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# Agronomic performance and protein content of *Pereskia aculeata* Mill. using organic chicken manure fertilizer

**José Rodrigo de Araújo Guimarães*, Jordany Aparecida de Oliveira Gomes, Daniela Aparecida Teixeira, Filipe Pereira Giardini Bonfim, Regina Marta Evangelista**

**Departament of Horticulture, São Paulo State University "Júlio de Mesquita Filho", School of Agriculture (FCA), José Barbosa de Barros, nº 1.780, ZIP: 18610-307, Botucatu, SP, Brasil**

***Corresponding author: rodrigoagronomoal@yahoo.com.br**

**Abstract**

*Pereskia aculeata* Mill is a rustic and perennial plant, developing well in various types of soil, both in the shade and in the sun. It is a plant with high nutritional potential indicated as an alternative food. The current study aimed to evaluate yield and protein content of *Pereskia aculeata* Mill. under different levels of organic chicken manure fertilizer. A completely randomized design was conducted with five treatments and four replicates. Replications consisted of two experimental plots that contained 'ora-pro-nobis' plant. The treatments referred to different levels of organic chicken manure fertilizer, such as T1_control (soil + 0 kg $m^{-2}$ of chicken manure); T2_(soil + 2 kg $m^{-2}$ of chicken manure); T3_(soil + 4 kg $m^{-2}$ of chicken manure); T4_(soil + 6 kg $m^{-2}$of chicken manure); T5_(soil + 8 kg $m^{-2}$ of chicken manure). The following traits were evaluated: shoot length (cm); total leaf area; leaf number; root length (cm); root volume (mL); root dry mass (g); shoot fresh mass (g); shoot dry mass (g) and crude protein content (g $100g^{-1}$). Results indicated significant increase in yield and protein content of *Pereskia aculeata* (12.21 g $100g^{-1}$) by enhancing the levels of chicken manure. Therefore, a recommendation of 3.5 kg $m^{-2}$ of chicken manure can be made on soils of average fertility using in organic farming systems under similar conditions to this experiment.

**Keywords:** *Pereskia aculeata* Mill.; cactaceae; non-conventional plant; alternative food; edible plants.
**Abbreviations:** SL_shoot length; TLA_total leaf area; LN_leaf number; RL_root length; RV_root volume; RDM_root dry mass; SFM_ shoot fresh mass; SDM_shoot dry mass; CPC_crude protein content.

## Introduction

In traditional medicine, *Pereskia aculeata* is used in the treatment of anti-inflammatory and cutaneous lesions caused by burns. The leaves are popularly used as emollient; and fruits used as expectorants and to treat syphilis (Duarte and Hayashi, 2005; Rosa and Souza, 2003; Sator et al., 2010). Organic food production has increased over recent years, mainly due to population demand for more sustainable alternatives and higher quality products; making it necessary to search new techniques to improve farming systems, focusing on the quality and efficiency of nutritional management. Nitrogen is crucial for plant growth and is available to them in the soil and organic animal fertilizers (Lisboa et al., 2007). In addition to providing nutrients that are released via mineralization process, organic fertilizers are the source of energy for microorganisms that improves soil structure and moisture-holding capacity.

Chicken manure is an example of organic fertilizer that contains high nitrogen content. Also, such fertilizer could in time complete or partially replace chemical fertilizer, since ensuring proper management (Blum et al., 2003). Besides, they have a regulatory effect on soil temperature; retarding phosphorus fixation; increasing cation exchange capacity of soil; and reducing nutrients leaching such as potassium, calcium and magnesium (Malavolta et al., 2002). Plant nutrition is among the factors that interfere with the chemical composition of the plant, since deficiency or excess nutrients can interfere in the production of biomass and in the quantity of active principle (Mapeli, 2005). Synthetic fertilizers when available in the soil can be lost much by leaching, volatilization or fixation. Organic fertilizers are gradually released nutrients for longer in the soil to meet the needs of vegetables. It is necessary that the vegetal material is well decomposed, for a greater effect of aggregation in the soil. For example, humus is an intermediate product of decomposition differentiating itself from vegetable remains that still need to pass through this process, that is, so that the plant remains reach the humus state must pass through the composting (Primavesi, 1987). Fertilization is responsible for improvement of production and quality of most plant species. As in other crops, medicinal and aromatic plants also need good nutrition for good agricultural productivity. It is in this sense that organic fertilization has importance in providing nutrients to plants and improvement of the physical, chemical and biological qualities of the soil

(Corrêia, 2010). The interest in organic products stimulated the search for studies in the development of new techniques for this production system, in the search for better efficiency mainly in nutrient management. It is also important to highlight the the availability of nitrogen from sources of plant or animal organic fertilizers or even what is available in soil organic matter (Lisboa et al., 2007). One of the most widely available and accessible organic materials among producers in several regions of Brazil is manure, which can be cattle, horses, pigs and chicken. Besides being an excellent source of organic matter, the daily production per animal has an expressive value. For example, a cow with a mean weight of 453 kg produces about 23.5 kg of manure per day, whereas a chicken with approximately 1.6 kg produces about 100 g of manure plus urine. In relation to the use of organic compost and bovine manure, the bed of chicken is the richest in nutrients, standing out in it has high content of nitrogen total, N-ammonium and phosphorus, as well as Ca, S and B (Silva, 2010). In addition, they act to reduce soil phytopathogens and improve the production of some crops (Blum et al., 2003). Thus, the use of chicken manure stands out among other organic fertilizers from animal origin, as it allows better production compared to commercial organic composts.
By considering the protein potential of *Pereskia aculeata* and the need for alternatives to organic farming systems, the current study aimed to evaluate yield and protein content of *Pereskia aculeata* Mill. under different levels of organic chicken manure fertilizer.

**Results and discussion**

***Agronomic performance of plants of Pereskia aculeatta***

Results indicated that there was a significant increase in the yield of *Pereskia aculeata* up to a certain level of chicken manure. The Table 3 shows a significant influence of different levels of organic chicken manure fertilizer through analysis of variance ($p \leq 0.01$), which can be attributed to the greater availability of organic matter in soil, meeting plants' nutritional needs.
Through a literature search on the use of organic fertilizers, similar results were found in other species. By using cattle manure, Oliveira et al. (2009) obtained higher yield of maxixe (*Cucumis anguria*) fruit. Therefore, organic matter is beneficial to supply nutrients to plants at proper level. The use of organic fertilizer also enhanced yield of onions according to Vidigal et al. (2010).
By applying 3.5 kg $m^{-2}$ of chicken manure, maximum yield was obtained in leaf number (LN), shoot fresh mass (SFM) and leaf area (LA): 12.19; 130.77g and 2504.05 $cm^2$, respectively (Fig. 1). Such results indicated that levels of chicken manure greater than 3.5 kg $m^{-2}$ are not recommended. Carvalho (2013) verified that shoot fresh mass of *Pereskia aculeata* differed statistically in the absence and presence of substrate and manure, i.e. 16.06g (soil + manure) and 15.78g (sand + manure). Also, highest mean was obtained in stem fresh mass, i.e. 23.08g (sand + manure) and 19.48g (soil + manure); while mean weight among treatments did not differ statistically in the absence of cattle manure. In the same study, Carvalho (2013) obtained 21.66g (sand + cattle manure) in root fresh mass, followed by 14.40g (soil + manure), such results differed statistically from treatments without cattle manure, as they presented the lowest mean weight , that is, between 2.80g and 5.80g.
For shoot length (SL), root volume (RV), shoot dry mass (SDM) and root dry mass (RDM), the application of 3.0 kg $m^{-2}$ provided the maximum yields, i.e. 72.2cm; 48.7mL; 20.9g and 10.84g (Fig. 2); showing the ideal level of organic fertilizer; consequently, meeting plant's nutritional needs and better yield per square meter harvested.
Regarding to the yield, similar results were found in plants from the same family (Cactaceae). Santos (2000) observed an increase of 81% in the shoot dry mass production of *Opuntia fícus-indica* L. Miil. by using 10 t $ha^{-1}$ of tanned manure corral; however, the level of 20 t $ha^{-1}$ of chicken manure was 59% higher in shoot dry mass than control treatment in the current study. The larger the leaf area or the size of the cladodes, the more important they are to Cactaceae, since they will capture more light; consequently, obtaining great water reserves per unit leaf area; producing more carbohydrates, vital for plant growth; greater photosynthetic capacity per unit leaf area; and higher yields per square meter area (Ramos, 2012).
In studies carried out with arracacha (*Arracacia xanthorrhiza* Bancroft), also an unconventional vegetable, it was recommended to use 10 t $ha^{-1}$ of semi-composted chicken manure to obtain higher yield and high-bromatological components, such as proteins, lipids, carbohydrates, fibres and total caloric value (Graciano et al., 2006).
Regarding to root length (RL), control treatment presented 42.42 cm (Fig. 3). However, root length was smaller (16.00cm) than other treatments at 8 kg $m^{-2}$ of chicken manure, this fact is due to the great availability of nutrients in the environment; consequently, roots do not grow in search of nutrients; besides that, over-fertilization results in root toxicity. Such results corroborates the one found by Gassi et al.(2009), who evaluated burdock (*Arctium lappa* L., Asteraceae) under different levels of phosphorus (4.3, 25.8, 43.0, 60.2 and 81.7 kg $ha^{-1}$) and organic chicken manure fertilizer (1; 6; 10; 14 and 19 t $ha^{-1}$); found that higher levels of chicken manure, regardless of phosphorus doses, increased in dry mass production of burdock leaves. However, the highest yields of root dry mass were obtained only with the highest levels of phosphorus and intermediate levels of chicken manure.

***Analysis of protein content***

Through analysis, it was observed that protein content increased at higher levels of chicken manure, obtaining a linear behaviour. The application of 8 kg $m^{-2}$ of organic compost in *Pereskia aculeata* contributed to produce 12.21g of protein per $100g^{-1}$ of dry mass. In control treatment (i.e. 0 kg $m^{-2}$), it was verified 6.34g of protein per $100g^{-1}$ of dry mass (Fig. 3).
Results indicated that the higher the dose of chicken manure, the higher the protein content in the leaves of 'ora-pro-nobis'. Moreover, there was enough available nitrogen in the soil; consequently, high protein content in leaves by increasing the levels of organic chicken manure fertilizer, which is an essential source of nitrogen.

**Table 1**. Chemical soil analysis used in the experiment.

| SAMPLE | pH | O.M. | $P_{resin}$ | $Al^{3+}$ | H+Al | K | Ca | Mg | SB | CEC | V% | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Int. | $CaCl_2$ | $g/dm^3$ | $mg/dm^3$ | ------------- | ------------- | $mmol_c/dm^3$ | ------------- | ------------- | | | | $mg/dm^3$ |
| 1 | 4.2 | 12 | 9 | 6 | 40 | 1.2 | 7 | 2 | 10 | 50 | 20 | 12 |

Source: Soil Laboratory FCA / UESP, Botucatu / SP. 2014.
OM - organic matter; P - phosphorus; Al 3+ aluminium; H + Al - potential acidity; K-potassium; Mg-magnesium; SB - sum of bases; CEC - cation exchange capacity; V - base saturation; S- sulphur.

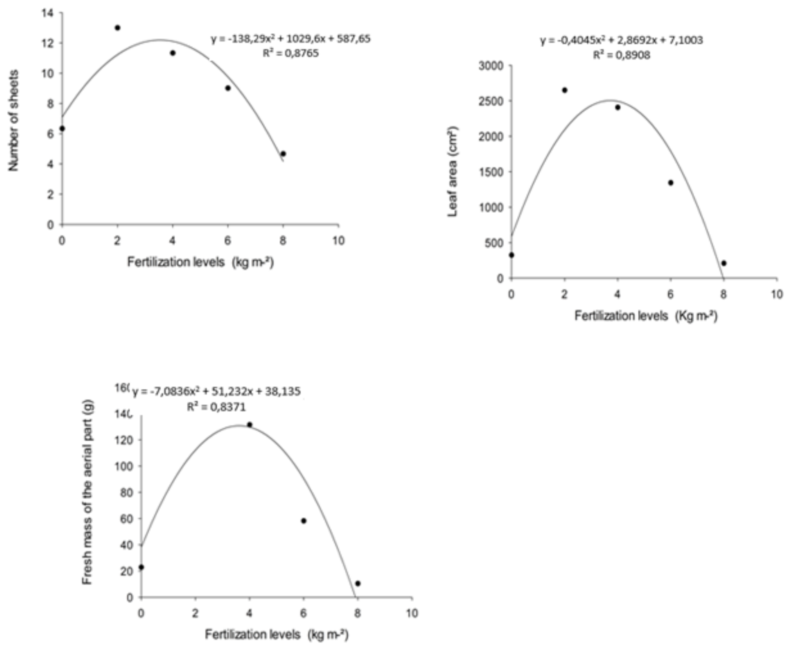


**Fig 1.** Leaf number, shoot fresh mass (g) and leaf area ($cm^2$) of ora-pro-nobis (*Pereskia aculeata* Mill.) under different levels of chicken manure fertilizer

**Table 2.** Chemical analysis of organic chicken manure fertilizer used in the experiment.

| SAMPLE | N | $P_2O_5$ | $K_2O$ | Ca | Mg | S | M | OM | C | pH | C/N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ------------ | ------------ | ------------ | % natural | ------------ | ------------ | | |
| | 4.1 | 5 | 4 | 3 | 1 | 0.7 | 10 | 61 | 34 | 7 | 8 |

Source: Soil Laboratory FCA / UESP, Botucatu / SP. 2014.
N – nitrogen; PsO5 – phosphorus; K2O – potassium; Ca – calcium; Mg – magnesium; S-sulphur; M – moisture; OM – organic matter; C – carbon; C/N – carbon nitrogen ratio.

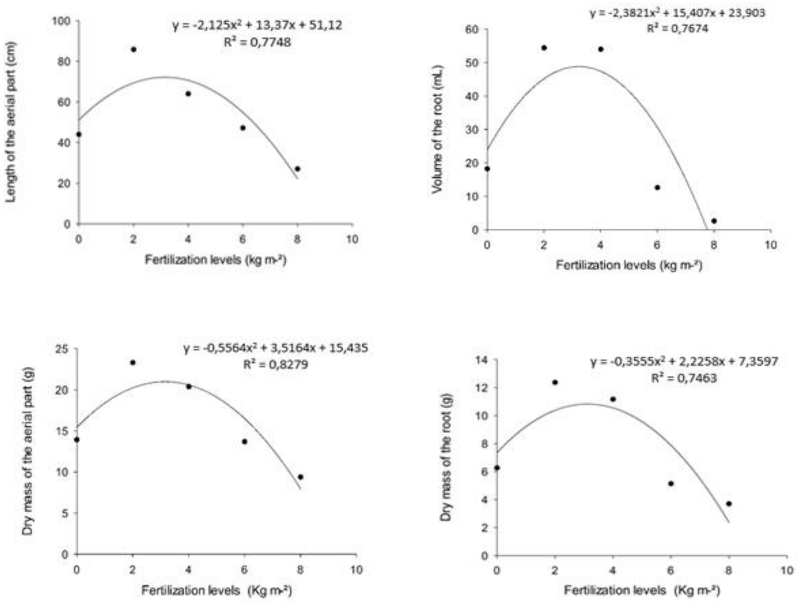


**Fig 2.** Shoot length (cm), root volume (mL), shoot dry mass (g) and root dry mass (g) of 'ora-pro-nobis' (*Pereskia aculeata* Mill.) under different levels of chicken manure fertilizer.

**Table 3.** Adjusted equations and coefficients of determinations for leaf number (LN), shoot length (SL), shoot fresh mass (SFM), leaf area (LA), root volume (RV), leaf length (LL), shoot dry mass (SDM), root dry mass (RDM) and crude protein (CP) under different levels of chicken manure.

| Adjusted equations | Coefficients of determinations |
|---|---|
| LF= $-0.4045x^2+2.8692x+7.1003$** | 0.89 |
| SL= $-2.125x^2 + 13.37x + 51.12$** | 0.77 |
| SFM= $-7.0836x^2$+51,232x+38,135** | 0.84 |
| LA= $-138.29x^2+1029.6x+587.65$** | 0.88 |
| RV=$-2.3821x^2+15.407x+23.903$** | 0.77 |
| LL= $-0.2071x^2-1.0429x+41.103$** | 0.77 |
| SDM=$-0.5564x^2+3.5164x+15.435$** | 0.83 |
| RDM=$-0.3555x^2+2.2258x+7.3597$** | 0.75 |
| CP= $0.1644x + 1.0612$** | 0.93 |

** Significant by F test at 1% probability.

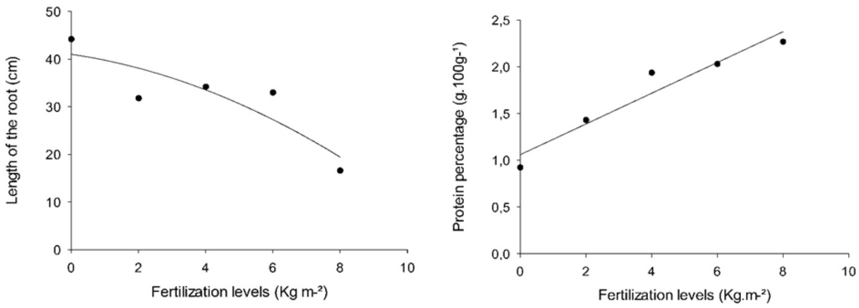


**Fig 3.** Root length and protein content in 'ora-pro-nobis' (*Pereskia aculeata* Mill.) under different levels of chicken manure fertilizer.

However, the protein content (Fig. 3) per dry mass of *Pereskia aculeata* was lower than those identified by Almeida et al (2014), who obtained 28.99g of protein per $100g^{-1}$ of dry mass; and by Takeiti et al. (2009), who verified 28.4g of protein per $100g^{-1}$ of dry mass. Although, the result of the current study was like that one observed by Queiroz (2012), i.e. 11.9 g of protein per $100g^{-1}$ of dry mass.

The current study corroborates with a study performed by Guimarães et al. (2013) on protein content of *Pereskia aculeata* under different levels of nitrogen by the Bradford and Kjeldahl methods. The total protein increased up to the level of 100 kg N $ha^{-1}$ by Kjeldahl method. Soluble protein increased in the doses of N between 50 and 200 kg $ha^{-1}$ by Bradford method. Furthermore, it was verified that total protein increased significantly from 0 to 50 kg N $ha^{-1}$ by Kjeldahl method; while variation was lower above 100 kg N $ha^{-1}$. These results show that there is an optimum fertilizer dose to obtain excellent levels of both yield and nutrients in plants.

The increase in protein content occurs in proportion to the amount of nitrogen absorbed, since it is reduced to the ammoniacal form and used in the synthesis of glutamic acid (Andrade et al., 2003).

*Pereskia aculeata* is considered an unconventional vegetable, which is source of protein, and may vary according to climatic factors, fertilization methods and growing period. In general, analysis indicated that organic chicken manure fertilizer provided higher biomass yields and protein yield.

**Materials and methods**

***Study area***

The current experiment was conducted in the experimental area of the Horticulture Department, School of Agriculture, Botucatu, UNESP (22°50'S, 48°26'W; 817m altitude). The climate of the region is hot and humid (Cwa), i.e. dry winter, hot and rainy summer, according to Köppen classification) and annual average temperature of 19,3 ºC. The mean annual rainfall is 1501.4 mm and 71% relative humidity (Cunha et al., 1999).

The soil of the experiment was classified as dystrophic Red Latosol (Embrapa, 2006) and collected in the ravine area of the orchard at the Department of Horticulture-UNESP in Botucatu.

***Experimental design***

The experiment was conducted from July to November 2014. Before its installation, soil samples were collected for the determination of chemical composition (Table 1) and the chemical analysis of the organic chicken manure fertilizer is shown in Table 2. The amount of organic fertilizer applied was calculated in fresh weight and contained 10% moisture.

The seedlings of *Pereskia aculeata* were obtained through vegetative propagation, taking live cuttings from a matrix in the medicinal plants orchard of Centroflora Company. Thus, apical cuttings were prepared with 15cm in length. The *Pereskia aculeata* cuttings have grown a period of 60 days, in polyethylene bibs covered by monofilament shade cloth 50% at the plant nursery. Afterwards, they were transplanted into 5L pots, in which contained the soil and treatments.

Harvest was performed at 120 days. Afterwards, plants were divided into root, stem and leaf. For shoot and root dry mass production, they were separated and packed in paper bags and dried in a forced-air-circulation oven at $65^oC$ until constant mass.

The experiment was performed in a complete randomized block design, with five treatments and four replicates. The treatments were composed of plants *Pereskia aculaeta*. Replicates consisted of two plots and each plot had *Pereskia* *aculeata*, being one for yield analysis and the other for protein analysis; therefore, a total of 20 plants per analysis. The treatments were obtained under different levels of organic chicken manure fertilizer, as follows: T1_control (soil + 0 kg $m^{-2}$ of chicken manure); T2 (soil + 2 kg $m^{-2}$ of chicken manure); T3_(soil + 4 kg $m^{-2}$ of chicken manure); T4_(soil + 6 kg $m^{-2}$ of chicken manure); T5_(soil + 8 kg $m^{-2}$ of chicken manure). Sprinkler irrigation was used.

### *Characteristics evaluated*

The following characteristics were evaluated: plant length (cm) were determined using a tape measure from basal end to the apex of the plant; total leaf area ($cm^2$) were determined using an area meter LI-3.100, LICOR; leaf number; root length (cm) were determined using tape measure; root volume (mL) were determined by volume change using 100 mL beaker; root dry mass (g), shoot dry mass (g) and shoot fresh mass (g) were weighed in digital scale; and leaf weight ratio were calculated according to Benincasa (2003).
Crude protein (%) was performed in dry mass of *Pereskia aculeata*. Sulfuric digestion and determination of the total N were carried out by steam stripping. The factor 6.25 was used to convert the nitrogen content into crude protein (CP), i.e. total nitrogen x 6.25, according to AOAC (2005).

### *Statistical analysis*

Data were submitted to analysis of variance through F test and polynomial regression. Means were compared by Tukey test 5% of probability, using the Assistat 7.7 Beta program.

## Conclusion

Results indicated that *Pereskia aculeata* presented excellent yield and high protein value by applying organic chicken manure fertilizer, being an alternative to organic food production. Yield and protein content were influenced by increasing the level of organic fertilizer. The level of 3.5 kg $m^{-2}$ of organic chicken manure fertilizer is sufficient for high quality and yield of *Pereskia aculeata*. Therefore, a recommendation may be done in organic farming systems under similar conditions.

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