Investigation note

Greenhouse trial of green manures on soil properties, chard production and environmental implications

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Abstract

In this trial, legumes dolichos beans (*Lablab purpureos* L. ex Sweet) and yorimon beans (*Vigna unguiculata* L. Walp) as green manures were studied. The objectives of the study were to evaluate the ability to capture carbon and fix nitrogen, as well as to measure the effects of its incorporation in the soil, the production of chard (*Beta vulgaris* var. cicla L.) and to analyze the environmental implications. In the results, *L. purpureos* produced 1 932 kg of dry matter in 90 days after sowing (869 kg ha^{-1} of C) and fixed 30 kg of N ha^{-1} in its tissues, while *V. unguiculata* produced 2 040 kg of dry matter in 80 days after sowing (918 kg ha^{-1} of C) and fixed 40 kg of N ha^{-1} in its tissues. The soil effects were not significant in both AV, compared to the control treatment (without AV). However, both promoted an increase in the weight of chard (40% in *L. purpureos* and 31% in *V. unguiculata*). Environmentally, green manures can reduce the carbon footprint by 280 kg ha^{-1} of CO_2 equivalent by dispensing with synthetic nitrogenous chemical fertilizers in the production of chard. Green manures, as a source of nutrients, especially N, could form part of the integral management of the soil in the horticultural production systems of semi-arid zones.

Keywords: *Lablab purpureos* L., *Vigna unguiculata* L., CO_2, GHG, horticulture.

Reception date: April 2020
Acceptance date: May 2020

It is essential to produce food in a way that preserves the environment and biodiversity, implementing sustainable practices that provide healthy and nutritious food, ecosystem services and resilience to climate change (Da Silva, 2018). Conventional agricultural production systems use synthetic chemical fertilizers (FQS) to provide plants with the necessary nutrients. However, such practice can have negative collateral effects of contamination and soil degradation, which are accentuated when combined with excessive tillage of the soil (García *et al*., 2010).

The use of green manures (AV) is part of a technological strategy that has been proven by the research and practice of farmers in temperate climates, showing itself to be efficient and economically viable and which aims to increase and conserve the organic matter of the soil and nutrients, especially nitrogen (N) (Kumar *et al*., 2013). Yield increases associated with the use of AV crops have been directly related to the nitrogen content of the cover and the total dry matter produced (Mehari *et al*., 2005).

The physical and chemical properties of soils have been used to evaluate the effects of applying different sources of organic matter to the soil during long-term experiments (Tejada and González, 2005). In addition to the aforementioned, the contributions of nitrogen to the soil, by green manures, mean an economic saving in nitrogen from FQS, in addition to a reduction in greenhouse gases (GHG) (mainly N_2O) that are emitted into the atmosphere in its manufacture and transportation.

The AV are one of the safest options to improve soil fertility, but it is necessary to evaluate different fertilizer species in each region in order to select the most favorable ones (Beltrán *et al*., 2006a). Prager *et al*. (2012), mention that the replication of trials in various agro-ecological zones and the incorporation of methodological tools that validate the character of AV as a multipurpose technology are required.

Due to the few studies on plant species that can be used as AV in semi-arid areas, the present study was carried out with the objective of evaluating the capacity of dolicho legumes (*Lablab purpureos* L. ex Sweet) and yorimon (*Vigna unguiculata* L. Walp) to capture carbon and fix nitrogen, as well as its effect on some physicochemical properties of the soil, in the production of chard (*Beta vulgaris* var. cicla L.) and analyze the environmental implications.

The trial was conducted at the Protected Agriculture Training Center of the Autonomous University of San Luis Potosí, Mexico. It was done in a protected environment and using pots with the intention of having a good control of the study variables. The AV were seeded in 600 caliber black polyethylene bags with a capacity of 12 L that contained agricultural soil (clay-sandy crumb). Each pot was considered a repeat, in total there were twelve pots per species.

Soil moisture was maintained in a range between field capacity and 70% available moisture throughout the trial. After forty-five days and until harvest, a weekly application of diluted chicken leachate was carried out during irrigation (pH and electrical conductivity of the organic solution was 6.5 and 1.3 dS m^{-1}, respectively). The AV were incorporated into the soil at the beginning of flowering, 80 days after planting (dds) for the yorimon bean and at 95 dds for the dolichos bean.

Prior to incorporation, five plants of each species were collected for analyzes of biomass production and nitrogen content. The nitrogen in the plant was determined by the Kjeldhal method and the carbon was estimated based on the relationship of the dry weight with the average carbon in the plants, which is 45% (Azcon and Talon, 2008). With the data of nitrogen and carbon concentration per plant, the values per hectare were calculated by means of a direct proportion, assuming a population density of 60 000 plants ha^{-1}.

At 30 days after the incorporation of the green manures, a composite soil sample was obtained for each AV, which was used to determine the apparent density (test tube method), pH (potentiometer, ratio 1:2 soil: water), the electrical conductivity in saturation paste (conductivity meter), organic matter (Walkley-Black method) and total nitrogen (microkjedhal) (Rodríguez and Rodríguez, 2011). After incorporation of the AV, the assay was performed with the subsequent culture. Chard seedlings (*Beta vulgaris* var. cicla L.), thirty-five days old and four true leaves, were transplanted into the same bags.

The treatments evaluated were: T1) soil without fertilization; T2) soil with synthetic chemical fertilizer (a single application of 1 g per triple 17 plant, seven days after transplanting); T3) soil with dolichos residues; T4) soil with bean and yorimon residues. The treatments were established under a completely randomized experimental design with twelve repetitions (bags that occupied the AV). From the six days after the transplant (ddt), and until the harvest, the SPAD units were measured every week with the SPAD-502 Minolta Plus equipment.

At 72 days ddt, the chard plants were harvested, and the following variables were evaluated: a) chlorophyll (spectrophotometry); b) fresh weight of leaves and roots; c) dry weight of leaf and root; d) height of the plant; e) nitrogen in plant tissue (Kjeldhal method). The data were submitted to an analysis of variance in the statistical package Statistical Analysis System with version 9.0 (SAS Institute, 2002). In the variables where significant differences were shown, a comparison of means was performed using the Tukey test ($p < 0.05$).

The results indicate that the AV were statistically equal in terms of dry matter production ($p < 0.05$). *L. purpureos* produced 1 932 kg of dry matter in 90 days after sowing (869 kg ha^{-1} of C), while *V. unguiculata* produced 2 040 kg of dry matter in 80 days after sowing (918 kg ha^{-1} of C). Regarding the percentage of nitrogen in plant tissue, both AV were statistically equal ($p < 0.05$), the average of N in plant tissue was 2% in the whole plant.

The amount of nitrogen contained in the biomass, and which can be incorporated into the soil, was 40 kg ha^{-1} in yorimon beans and 37 kg ha^{-1} in dolichos beans, obtained in 80 and 95 dds, respectively. 75% and 65% would correspond to the aerial part of the plants for yorimon and dolichos, respectively. Beltran *et al*. (2009), reported values of 4 and 3.96% N in whole plant tissue for dolichos and yorimon, respectively.

Beltrán *et al*. (2006b) mention that the incorporation of dolichos beans as green manure, in doses of 6 t of dry matter, has a nitrogen contribution to the soil of 240 kg ha^{-1} at 160 days of establishment. García *et al*. (2010), mention that the decomposition of the compost and the subsequent release of N depend, to a greater extent, on the quality and quantity of the residues, the humidity and temperature of the soil, the mineralization and the pH.

The effects of AV in the soil were not statistically different in relation to the control treatment ($p< 0.05$). However, AV soils increased 5% bulk density, 11% organic matter and 17% inorganic nitrogen (Table 1).

Table 1. Effect of green manures on soil properties 30 days after incorporation.

Property of the soil	Soil before green fertilizers	Soil with dolichos	Soil with yorimón
Density (g cm^{-3})	1.26 ±0.02*	1.2 ±0.02	1.2 ±0.1
pH	8 ±0.15	8.1 ±0.25	8 ±0.08
EC (dS m^{-1})	1.1 ±0.17	1 ±0.05	1.1 ±0.15
Organic material (%)	0.7 ±0.1	0.78 ±0.05	0.76 ±0.03
Organic carbon (%)	0.41 ±0.06	0.45 ±0.03	0.44 ±0.03
Inorganic nitrogen (ppm)	28.22 ±1.07	32.5 ±0.09	33 ± 0.08

*= values are means ± standard error.

The effects of AV on soil properties, in a first incorporation cycle, have been reported by various authors. Beltrán *et al*. (2006b), found that, in plots with AV incorporation, organic matter increased only 0.17%. The gradual changes in soil by AV can be explained by the close C/N ratio of AV, which in this trial had an average of 23.

This value indicates that the materials can decompose rapidly in the soil, be mineralized (which would provide nutrients in the subsequent cultivation) and provide a labile carbon (which is an energy source for soil microorganisms, the vast majority beneficial for agriculture) (Prager *et al*., 2012). Johnston *et al*. (2009), mention that the increase in organic matter, during the transition period in which AV contributions are made, occurs slowly and takes several years.

Therefore, it is possible that the significant effects on soil productivity are long-term (Brechelt, 2004; García *et al*., 2010). Other studies have reported significant changes in organic matter from the first year, although the accumulation rate of organic matter is very slow (Herencia *et al*., 2008). Regarding the effects of AV on subsequent cultivation, the results are shown in Table 2. The AV significantly promoted a higher fresh and dry weight in chard plants ($p< 0.05$). The SPAD units were the same between the four treatments ($p< 0.05$) and the chlorophyll was lower in the plants to which neither fertilizer nor AV (T1) was applied.

For an AV to be considered as an effective source of nitrogen for horticultural crops, it must provide sufficient N according to the requirements of the crop, and the release of available N must be synchronized with the phenology of said crop (Hernández-Mendoza *et al*., 2007). The results of this study confirm that AV promoted better nitrogen nutrition than the control treatment with FQS (Table 2).

Table 2. Results of the study variables in the culture of chard (*Beta vulgaris* var. cicla L.).

Variable	T1 without fertilizer	T2 with FQS	T3 Dolichos	T4 Yorimon
PFPA (g)	27.12±5.47 c	42.2 ±6.34 b	61.22 ±3.81 a	59.02 ±8.45 a
PFR (g)	8.78 ±2.16 c	23.62 ±3.01b	31.7 ±3.99 a	27.62 ±3.08 b
PFT (g)	35.9 ± 6.59 c	65.82 ±6.68 b	92.92 ±6.7 a	83.64 ±12.39 a
PSPA (g)	3.94 ±0.95 c	5.62 ±0.73 b	7.94 ±1.56 a	7.54 ±1.04 a
PSR (g)	1.52 ±0.25 c	3.44 ±1 b	4.52 ±1.17 a	4.34 ±1.36 a
PST (g)	5.46 ±1.03 c	9.06 ±1.14 b	12.46 ±3.63 a	11.88 ±3.22 a
Cl (mm L^{-1})	1.16 ±0.19 b	1.52 ±0.09 ab	1.68 ±0.01 a	1.43 ±0.2 ab
SPAD	44.58 ±1.19 a	45.81 ±1.39 a	45.92 ±1.73 a	45.94 ±2.13 a
N (%)	2.33 ±0.11 a	2.49 ±0.09 a	2.52 ±0.1 a	2.48 ±0.11 a

Values are mean ±standard error. Means with the same letter in the same row are not significantly different according to Tukey's test ($p < 0.05$), (n= 6). Synthetic chemical fertilizer (FQS), fresh weight aerial part (PFPA), fresh weight root (PFR), total fresh weight (PFT), dry weight aerial part (PSPA), root dry weight (PSR), total dry weight (PST), chlorophyll (Cl), SPAD units (SPAD), nitrogen (N).

The possible causes of the higher yield of chard plants, due to the effect of AV, are the difference in organic matter of the soils due to the effect of AV (Table 1), which was 11% more than the soils without AV (T1 and T2), which could have positively affected other soil processes: cation exchange capacity, porous space, moisture retention, increased biological activity, etc. (Martínez *et al*., 2008).

In addition to the above, the higher inorganic nitrogen content of the soil from the treatment with yorimon beans (17%), even though this difference was not shown in the nitrogen content in the plant, could have contributed to this increase in yield. Singh *et al*. (2010), report that the yorimon bean showed a similar result to the application of 30 kg ha^{-1} of nitrogen fertilizer, with which it was possible to increase the production of mint dry matter by 23.4% and the production of essential oil by 25.2%.

In our study, the application of 1 g $plant^{-1}$ of T17 fertilizer is equivalent to 80 kg of N ha^{-1} (assuming a density of 80 thousand plants of chard ha^{-1}). The environmental implications of AV use are considered positive in the sense of not using FQS, or significantly reducing it, as was particularly demonstrated in this trial. Nitrogen fertilizers (such as ammonium nitrate and urea), in their production process, have a carbon footprint of 3.5 kg of CO_2 equivalent for each kg of N produced (which is the case of fertilizers manufactured in the European Union).

However, the carbon footprint may be greater, as in the case of fertilizers produced in China, where the carbon footprint may be greater than 10 kg of CO_2 equivalent (Bentrup, 2016). In this way, the dispensing of 80 kg of N ha^{-1} of nitrogen fertilizer (which was applied in the form of triple 17 in the control treatment for the production of chard), would be avoiding, at least, the emission into the atmosphere of 280 kg ha^{-1} of CO_2 equivalent (considering the carbon footprint of 3.5 kg CO_2 eq).

This is significant, if we consider that most crops of economic importance have nutrient requirements from 150 kg of N ha^{-1} in the case of wheat and up to more than 1000 kg of N ha^{-1} in greenhouse tomatoes, which would represent 540 and 3 600 kg ha^{-1} of CO_2 equivalent, respectively. The FAO (2014) points out that greenhouse gases have doubled in agricultural activities in Latin America in the last fifty years, so it is essential to promote the absorption and retention of these gases.

Another relevant implication is the incorporation of carbon into the soil (in this test *V. unguiculata* captured 918 kg ha^{-1} of C), where a part can become part of the stable carbon of the soil. Reicosky (2002) mentions that, globally, organic carbon losses from agricultural soils fluctuate between 30 and 50% of their initial level and that few practices are intentionally carried out for their conservation within conventional agriculture.

For his part, Lal (2004) mentions that 58% of the CO_2 emissions emitted into the atmosphere is attributed to the loss of organic matter caused by primary tillage and conventional cultivation techniques. Lal (2011), points out that agricultural soils can be important carbon sinks if proper use is carried out.

Conclusions

Dolicho legumes (*Lablab purpureos*) and yorimon (*Vigna unguiculata* L.) did not significantly improve the physical and chemical properties of the soil. However, they promoted a higher yield of chard in relation to the production with synthetic chemical fertilizer (40% in *L. purpureos* and 31% in *V. unguiculata*). The use of green manures contributes to supplement the use of synthetic chemical fertilizers in this vegetable and, in the case of nitrogenous ones, to decrease the carbon footprint generated by its manufacture.

Cited literature

Azcón, B. J. y Talon, M. 2008. Fundamentos de la fisiología vegetal. 2nd Edición. Ed. McGrawHill. 631 p.

Beltrán-Morales, F. A.; García-Hernández, J. L.; Zamora-Salgado, S.; Fenech-Larios, L. y Murillo-Marcial, R. 2006a. El uso de abonos verdes en la agricultura orgánica. 159-205 pp.

Beltrán-Morales, F. A.; García-Hernández, J. L.; Valdez-Cepeda, R. D.; Murillo-Amador, B.; Troyo-Diéguez, E.; Larrinaga, J. y Beltrán-Morales, L. F. 2006b. Efecto de sistemas de labranza e incorporación de abono verde (*Lablab purpureus* L.) sobre la respiración edáfica en un yermosol háplico. Interciencia. 31:226-230.

Beltrán-Morales, F. A.; García-Hernández, J. L.; Ruiz-Espinoza, F. H.; Fenech-Larios, L.; Murillo-Amador, B.; Palacios, A y Troyo-Diéguez, E. 2009. Nutrimental potential of red dolichos, brown dolichos and cowpea for green manure produced under three tillage Systems. Trop. Subtrop. Agroecosystems. 10:487-49.

Bentrup, F.; Hoxha, A. and Christiensen, B. 2016. Carbon footprint analysis of mineral fertilizer production in Europe and other world regions. *In*: Conference: the 10th International Conference on Life Cycle Assessment of Food (LCA Food 2016). At: University College Dublin (UCD), Dublin, Ireland.

Brechelt, A. 2004. El manejo ecológico del suelo. Fundación Agricultura y Medio Ambiente (FAMA). Red de acción en plaguicidas y sus alternativas para América Latina (RAP-AL) (Ed.). Santiago de Chile, Chile.

Da Silva, J. G. 2018. Time to embrace sustainable agriculture-FAO. https://www.standarmedia.co.ke/farmers/article/2001297618/time-to-embrace-sustainable-agriculture-fao.

FAO. 2014. Latinoamérica duplicó sus emisiones agrícolas de gases de efecto invernadero en los últimos 50 años. http://www.fao.org/ americas/noticias/ver/es/c/240450/.

García-Hernández, J. L.; Murillo-Amador, B.; Nieto-Garibay, A.; Fortis-Hernández, M.; Márquez-Hernández, C.; Castellanos-Pérez, E.; Quiñones-Vera, J. J. y Ávila-Serrano, N. Y. 2010. Avances en investigación y perspectivas del aprovechamiento de los abonos verdes en la agricultura. Terra Latinoam. 18(4):391-399.

Herencia, J.; Ruiz, J.; Melero, S.; García, P. and Maqueda, C. 2008. A short-term comparison of organic v. conventional agriculture in a silty loam soil using two organic amendments. The Journal of Agricultural Science. 146(6):677-687. Doi: 10.1017/S0021859608008071.

Hernández-Mendoza, T. M.; Salcedo-Pérez, E.; Arévalo-Galarza, G. y Galvis-Spinola, A. 2007. Evaluación de la concentración de lignina como indicador de la capacidad de aporte de nitrógeno de residuos orgánicos. Rev. Chapingo Ser. Cienc. Fores. Amb. 13:5-13.

Johnston, A. E.; Poulton, P. R. and Coleman, K. 2009. Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. Adv. Agron. 101:1-57.

Kumar, A.; Kumar, G. D. and Mukesh, K. 2013. Green manure crops: A boon for agricultural soil. International J. Agr. Environ. Biotech. 6:193-198.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. Science. 304(5677):1623-1627.

Lal, R. M 2011. Sequestering carbon in soils of agro-ecosystems. Food Policy. 36(1):S33-S39.

Martínez, E. H.; Fuentes, E. J. P. y Acevedo, E. 2008. Carbono orgánico y propiedades del suelo. J. Soil Sc. Plant Nutr. 8(1):68-96.

Mehari, A. F.; Mrema, A. and Weih, M. 2005. The suitability of *Acacia tortilis* as an alternative tree manure crop to *Leucaena leucocephala* in sub-Saharan Africa. Afr. J. Ecol. 43(2):162-165.

Prager-Mosquera, M.; Sanclemente-Reyes, O.; Sanchez-de-Prager, M.; Miller-Gallego, J. y Ángel-Sánchez, D. 2012. Abonos verdes: tecnología para el manejo agroecológico de los cultivos. Agroecología. 7:53-62.

Reicosky, D. C. 2002. Tillage and gas exchange. *In*: Lal, R. (Ed.). Encyclopedia of soil science. Boca Raton, FL: Taylor & Francis. 1333-1335 p.

Rodríguez, X. y Rodríguez X. 2011. Métodos de análisis de suelos y plantas: criterios de interpretación. 2ª. (Ed.). Trillas, México, DF. 239 p.

SAS Institute. 2002. SAS/STAT user's guide, version 5.0. 4th (Ed.) SAS Inst., Cary, NC.

Singh, M.; Singh, A.; Singh, S.; Tripathi, R. S.; Singh, A. K. and Patra, D. D. 2010. Cowpea (*Vigna unguiculata* L. Walp.) as a green manure to improve the productivity of a menthol mint (*Mentha arvensis* L.) intercropping system. Indust. Crops Prod. 31:289-293.

Tejada, M. and González, J. L. 2005. Beet vinasse applied to wheat under dryland conditions affects soil properties and yield. Eur. J. Agron. 23:336-347.