0.1
Fe (mg kg^{-1})	Atomic absorption	104
Zn (mg kg^{-1})	Atomic absorption	1.0
Al (meq$_c$ 100 g)	Exchangeable acidity	0.3
CEC (meq$_c$ 100 g)	Atomic absorption	8.3
NO_3 (mg kg^{-1})	Steam entrainment	28
NH_4 (mg kg^{-1})	Steam entrainment	20
Sand (%)	Bouyoucos	55
Silt (%)	Bouyoucos	19
Clay (%)	Bouyoucos	26
Textural classification		Sandy-clay loam

Sample collection

The plants were harvested 30, 61, 92, 123, and 155 DAS and the following quantified in each sample: green and dry biomass yield, number of leaves, plant height, and canopy coverage. Both fresh and dry biomass yield were determined by the square method (1 m^2); the fresh biomass was quantified after cutting and extracting all plants located on the indicated surface, whereas for dry biomass the samples were taken to the Animal Nutrition laboratory of the Faculty of Agronomic Sciences of the UNACH, where they were cleaned of impurities and placed in an air circulation oven at 70°C for 48 hours. After obtaining the dry weight of the biomass, the samples were ground for further analysis of N, P, and K. In the last sampling (155 DAS), the root system was extracted in order to quantify the nodules. Ten plants located in the central furrows of the experimental plots were measured by a flexometer to obtain the plant length and the total number of leaves per plant, whereas the canopy coverage was obtained by the square method (1 m^2) subdivided into 100 squares of 10 cm each.

Nutrient analysis

The Kjedahl method was used (Bremner, 1996) to determine the N in whole plants of *C. ensiformis* and *M. deeringiana*, whereas P and K were obtained by colorimetric (Chapman et al., 1984) and atomic absorption spectrophotometry (Fishman and Downs, 1966), respectively. The accumulation of N, P, and K in dry biomass was calculated as follows:

$$\text{Accumulation x (g plant)} = \frac{(\text{g dry matter})(\text{x \% NPK total in the plant})}{100}$$

Statistical analysis

All data were subjected to an analysis of variance according to the selected experimental design. Subsequently, a comparison of means was carried out (Tukey test 0.05) using the statistical program Statgraphics Centurión XVII (Statgraphics, 2014).

Results and Discussion

The variance analysis for the fresh and dry biomass yield variables only showed significant differences ($p<0.05$) between treatments in the last two samplings (123 and 155 DAS). At the evaluated densities (T1 and T2), the *C. ensiformis* species registered the highest accumulation of biomass at 92 DAS with 22,320 and 26,390 kg ha^{-1} in fresh biomass and 11,080 and 13,150 kg ha^{-1} in dry biomass, respectively, but a decrease in biomass was observed at 155 DAS (Table 3). These biomass yields are within the values reported by Precoppe (2005), who recorded *C. ensiformis* yields of 20.00 to 40.00 t ha^{-1} for fresh matter and 3.00 to 6.00 t ha^{-1} for dry matter. In treatments T3 and T4, the biomass yields gradually increased from 123 and 155 DAS, with maximum values of 20,833 and 33,160 kg ha^{-1} for fresh biomass and 10,323 and 15,890 kg ha^{-1} for dry biomass, respectively (Table 3). The fresh biomass production recorded at T3 and T4 is within the yields reported by Costa-Mello et al. (2018) in *M. deeringiana* at 75 DAS; they recorded yields from 20.29 to 44.79 t ha^{-1}. However, the dry biomass yield found in this study was higher than that recorded by those authors (9.47 t ha^{-1}). The dry biomass yields obtained in this study are above the values recommended as cover/green manure.

The high biomass yields in both legume species sowed at different population densities confirm its potential as green manure for the improvement of degraded soils in the tropical and subtropical regions of south-southeast Mexico. In this regard, Aguilar (2014) reported that one of the desirable characteristics of the species used as green manures is that they can grow in poor soils with little or no management.

Table 3. Accumulation of fresh and dry biomass, plant length plant, and number of leaves in *C. ensiformis* and *M. deeringiana* at different growth periods.

Treatments	Days After Sowing				
	30	61	92	123	155
			Fresh biomass (kg ha^{-1})		
T1	13373 a	20950 a	22320 a	21280 b	9867 c
T2	9343 a	25946 a	26390 a	21560 b	13667 bc
T3	9150 a	18463 a	19723 a	20250 b	20833 b
T4	12270 a	23080 a	25103 a	26756 a	33160 a
CV %	25.04	19.48	19.33	19.65	23.38
			Dry biomass (kg ha^{-1})		
T1	7237 a	10337 a	11080 a	10447 b	4873 c
T2	4513 a	12647 a	13150 a	10623 b	6957 bc
T3	4490 a	8980 a	9843 a	10323 b	10270 b
T4	6170 a	11613 a	12547 a	13540 a	15890 a
CV %	27.63	18.67	19.53	18.55	21.69
			Plant length (cm)		
T1	60.67 b	113.33 b	147.67 b	149.00 b	146.67 b
T2	63.00 b	134.33 b	155.00 b	152.67 b	152.33 b
T3	126.00 a	320.67 a	407.33 a	470.00 a	512.33 a
T4	134.67 a	325.00 a	409.67 a	474.33 a	513.00 a
CV %	6.72	4.03	2.16	1.67	1.31
			Number of leaves		
T1	42 b	133 b	309 ab	265 c	94 b
T2	43 ab	147 a	322 a	277 b	97 b
T3	46 a	153 a	282 b	292 a	350 a
T4	47 a	155 a	286 b	299 a	353 a
CV %	4.62	3.64	3.97	1.27	2.84

Values in the same column with a different letter indicate significant ($p < 0.05$) differences between the means. CV=coefficient of variation.

Although the soil presented low fertility levels (Table 2), its physical and chemical characteristics and climatic conditions (Figure 1) were favourable for the production of biomass in the four treatments evaluated. This is probably due to the fact that these species adapted in tropical and subtropical zones, where agroclimatic conditions are favourable for their cultivation. A study under conditions of Kenia showed that the most outstanding legumes as green manure were *C. ensiformis* and *M pruriens*, based mainly on biomass accumulation (Murehiti et al., 2003). The length of the plant and the number of leaves showed a relationship with the accumulation of biomass; in the four treatments, the length of the plant gradually increased as the vegetative development of each species progressed. At 123 DAS, the maximum plant length was 149.00 cm and 155.00 cm for T1 and T2 at 92 DAS, whereas the longest plant length for T3 and T4 was 512.33 cm and 513.00 cm at 155 DAS (Table 3). The highest number of leaves accumulated for T1 and T2 was 309 and 322 at

92 DAS, respectively, whereas the maximum number of leaves for T3 and T4 was 350 and 353 up to 155 DAS (Table 3). These results show that the species evaluated in this study as green manures have suitable capacities to accumulate fresh matter and dry matter in their different organs, such as the leaves and stems, ensuring a proportional increase in biomass yield, which is essential in crop production (Barrientos-Llanos et al., 2015). The growth of these organs implies a physiological process, which depends on several factors, such as photosynthesis, respiration, elongation, and cell division (Gamage et al., 2018). In addition, other components that influence growth are the sowing date, planting density, fertilization, irrigation, pest control, and the duration of the crop (Vanek, 2009). The higher production of fresh and dry matter, higher plant height, and greater number of leaves with *M. deeringiana* during its growth (T3 and T4) was probably due to the fact that the flowering stage started earlier in *C. ensiformis* than in *M. deeringiana* (90 vs. 120 DAS). Thus, both densities of *M. deeringiana* showed a tendency to continue vegetative growth after 90 days (its life cycle was longer than that of *C. ensiformis*).

According to Rajwade et al. (2000), this behaviour is part of the typical characteristics of each species and the interaction with the environment. The canopy coverage presented a similar pattern (Figure 2), as *C. ensiformis* at the two densities (T1 and T2) showed a gradual increase of 30% and 27% at 30 DAS, respectively, 79% at 61 DAS, and 100% canopy coverage at 92 and 123 DAS, respectively. At 155 DAS, a small decrease of 92% was observed for T1 and 97% for T2. In contrast, *M. deeringiana* (T3 and T4) increased its canopy coverage from 30 DAS, exceeding 80%, and then remained constant at 100% until 155 DAS. This shows the efficiency of plants to accumulate biomass and translocate nutrients at certain times during growth to the organs with the highest demand. The decrease in biomass yield for T1 and T2 can be explained as a function of plant maturity, in which biomass production begins to decline due to senescence, which occurs mainly in mature leaves and with translocation of nutrients to the fruits (Zapata et al., 2019). Canopy coverage represents one of the most important characteristics of the species used as green manures in the tropical regions of Mexico. The orography in these regions makes it necessary to practice agriculture in hillside conditions, favouring erodibility, which requires maintenance of the surface area of agricultural soils due to the erosivity typical of these regions. The soil is covered to reduce hydric erosion (García-Hernández et al., 2010) and represents a fundamental principle of the ecological management of agricultural soils.

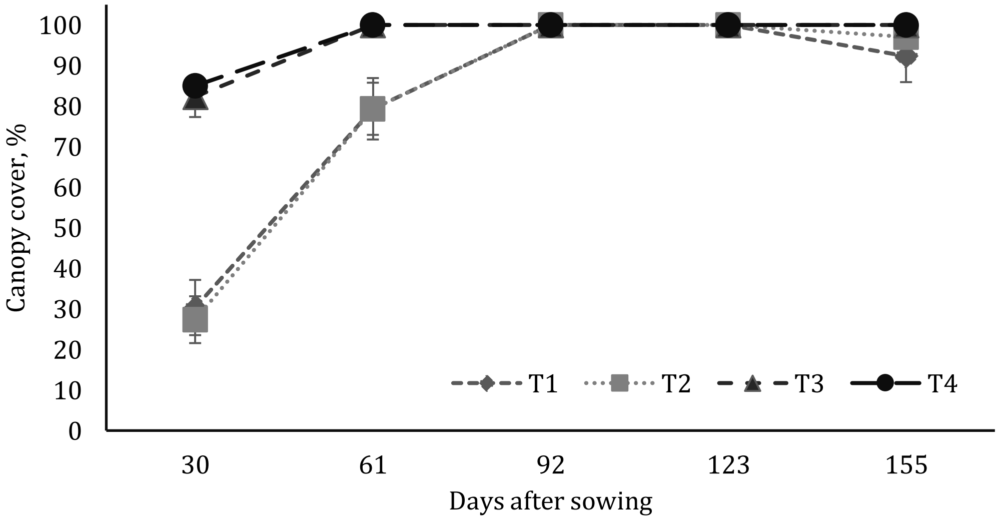

Figure 2. Percentage of canopy coverage during the growing season of *C. ensiformis* and *M. deeringiana* at different growth periods.

In practically all samples, the N, P, and K that accumulated in the biomass of the species under study had significant differences between treatments ($p<0.05$). The total N accumulated in *C. ensiformis* at the lowest density (T1) varied from 188 to 288 kg ha^{-1} from 30 to 90 DAS, whereas with the highest density (T2) the values ranged from 117.35 to 341.90 kg ha^{-1} during the same period. Subsequently, the N accumulation tended to decrease significantly for both treatments (Table 4). In *M. deeringiana*, a different behaviour was observed; the total N accumulated in the biomass of both densities (T3 and T4) increased until the last sampling period (150 DAS), but with a tendency to be significantly higher for T4 than T3 (463.99 vs. 301.44 ha^{-1}). With respect to accumulated P, a trend very similar to that recorded for N was observed, but in smaller amounts. For example, with *C. ensiformis* at the lowest density (T1), the total accumulated P varied from 23.8 to 36. 56 kg ha^{-1} from 30 to 90 DAS, whereas at the highest density (T2) the values ranged from 14.8 to 43.4 kg ha^{-1} during the same period. After 90 DAS, a tendency was also observed in both treatments for the accumulation to decrease significantly to 16.8 and 22.9 kg ha^{-1}, respectively. The P in the biomass of *M. deeringiana*, regardless of density (T3 and T4), also increased as the growth period progressed (from 30 to 150 DAS), with values from 23.80 to 54.43 kg ha^{-1} and 32.70 to 84.32 kg ha^{-1}, respectively, but with a tendency to be significantly higher for T4 than T3 in each sampling carried out (Table 4). K was the mineral that accumulated the least in the biomass of both legume species, with values from 20.26 to 31.04 kg ha^{-1} (T1), 12.64 to 36.82 kg ha^{-1} (T2), 13.92 to 32.00 kg ha^{-1} (T3), and 19.13 to 49.26 kg ha^{-1} (T4), but only the latter treatment gradually accumulated K until the end of the evaluation period (from 30 to 155 DAS).

Table 4. Nitrogen, phosphorus, and potassium accumulated in the dry biomass of *Canavalia ensiformis* and *Mucuna deeringiana* at different growth periods.

Treatments	Days After Sowing				
	30	61	92	123	155
			Accumulated N (kg ha^{-1})		
T1	188.15 a	268.75 b	288.08 b	271.61 b	126.71 c
T2	117.35 a	328.81 a	341.90 a	276.21 b	180.87 bc
T3	131.11 a	262.22 b	287.43 b	301.44 ab	299.88 b
T4	180.16 a	339.11 a	366.36 a	395.37 a	463.99 a
CV %	27.54	19.22	20.12	19.02	21.04
			Accumulated P (kg ha^{-1})		
T1	23.88 ab	34.11 b	36.56 b	34.47 b	16.08 c
T2	14.89 b	41.73 ab	43.40 ab	35.06 b	22.96 c
T3	23.80 ab	47.59 ab	52.17 ab	54.71 ab	54.43 b
T4	32.70 a	61.55 a	66.50 a	71.76 a	84.22 a
CV %	27.83	21.18	22.20	20.44	20.99
			Accumulated K (kg ha^{-1})		
T1	20.26 a	28.94 a	31.04 b	29.25 b	13.65 c
T2	12.64 b	35.41 a	36.82 a	29.75 b	19.48 bc
T3	13.92 b	27.84 a	30.51 b	32.00 b	31.84 b
T4	19.13 a	36.00 a	38.89 a	41.97 a	49.26 a
CV %	27.54	19.15	20.03	18.91	21.44

Values in the same column with different letters indicate significant ($p < 0.05$) differences between the means. CV=coefficient of variation.

In the edaphoclimatic conditions of this study, the amount of N accumulated in the aerial biomass of the legumes was higher than the amount of P and K, which is similar to previous reports (Mangaravite et al., 2014). This can be explained in terms of an adequate symbiosis between the roots of both *M. deeringiana* and *C. ensiformis* with bacteria of the genus *Rhizobium* or *Bradyrhizobium* to fix N_2 (Starovoytov et al., 2010; Saldaña-Acosta, 2017). According to Gerónimo et al. (2002), *M. deeringiana* can accumulate up to 260 kg ha^{-1} from fixation, whereas *C. ensiformis* can fix from 240 to 318 kg ha^{-1} (Vera-Núñez et al., 2008; Renté-Martí et al., 2018). Furthermore, approximately 80-85 % of the total N accumulated in biomass is derived from biological N fixation (Partelli et al., 2011). Although biological N fixation was not quantified in this study, it was possible to observe and quantify nodules on plant roots at 155 DAS in all treatments (Figure 3), with a greater tendency to find effective nodules (73 to 83) than nodules with no activity (36 to 39).

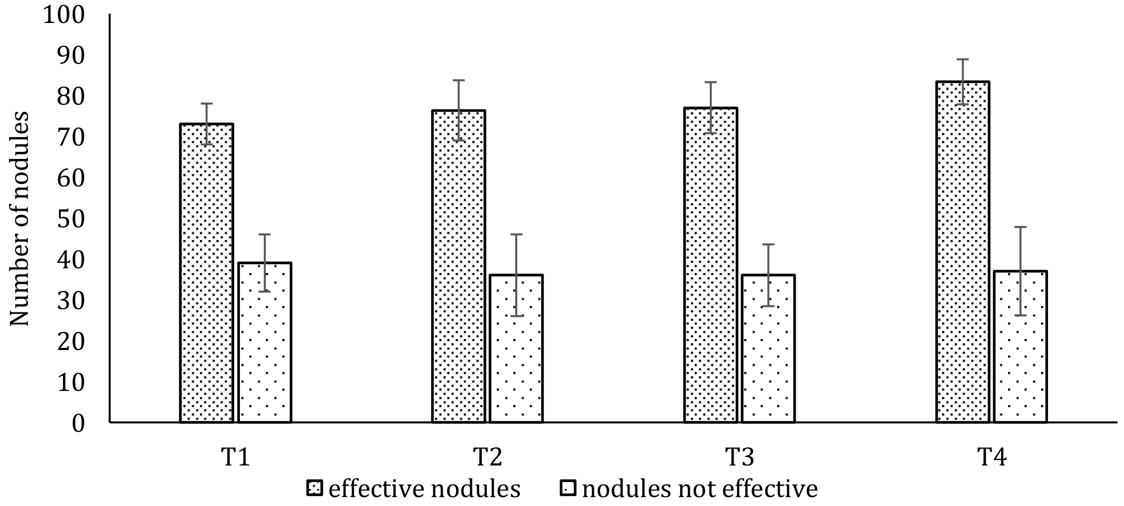

Figure 3. Number of effective and ineffective nodules in *C. ensiformis* and *M. deeringiana* at 155 DAS.

Fewer effective nodules were reported by Córdova-Sánchez et al. (2011), who recorded an average of 59 nodules in *C. ensiformis* and 61 nodules in *M. deeringiana*. Nodulation depends on other factors than the presence of effective rhizobia in the Fabaceae rhizosphere for an optimal interaction between the plant and bacteria (Bianco, 2020). Although P and K did not accumulate in large amounts in the present study, the values were generally higher than those reported by other authors for the same species, but with lower biomass yields, which may be related to differences in edaphoclimatic and management conditions during cultivation. For example, under the conditions in Brazil, Mangaravite et al. (2014) reported P and K values of 16 kg and 192 kg ha^{-1} for *C. ensiformis* biomass harvested at 74 DAS, whereas biomass from *M. deeringiana* cut at 104 DAS had values of 11 and 86 kg ha^{-1}, respectively. On the other hand, P and K availability is mainly related to the quantity and quality of effective nodules (Weisany et al., 2013; Sulieman et al., 2013; Divito and Sadras, 2014). The low accumulation of P and K in the biomass of the studied legumes indicates that the amount that will return to the soil after its incorporation as green manure is low; however, after decomposition and mineralization, these nutrients will be found in an easily accessible form for the next crop.

Conclusion

Overall, our results indicate that both *C. ensiformis* and *M. deeringiana* successfully established in tropical soils of Chiapas, Mexico; however in this study *M deeringiana* showed the greatest potential as a green manure in terms of biomass yield and minerals accumulation.

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