International Journal of Phytocosmetics and Natural Ingredients 2015;2:11

doi 10.15171/ijpni.2015.11

Original Article

Effect of Organic Fertilization on Biomass Production and Bioactive Compounds in *Passiflora incarnata* L.

Marcio Campos[1], Maíra Uliana[2], Daniel V. Montero[1*], Giuseppina Lima[2], Lin Chau Ming[1]

[1]Laboratory of Medicinal Plants and Ethnobotany, Department of Horticulture, Faculty of Agronomic Science, State University of São Paulo (UNESP), Botucatú, Brazil
[2]Laboratory of Chemistry and Biochemistry, Institute of Bioscience, State University of São Paulo (UNESP), Botucatú, Brazil

Correspondence to
Daniel V. Montero
Email:
dvillamontero@gmail.com

Received 30 July 2015
Accepted 27 August 2015
ePublished 2 September 2015

Abstract

The objective of this study was to evaluate the growth of biomass of Passiflora incarnata L. in response to different levels of organic fertilizer and compare the content of total polyphenols, total flavonoids and TEAC in leaves and stems. The experiment was conducted with family farmers in Botucatu (Brazil), between October 2013 and February 2014. Seedlings were grown in a greenhouse and then transplanted to the field with spacing 0.30 m x 0.60 m without staking and irrigated by sprinkler. The experimental design was a randomized block design with 5 treatments and four blocks. The organic fertilization and liming were made in the row with rabbit manure, chicken manure, organic waste and dolomitic limestone 30 days before transplanting. Leaves and stems of P. incarnata were dried in oven with air circulation at 40°C/48 h. The analysis of total flavonoids and polyphenols were performed according to the spectrophotometric method using the Folin-Ciocalteau reactive. Results showed that organic fertilization had a positive effect on the plant´s development, incising the biomass production (dry matter). However, the production of bioactive compounds such as polyphenols, total flavonoids and the antioxidant capacity were not influenced by the effect of organic fertilization.

Keywords: Medicinal plant, Passion fruit, Phytochemistry, Flavonoids, Polyphenols, Antioxidant

Introduction

Passiflora incarnata L. is a fast-growing perennial vine native to Mexico and the southern United States. Preserved seeds found in burial chambers 3500-800 b.c confirmed its importance and use among people since pre-columbium time. Europeans named the plant "*flos passionis*" in reference to the passion of Christ, which they saw metaphorically represented in the floral anatomy. Ethno-pharmacological survey of *P. incarnata* reported principally a potent therapeutic action on the central nervous system. Sedative, antispasmodic and anxiolytic properties also have been described.[1,2]

Clinical applications of *P. incarnate* are known worldwide and the species is found in the official British Herbal Pharmacopoeia since 1983, Homoeopathic Pharmacopoeia of India (1974), United States Homoeopathic Pharmacopoeia (1981), Pharmacopoeia Helvetica (1987) and the Pharmacopoeias of Egypt, France, Germany and Switzerland[3] coming to be an efficient alternative to treat several health disorders.[4]

In Brazil, an extraordinary expansion of the area cultivated with *P. incarnate* has been observed in recent years due to the emergence of a growing interest for herbal medicines in national and international markets that are transforming *P. incarnata* in a promising medicinal plant.

Main bioactive substances identified from *P. incarnata* include polyphenols, flavonoids, saponines, alkaloids, carotenoids, anthocyanins, sugars and *C*-glucoside.[5] It´s believed that commonly found flavonoids like quercetin, vitexin and rutin are critical factors for maintaining optimum health and well being conditions because they interfere within several processes like molecular signals in symbiotic systems mechanisms and as a defense against free radical damage.[6]

The antioxidant capacity of *Passiflora* has been mentioned to be related with the content of polyphones and flavonoids by several authors.[7-15] Extract of *Passiflora* seeds,[8] leaves,[9-11,13] fruits[12,14] and rinds[15] were found to be effective against the antioxidant test models (free radical reducing power methods) exhibiting strong potential antioxidant activity. Infusions of *Passiflora* also showed different degrees of suitability as acceptable functional beverage[10] or as a possible source of natural flavonoids.[15] However, despite its nutritional importance very little is known regarding the biochemical and physiological performance of *P. incarnata* when organic fertilization is applied at the

Please cite this paper as: Montero DV, Campos M, Uliana M, Lima G, Ming LC. Effect of organic fertilization on biomass production and bioactive compounds in Passiflora incarnata L. *International Journal of Phytocosmetics and Natural Ingredients*. 2015;2:11. doi:10.15171/ijpni.2015.11.

crop. Thus, the purpose of this study was to compare the effect of organic fertilization on the content of bioactive compounds found in leaves and stem of *P. incarnate*; to better understand the performance of the crop at different levels of organic fertilization.

Materials and Methods

Seeds of *P. incarnata* were immersed in water for 24 hours and then planted 0.5 cm deep in trays with 200 cells containing commercial substrate Plantmax®, in September 2013 at the greenhouse of medicinal plants, Department of Horticulture, State University of Sao Paulo (UNESP-Botucatu). The emergence of the seedlings took places seven days later and 45 days after, seedlings were transplanted to the field. The spacing used between plants and between rows was 0.3 m × 0.6 m, totaling 8 m^2 of total area, including borders. To compare the effect of organic fertilization we conducted a completely randomized block experiment with 5 treatments and 4 repetitions totaling twenty eight randomized blocks in an area of 300 m^2. Each block had 12 useful plants and 24 surround plants.

Statistical Analysis

For the statistical analyses we used 10 randomized working plants from each experimental block. The treatments were as follow:
Treatment 1: 0 ton of organic fertilizer;
Treatment 2: 1.5 $ton.ha^{-1}$ of dolomitic limestone;
Treatment 3: 16.5 $ton.ha^{-1}$ of organic fertilizer (5 $ton.ha^{-1}$ of plant residue, 10 $ton.ha^{-1}$ of rabbit manure, 1.5 $ton.ha^{-1}$ and dolomitic limestone);
Treatment 4: 26.5 $ton.ha^{-1}$ of organic fertilizer (5 $ton.ha^{-1}$ of plant residue, 10 $ton.ha^{-1}$ of rabbit manure, 10 $ton.ha^{-1}$ of manure and chicken 1.5 $ton.ha^{-1}$ dolomite limestone). Herbaria samples were deposited at the faculty's herbarium (Figure 1).

Dry Matter

The plant materials were dried in forced air oven at 47°C for 48 hours. After the drying process leaves and stems were separated, powdered and weighed in Analytical Balance.

Total Polyphenols

The total phenols analysis was conducted according to the spectrophotometric method.[16] Samples were weighed and placed into centrifuge tubes containing 50% acetone. They were then brought into ultrasonic bath for 20 minutes and then centrifuged at 6000 xg for 10 minutes and the supernatant was collected. The precipitate was reextracted and the supernatants were combined. Aliquots of the combined supernatants (0.1 ml) were transferred to test tubes, along with 0.5 ml of Folin-Ciocalteu reagent and 2.5 mL saturated Na_2CO_3 solution. After 1 hour of reaction (complete carbonate precipitation) the absorbance was read at 725 nm.

Figure 1. *Passiflora incarnate* L.

Total Flavonoid

The extraction and analysis of total flavonoid contents were carried out according to the spectrophotometric method. Samples were taken to ultrasonic bath for 30 minutes and added aluminum chloride 5% and centrifuged for 20 minutes at 10000 xg. Then, the samples were filtered and the absorbance was read at 425 nm.

Antioxidant Capacity (TEAC)

To determine the antioxidant capacity we modified the methodology proposed by Brand-Williams et al[16] as follows: 0.300 g of each dried sample were diluted in 10 ml of ethanol. The samples solutions were centrifuged at 2000xg for 10 minutes at 5°C. 0.500 uL aliquot of the supernatant was combined with 3 ml of ethanol for analysis added 300 uL TEAC 1 in 2×10^{-4} g mL-test tubes. After homogenization, the samples were stored in the dark for 60 minutes. A negative control was performed with 0.3 mM TEAC ethanol to observe the decay of the radical donor against antioxidants. A calibration curve was prepared with 20, 40, 80, 120 and 160 micromol Trolox and the results were expressed as mM of Trolox equivalent/mg/g^{-1} of the sample (TEAC).
The results of dry matter, polyphenol, flavonoid and TEAC were subjected to statistical analysis of variance and means were compared by Tukey test ($P \leq .05$) using statistical software RStudio.

Results and Discussion

Biomass productivity (leaves and stems) of *Passiflora incarnata* was influenced by the effect of organic fertilization, and this could be very interesting for local development since farmers could be encourage adopting a more sustainable agriculture, replacing the use of synthetic Agrochemicals and petroleum derivate fertilizers. According to Table 1, leaves and stems showed the highest values of dry matter in the treatment 3, followed by treatments 5 and 4, respectively. The application of organic fertilizers favors the conditions for vegetative growth, thus more value is added to the crop. In practical terms, the abundant foliage would produce better yield and this represent more income for square meter in a rural property.

Flavonoids and polyphones were more abundant in the leaves of *P. incarnata* (Table 2), the main source of these substances in the plant.[1-5] The antioxidant capacity of *Passiflora* leaves has been mentioned to be related with the content of polyphones in amount around 342.80-382.00 mg gallic acid equivalent L^{-1}.[7,10] However, analyses comparing the experimental treatments showed no significant difference among the flavonoid contend. The production of polyphenols and antioxidant capacity also showed no significant difference among treatments and this means that the effect of organic fertilization did not increase the production of bioactive compounds in *P. incarnata*. However all the treatments with organic fertilization maintained the average standard require for commercialization with more than 0.2% concentration of bioactive compounds (Table 2).

Conclusions

Organic fertilization at different levels has a significant effect on the biomass production in *Passiflora incarnata*, which represents more productivity and better yields. However, the total amount of flavonoids, polyphenols and antioxidant activity were not influenced by the organic fertilization which maintained the average commercial quality of the product in all the experimental treatments. More studies should be performed in order to determine if the production of bioactive compound could be more influenced by genotype rather than nutritional factors in *P. incarnata*.

Acknowledgments

Thanks to Faculty of Agronomic Science and the Institute of Biosciences, State University of São Paulo (Unesp-Botu-

Table 1. Results of the Dry Mass Matter in Leaves and Stems

	Leaves	Stems	Total (Leaves and Stems)
	Dry Mass (g)	Dry Mass (g)	Dry Mass (g)
T1	45.65 bc	12.43 c	58.07 bc
T2	54.00 abc	16.70 bc	70.70 abc
T3	81.20 a	27.60 a	108.80 a
T4	74.88 ab	21.55 ab	96.40 ab
T5	76.63 ab	26.85 a	103.50 a
T6	32.45 c	10.42 c	42.88 c
T7	25.83 c	9.90 c	35.73 c
CV (%)	25.94	21.74	24.07

Means followed by the same letter are not statistically different by Tukey test ($P \leq .05$). T1: control; T2: lime; T3: lime + organic matter; T4: lime + organic matter + manure; T5: lime + organic matter + manure + Yoorin; T6: organic commercial production 1; T7: organic commercial production 2.

Table 2. Results of the Polyphenols, Flavonoids and TEAC in Leaves and Stem

	Leaves			Stems		
	Polyphenols (g gallic acid eq. 100 g^{-1})	Flavonoids (mg quercetin eq. 100 g^{-1})	TEAC (μM TROLOX eq. 100 g^{-1})	Polyphenols (g eq. gallic acid 100 g^{-1})	Flavonoids (mg quercetin eq. 100 g^{-1})	TEAC (μM eq. TROLOX 100 g^{-1})
T1	2.30 a	272.1 ab	416.10 a	0.90 a	50.70 a	146.20 a
T2	2.27 a	299.1 ab	318.20 a	0.72 a	46.71 a	100.80 a
T3	2.32 a	233.54b	341.90 a	0.80 a	38.19 a	155.80 a
T4	2.13 a	274.1 ab	278.00 a	0.73 a	37.25 a	104.00 a
T5	2.33 a	301.8 ab	364.10 a	0.85 a	44.01 a	169.90 a
T6	2.47 a	375.6 a	436.00 a	0.91 a	47.60 a	134.60 a
T7	2.46 a	331.5 ab	436.50 a	0.80 a	53.35 a	155.60 a
CV (%)	8.00	16.62	21.43	15.22	24.24	33.47

Means followed by the same letter are not statistically different by Tukey test ($P \leq .05$). T1: control; T2: lime; T3: lime + organic matter; T4: lime + organic matter + manure; T5: lime + organic matter + manure + Yoorin; T6: organic commercial production 1; T7: organic commercial production 2.

catu) and the APL project for the financial assistance. Also, would like to thanks the PEC-PG/Capes (Brazil).

References

1. Speroni E, Minghetti A. Neuropharmacological activity of extracts from Passiflora incarnata. *Planta Med.* 1988;54(6):488-491. doi:10.1055/s-2006-962525.
2. Dhawan K, Dhawan S, Sharma A. Passiflora: a review update. *J Ethnopharmacol.* 2004;94(1):1-23. doi:10.1016/j.jep.2004.02.023.
3. Dhawan K, Kumar S, Sharma A. Anti-anxiety studies on extracts of Passiflora incarnata Linneaus. *J Ethnopharmacol.* 2001;78(2-3):165-170. doi:10.1016/s0378-8741(01)00339-7.
4. Müller SD, Vasconcelos SB, Coelho M, Biavatti MW. LC and UV determination of flavonoids from Passiflora alata medicinal extracts and leaves. *J Pharm Biomed Anal.* 2005;37(2):399-403. doi:10.1016/j.jpba.2004.10.047.
5. Patel SS, Soni H, Mishra K, Singhai AK. Recent updates on the genus Passiflora: a review. *Int J Res Phytochem Pharmacol.* 2011;1(1):1-16.
6. Lynn DG, Chang M. Phenolic signals in cohabitation: implications for plant development. *Annual Review of Plant Physiology and Plant Molecular Biology.* 1990;41:497-526.
7. Devi Ramaiya S, Bujang JS, Zakaria MH, King WS, Shaffiq Sahrir MA. Sugars, ascorbic acid, total phenolic content and total antioxidant activity in passion fruit (Passiflora) cultivars. *J Sci Food Agric.* 2013;93(5):1198-1205. doi:10.1002/jsfa.5876
8. Lourith N, Kanlayavattanakul M. Antioxidant activities and phenolics of Passiflora edulis seed recovered from juice production residue. *J Oleo Sci.* 2013;62(4):235-240. doi:10.5650/jos.62.235.
9. Montefusco-Pereira CV, de Carvalho MJ, de Araújo Boleti AP, Teixeira LS, Matos HR, Lima ES. Antioxidant, anti-inflammatory, and hypoglycemic effects of the leaf extract from Passiflora nitida Kunth. *Appl Biochem Biotechnol.* 2013;170(6):1367-1378. doi:10.1007/s12010-013-0271-6.
10. Pineli Lde L, Rodrigues Jda S, Costa AM, de Lima HC, Chiarello MD, Melo L. Antioxidants and sensory properties of the infusions of wild passiflora from Brazilian savannah: potential as functional beverages. *J Sci Food Agric.* 2015 May;95(7):1500-6. doi:10.1002/jsfa.6852
11. Sathish R, Sahu A, Natarajan K. Antiulcer and antioxidant activity of ethanolic extract of Passiflora foetida L. *Indian J Pharmacol.* 2011;43(3):336-339.
12. Simirgiotis MJ, Schmeda-Hirschmann G, Bórquez J, Kennelly EJ. The Passiflora tripartita (Banana Passion) fruit: a source of bioactive flavonoid C-glycosides isolated by HSCCC and characterized by HPLC–DAD–ESI/MS/MS. *Molecules.* 2013;18(2):1672-1692.
13. Sunitha M, Devaki K. Antioxidant Activity of Passiflora edulis Sims Leaves. *Indian J Pharm Sci.* 2009;71(3):310-311. doi:10.4103/0250-474x.56038
14. Talcott ST, Percival SS, Pittet-Moore J, Celoria C. Phytochemical composition and antioxidant stability of fortified yellow passion fruit (Passiflora edulis). *J Agric Food Chem.* 2003;51(4):935-941. doi:10.1021/jf020769q
15. Zeraik ML, Serteyn D, Deby-Dupont G, Wauters JN, Tits M, Yariwake JH, et al. Evaluation of the antioxidant activity of passion fruit (Passiflora edulis and Passiflora alata) extracts on stimulated neutrophils and myeloperoxidase activity assays. *Food Chem.* 2011;128(2):259-265. doi:10.1016/j.foodchem.2011.03.001
16. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology.* 1995;28(1):25-30.