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CAN PUMPKIN BE USED AS A MELLIFEROUS PLANT?[1]

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ABSTRACT - The aim of this paper was to study nectar in male and female flowers of pumpkin (*Cucurbita moschata* Duch) accessions, aiming at evaluating the species potential as a melliferous plant. Two experiments were conducted in an experimental area of the State University of Bahia, at the Department of Technology and Social Sciences, in Juazeiro-BA, in the period from January to December 2017. Nectar volume, soluble solids, and pH were evaluated in the two floral types using 32 accessions and Tetsukabuto hybrid, which was used as check. Using a sample of accessions, the types of sugar present in the nectar were studied. Male flowers did not have significant differences in nectar traits; however, there was significant variation in female flowers in the first experiment and in both floral types in the second experiment. In male flowers in both experiments, the maximum volume of nectar was 310 µL and in female flowers it was 803 µL. The sugar concentration was around 24 °Brix. Sucrose was similar in both floral types and was higher than glucose and fructose contents, showing mean values of 1,306.13, 357.14 and 351.88 g.mL^{-1}, respectively. Obtaining progeny with contrasting characters in the accessions will be important for species improvement. These results indicate that pumpkin might be considered as a melliferous plant with great potential.

Keywords: *Cucurbita moschata. Apis mellifera*. Nectar. Germplasm. Variability.

A ABÓBORA PODE SER UTILIZADA COMO PLANTA MELÍFERA?

RESUMO - O trabalho teve como objetivo estudar o néctar em flores masculinas e femininas de acessos de abóbora (*Cucurbita moschata* Duch), visando avaliar o potencial da espécie como planta melífera. Foram realizados dois experimentos, no campo experimental da Universidade do Estado da Bahia, no Departamento de Tecnologia e Ciências Sociais, em Juazeiro-BA, no período de janeiro a dezembro de 2017. Foram avaliados volume, sólidos solúveis e pH do néctar em 32 acessos e no híbrido Tetsukabuto, como testemunha, nos dois tipos florais. Em uma amostra de acessos, foram estudados os tipos de açúcares presentes no néctar. As flores masculinas não apresentaram diferença significativa para as características do néctar, porém, as flores femininas, no primeiro experimento, e os dois tipos florais no segundo experimento apresentaram variação significativa. Nas flores masculinas dos dois experimentos, o máximo de volume de néctar foi de 310 µL e nas femininas foi de 803 µL. A concentração de açúcar ficou ao redor de 24 °Brix. A sacarose se mostrou semelhante nos dois tipos florais e foi superior ao conteúdo de glicose e de frutose, apresentando valores médios de 1.306,13, 357,14 e 351,88 g.mL^{-1}, respectivamente. A obtenção de progênies com caracteres contrastantes dos acessos será importante para o melhoramento da espécie. Esses resultados indicam que a abóbora pode ser considerada como uma planta com um grande potencial melífero.

Palavras-chave: *Cucurbita moschata. Apis mellifera.* Néctar. Germoplasma. Variabilidade.

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INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch.) belongs to the family Cucurbitaceae and is of great importance in human and animal diet, mostly in family farming in the Northeast of Brazil (FERREIRA et al., 2016), considering its use in the generation of direct and indirect jobs and for being an important source of nutrients, antioxidants, vitamins, fibers and minerals (DARRUDI et al., 2018).

Species of the genus Cucurbita are monoecious, having male and female flowers (MÉLO et al., 2010); both flowers have nectar. However, nectaries are different between floral types (VIDAL et al., 2010). In the male flower, the nectary is at the base of the stem, accessible by three openings, and in the female flower it is ring shaped and circulates the base of the stem (NEPI; PACINI, 1993). According to Vidal et al. (2010), the shape of the nectary is a facilitator for bees to access nectar and perform the collection. In the case of pumpkins, considering that there is variability in many plant characters, it is reasonable to assume that there is also variation in floral nectar traits, including the shape of the nectary.

It is important to note that the Brazilian Northeast has a wide genetic variability in pumpkin fruits in family farming (FERREIRA et al., 2016) and the plant has a good adaptation to the edaphoclimatic conditions of the region (BLANK et al., 2013), and can be cultivated throughout the year, mainly in irrigated crops, thus favoring the use of the species potentially as an auxiliary plant in the production of honey. However, there are very few reports of pumpkin as honey producer. Nevertheless, its flowers, either male or female, are large and imposing, which is a factor of great attractiveness for honey bees (PINTO et al., 2016), and considering flower size, these flowers probably have high amounts of nectar and pollen, and nectar is the major ingredient in honey production and the main source of carbohydrates for plants. Two studies carried out by Nepi, Guarnieri and Pacini (2001) and Ashworth and Galetto (2002) reported quantitative and qualitative aspects of nectar in germplasm of *C. pepo* L. and *C. maxima* ssp. *andreana* (Naudin), respectively, non-cultivated botanical varieties, in which female flowers were observed to produce a more concentrated nectar and in higher amounts than male flowers. Honey bees have preference for more concentrated nectar because its dehydration takes less time and requires less work, so it is easier to change it into honey (ALMEIDA et al., 2003).

According to Vidal (2017), the exploitation of honey bees is still considered recent in the Brazilian Northeast, and this practice might be considered an increase in income for small farmers, as its implementation and maintenance is low cost, has fast financial return, and there is a favorable market for honey commercialization.

However, no studies with *C. moschata* focused on floral nectar traits and considering this crop as a melliferous plant have been found in literature. Therefore, and in face of the need for studies on nectar traits to be used as apicultural pasture by honey bees in honey production, the aim of this study was to analyze quantitative and qualitative aspects of nectar in male and female flowers of pumpkin accessions, to evaluate this species potential as a melliferous plant.

MATERIAL AND METHODS

The present study was conducted in two crop seasons in the experimental field of the State University of Bahia, at the Department of Technology and Social Sciences (UNEB/DTCS), Campus III, in Juazeiro-BA (latitude 9°25'43.6" S, longitude 40°32'14" W, and altitude 384 m). According to Köppen's classification, climate in the region is semiarid, with mean annual rainfall of 484 mm, and rains concentrated in the period from November to April. The first experiment was conducted from January to May 2017 and the second experiment was conducted from August to December 2017.

Treatments and Experiments Conducted

In the first experiment, 14 pumpkin accessions were evaluated (Table 1), obtained by collecting fruits in supermarkets and street markets of Petrolina-PE and using Tetsukabuto hybrid as check.

The second experiment was comprised of 23 pumpkin accessions (Table 1), derived from seeds collected in Rio Grande do Norte, Pernambuco and Bahia (Farmers' Market, Juazeiro-BA). The hybrid Tetsukabuto was also used as check, as well as five accessions from the first experiment, which were chosen at random.

Seedlings from both experiments were produced in expanded polystyrene trays with 128 cells, containing Tropstrato® commercial substrate and transplanted to the field in the stage of first fully expanded leaf, with spacing of 4.0 x 3.0 m. Soil was previously prepared by harrowing and plowing. Fertilization was performed based on soil analysis and according to the fertilization recommendations for this crop (COSTA; FARIA; PEREIRA, 2008).

Table 1. Accessions with the respective codes, regions of origin and years of collection that were used in both experiments.

Code	Region of Origin	Year of collection
A01RN	Mossoró -RN	2011
A02RN	Mossoró -RN	2011
A03RN	Mossoró -RN	2011
A05RN	Mossoró -RN	2011
A07RN	Mossoró -RN	2011
A08RN	Mossoró -RN	2011
A10RN	Mossoró -RN	2011
A12RN	Mossoró -RN	2011
A13RN	Mossoró -RN	2011
A14RN	Mossoró -RN	2011
A15RN	Mossoró -RN	2011
A16RN	Mossoró -RN	2011
A17RN	Mossoró -RN	2011
BGAbo-01	Petrolina-PE*	2014
BGAbo-03	Petrolina-PE	2014
BGAbo-04	Petrolina-PE	2014
BGAbo-05	Petrolina-PE	2015
BGAbo-06	Petrolina-PE	2015
BGAbo-07	Petrolina-PE	2015
BGAbo-08	Petrolina-PE	2016
BGAbo-09	Petrolina-PE	2016
BGAbo-10	Petrolina-PE	2016
BGAbo-11	Petrolina-PE	2016
BGAbo-12	Petrolina-PE	2016
BGAbo-13	Petrolina-PE	2016
BGAbo-14	Petrolina-PE	2016
BGAbo-15	Petrolina-PE	2016
A04MP**	Casa Nova - BA	2017
A05MP	Casa Nova - BA	2017
A10MP	Santa Maria da Boa Vista - PE	2017
A16MP	Curaçá - BA	2017
A17MP	Curaçá - BA	2017
Tetsukabuto	Agristar***	2017

*Fruits obtained in open markets and supermarkets in Petrolina-PE; **MP= Fruits produced in different municipalities acquired in the Farmers' Market, Juazeiro-BA; ***Hybrid Seed Supplier Company.

The treatments in both experiments were allocated to a complete randomized block design, with three replicates, and each plot comprised of five plants, considering the three central plants for the analyses.

Farming practices were those recommended for pumpkin crops, according to Filgueira (2013).

Evaluations of Nectar Volume

One male flower and one female flower were used from three plants inside the plot of each accession and from the check in both experiments, totaling six flowers per plot of each accession and also six flowers for the control. Both floral types were protected one day prior to collection, in pre-anthesis, to avoid interference from pollinating agents. The next day, flowers were collected between 6:00 AM and 7:00 AM and taken to the Laboratory of Molecular Biology of the State University of Bahia, Campus III, Juazeiro-BA.

For evaluations of nectar volume, the

methodology proposed by Kearns and Inouye (1993) was used, with some adaptations, where graded syringes with volumetric capacity of 5 mL were used in the first experiment, and 3 mL syringes were used in the second experiment, as they were more accurate. Sampling consisted in removing petals from both floral types and also anthers from male flowers as the pollen present in anthers causes clogging of syringes. After removing petals and anthers, the syringe was inserted in the nectary of flowers to collect the nectar and for subsequent quantification of volume.

Evaluation of Sugar Concentration in the Nectar

The nectar extracted from male and female flowers, which was used to check volume, expressed in milliliters, was also used to estimate sugar concentration in ºBrix. For that, one drop of the nectar was deposited in the refractometer. A manual RHB90 analog refractometer (0 to 32 ºBrix) was used in the first experiment, and a digital Atago PAL-1 refractometer was used in the second experiment, as it was more precise (0 to 53 ºBrix).

Analysis of Nectar pH

Universal pH strips (KASVI) were used for pH analysis both in the first and in the second experiments. Each strip was comprised of five acid-base indicators with differentiated colors. Nectar was collected from male and female flowers using a syringe and subsequently deposited in a beaker. After that, the pH strip was inserted in the liquid content so that that the five acid-base indicators were in contact with the nectar for one second, and afterwards, the results were compared with a table of colors indicated by the product manufacturer, which had a pH variation of 0-14.

Determining Sugars in the Nectar

Nectar was collected from eight male flowers and eight female flowers from seven different accessions chosen randomly and from the check, in the second field experiment. After being collected, the nectar was deposited in adequately identified microtubes, and after that, they were taken to the freezer and kept at a temperature of -14 °C, for the preservation of sugars present in the nectar.

After nectar collection was completed, samples were taken to the Laboratory of Water of IF Sertão-PE, where they were thawed. After being thawed, 150 µL were removed from the sample with a micropipette and diluted in 1,350 µL of ultrapure water (deionized and passed through a 45-mesh membrane).

Samples were diluted in microtubes and, after that, they were agitated for subsequent homogenization. Using a syringe, these samples were removed and transferred to a vial using a nylon syringe filter, with filter diameter of 13 mm and 0.45 µm pore.

Sugars were determined simultaneously using High Power Liquid Chromatography (HPLC) using an AGILENT chromatograph (1260 Infinity LC model, Agilent Technologies, Santa Clara, CA, USA), coupled to a Refraction Index Detector (RID) (G1362A model). The column used was Agilent Hi-Plex H (300 x 7.7 mm) with internal 8.0 µm particles protected with a 5 x 3 mm PL Hi-Plex H guard column (Agilent Technologies). Column and RID furnace temperatures were kept at 25 °C and 30 °C, respectively. Sample injection volume (previously filtered through a 0.45 µm membrane) was 10 µL, with a 0.6 mL min^{-1} flow, and running time of 6 minutes. The phase used was 4.0 mM L^{-1} H_2SO_4 in ultrapure water. The data obtained were processed using OpenLab CDS ChemStation EditionTM software (Agilent Technologies). Analytical procedure followed the application notes described in the analysis *compendium* by Agilent Technologies (BALL et al., 2011).

Statistical Analysis

The data were submitted to an individual analysis of variance (ANOVA) for each experiment, and mean values were compared by Tukey's test with a significance level of 5%, using the computer program in genetics and statistics: Genes (CRUZ, 2016).

The ANOVA assumptions were tested and those descriptors that do not meet the assumptions were transformed.

RESULTS AND DISCUSSION

Nectar Evaluations

Nectar volume and sugar concentration (° Brix) did not show significant differences for male flowers in the ANOVA using transformed data. However, maximum values of 300 µL for the volume of nectar and 24.25 (°Brix) for the concentration of sugars were observed, and in all accessions evaluated, the male pumpkin flower had amounts of nectar and sugars that are of great importance for the attractiveness of honey bees. As for female flowers, significant differences were observed between accessions (Table 2). In the same Table, it can also be seen that there was a variation from 133 to 803 µL, among the accessions for the volume of nectar of female flowers.

Table 2. Mean nectar volumes (µL) and sugar concentrations (ºBrix) in female flowers, analyzed in different pumpkin accessions and in Tetsukabuto hybrid, in the first experiment in 2017, Juazeiro, BA, Brazil.

Female Flower		
Accession	Nectar Volume (µL)	Sugar Concentration (°Brix)
BGAbo – 03[(2)]	400.00 abc[(1)]	16.22 ab
BGAbo – 04	133.00 c	12.38 ab
BGAbo – 05	400.00 abc	16.04 ab
BGAbo – 07	233.00 bc	20.69 a
BGAbo - 08	600.00 ab	16.00 ab
BGAbo - 09	200.00 bc	12.69 ab
BGAbo -10	250.00 bc	18.54 ab
BGAbo - 11	366.00 abc	10.36 b
BGAbo - 12	376.00 abc	17.59 ab
BGAbo - 13	803.00 a	15.91 ab
BGAbo - 14	363.00 abc	16.01 ab
Tetsukabuto	650.00 ab	20.12 a
Mean	397.83	16.04
Amplitude	670.00	10.33
CV (%)	38.77	18.41
F	4.92**	3.24**

[1]Means followed by the same lowercase letters do not differ statistically from each other using Tukey's test at 5% significance level. [2]Accession used in the first experiment, derived from the State of Pernambuco.

Nectar volume is a feature of great importance in the definition of a melliferous plant, because the high availability of nectar in flowers allows an increase in the visitation rate for nectar collection by bees (EDGE et al., 2011). The results indicate that pumpkin can be considered a honey plant, since both floral types showed promise for use as bee pasture.

Regarding soluble solids, a variation from 10.36 to 20.69 ºBrix was found for female flowers (Table 2), but the pH means did not vary, remaining around 6.00 for both floral types. The appearance of empty flowers was observed in the accessions BGAbo-01 and BGAbo-06 in female flowers and in the accession BGAbo-15, in both floral types. However, those accessions that had flower without nectar will not be attractive to pollinators.

Regarding mean nectar volume of female flowers, the accession that stood out the most was BGAbo-08. The accessions BGAbo-04, BGAbo-07, BGAbo-09 and BGAbo-10 are different from the accession with best performance. The other accessions and the Tetsukabuto were similar when compared to the highlighted accession. For the concentration of sugar in the nectar, all accessions were similar, except for the BGAbo-07 accession and the Tetsukabuto hybrid, which had the highest means, but BGAbo-11 had the lowest concentration (Table 2). It is important to emphasize that the nectary of female flowers in pumpkin has a larger secreting surface and a higher density of stomata (NEPI; CIAMPOLINI; PACINI, 1996). The coefficients of variations (CVs) in nectar volume and sugar concentration for female flowers were high (Table 2), particularly for nectar volume. The high values of CVs are normal, as the germplasm used was not improved, which indicates the variation among plants within the accessions.

In the second experiment, mean nectar volumes of male flowers varied significantly and the means of accessions ranged from 30 to 310 µL. The female flowers did not show significance for the

volume of nectar, even so, they had a maximum volume of 470 µL, showing their potential as a honey plant. There was a significant variation for soluble solids of both floral types, with means from 4.01 to 21.42 (°Brix) for male flowers and from 0 to 22.64 (° Brix) for female flowers (Table 3).

Table 3. Mean nectar volumes (µL) in male flowers and sugar concentrations (ºBrix) in male and female flowers, derived from different pumpkin accessions and from Tetsukabuto hybrid, in the second experiment in 2017 and 2018, Juazeiro, BA, Brazil.

	Male Flower		Female Flower
Accession	Nectar Volume (µL)	Sugar Concentration (°Brix)	Sugar Concentration (°Brix)
A01RN [2]	100.00 ab[1]	20.33 a	19.51 ab
A02RN	210.00 ab	14.75 ab	19.03 ab
A03RN	197.00 ab	18.11 a	19.65 ab
A05RN	130.00 ab	19.36 a	22.64 a
A07RN	130.00 ab	18.29 a	0.00 b
A08RN	140.00 ab	16.22 ab	7.27 ab
A10RN	77.00 ab	10.79 ab	0.00 b
A12RN	110.00 ab	14.98 ab	17.22 ab
A13RN	140.00 ab	15.73 ab	16.79 ab
A14RN	210.00 ab	14.78 ab	20.72 a
A15RN	150.00 ab	17.00 a	16.10 ab
A16RN	170.00 ab	20.67 a	20.95 a
A17RN	30.00 b	4.01 b	4.33 ab
BGAbo-01[3]	250.00 ab	17.00 a	13.36 ab
BGAbo-05	300.00 a	15.82 ab	9.33 ab
BGAbo-06	230.00 ab	17.04 a	10.87 ab
BGAbo-12	180.00 ab	16.66 a	5.07 ab
BGAbo-14	200.00 ab	16.53 ab	10.88 ab
A04MP [4]	300.00 a	17.17 a	15.15 ab
A05MP	310.00 a	17.74 a	4.83 ab
A10MP	230.00 ab	21.42 a	6.77 ab
A16MP	280.00 ab	19.14 a	17.60 ab
A17MP	190.00 ab	19.60 a	12.38 ab
Tetsukabuto	100.00 ab	18.88 a	21.68 a
Mean	181.83	16.75	13.00
SFV2 Mean			14.19
Amplitude	280.00	17.41	22.64
CV (%)	45.82	23.71	56.63
F	2.35**	2.41**	3.15**

[1]Means followed by the same lowercase letters do not differ statistically from each other using Tukey's test at 5% significance level. ** - significant at 1% probability level. Accessions collected at: [2]RN= Rio Grande do Norte; [3]Accession used in the first experiment, derived from the State of Pernambuco; [4]MP= Farmers' Market, Juazeiro-BA.

A second character of great importance in defining a melliferous plant is the sugar concentration, as this determines the viscosity of the nectar, which is one of the attractions to bees and consequently can influence their feeding behavior (PAMMINGER et al., 2019). The male flowers of both experiments in the present study had absolute values slightly higher than those of the female flowers, among the accessions evaluated.

Similar results were found for nectar volume in studies conducted by Nepi, Guarnieri and Pacini (2001), with *Cucurbita pepo*, and by Ashworth and Galetto (2002) with *Cucurbita maxima* ssp. *andreana*. However, for sugar concentrations, the authors found higher values in female flowers, which was not corroborated in the present study. It is noteworthy that these studies were carried out under environmental conditions similar to those of the work in question. Therefore, since pumpkin plants have a higher number of male flowers, these flowers are expected to also indicate whether pumpkin has potential to be recommended as melliferous plant.

Mean nectar volume of male flowers was highest in accessions BGAbo-05, A04MP and A05MP, with values ranging from 300 to 310 µL. The A17RN accession was the one that produced the least, showing an average amount of 30 µL of nectar (Table 3). Regarding sugar concentration in the nectar, all accessions showed promise (varying from 14.78 to 21.42 ºBrix), except for male flowers of accessions A10RN and A17RN. However, eight accessions with female flowers were inferior, with contents varying from 4.33 to 10.88 (ºBrix), while the highest values varied from 13.36 to 22.64 (ºBrix) in the 14 remaining accessions. Accessions A07RN and A10RN for nectar concentration in female flowers had empty flowers (Table 3). The CVs of nectar volume were high, similar to those of the first experiment, and CV of sugar content in female flowers was also high in this experiment. These high CVs are likely to be a result of the high variation in the referred characters among plants within the accessions and this variation may be increased by strong biotic stresses to which the plants were submitted, notably in the second season.

Another important characteristic of a melliferous plant is nectar pH, and the results obtained in both floral types of both experiments indicated that pH is almost neutral (6 to 6.7). Floral nectar pH can be of great importance regarding honey pH (ALVES et al., 2005), and it might have a great protective effect against honey-deteriorating microorganisms (MEIRELES; CANÇADO, 2013). Evangelista-Rodrigues et al. (2005), studying the characteristics of honey made from algarroba and in wild plants in the State of Paraíba, Brazil, found pH of 3.85 and 4.66, respectively. Typically, honey pH is lower than nectar pH, as mandibular substances of honey bees in contact with the nectar that is carried to the beehive help change honey pH (ALVES et al., 2005), thus causing it to become more acidic.

Empty flowers were also found in the second experiment, which was similar to the first. However, this phenomenon was observed only in the female flowers of accessions A07RN and A10RN (Table 3). There was also high variation in nectar production, in either female or male flowers, in some accessions, both within each season and between seasons. On the other hand, temperature in both seasons was around 27 ºC. According to Almeida et al. (2003), optimal temperature for a good nectar production is between 25 °C and 32 °C and differences in environmental conditions also cause higher or lower nectar secretion (PACINI; NEPI; VESPRINI, 2003). Therefore, it is very important to obtain the progeny of these accessions, comparing them to some contrasting accession (with consistent production in both seasons) to examine whether the character has genetic control or if it is only related to environmental variation.

When considering the performance of accessions in both seasons, mean nectar volume in male flowers showed a variation of only 1.67 µL. However, there was an increase in the volume in the second season in some accessions (BGAbo-05, with an increase of 50%, and BGAbo-06, with an increase of more than 40%). The others had their volume reduced by little over 15%, and Tetsukabuto hybrid had the highest reduction (of approximately 40%) in male flowers of the second experiment. Regarding female flowers, accessions had high variation in performance of nectar volume, with some accessions having empty flowers in the first experiment, but with nectar in the second experiment (accessions BGAbo-05 and BGAbo-06), while the other accessions, including Tetsukabuto hybrid, had a lower nectar production in the second season (Table 4).

Female flowers in the first experiment had a higher mean volume of nectar (298.33 µL); however, in the second experiment, the two floral types had similar mean values for this character (190.83 and 211 µL) (Table 4). However, Vidal et al. (2006), studying nectar traits in *C. pepo*, found quite lower nectar volumes (18.50 µL to 79.20 µL) than the values found in the present study. Thus, female flowers, even in lower numbers per plant, are indicative that pumpkin is a melliferous plant due to nectar volume.

Table 4. Mean nectar volumes (µL) and sugar concentrations (ºBrix) in different *C. moschata* accessions and in Tetsukabuto hybrid, and evaluation of the accession x environment interaction (Ac x E), present in both experiments in 2017, Juazeiro, BA, Brazil.

Male Flower				
	Nectar Volume (µL)		Sugar Concentration (°Brix)	
Accession	Experiment 1	Experiment 2	Experiment 1	Experiment 2
BGAbo-01	300.00 a	253.00 a	17.04 a	17.01 a
BGAbo-05	203.00 a	300.00 a	20.60 a	15.82 a
BGAbo-06	157.00 a	230.00 a	19.48 a	17.04 a
BGAbo-12	213.00 a	183.00 a	17.03 a	16.66 a
BGAbo-14	233.00 a	200.00 a	18.24 a	16.53 a
Tetsukabuto	167.00 a	100.00 a	17.70 a	18.88 a
Mean	212.16	211.00	18.35	16.99
Flower Female				
	Nectar Volume (µL)		Sugar Concentration (°Brix)	
Accession	Experiment 1	Experiment 2	Experiment 1	Experiment 2
BGAbo-01	0.00 b	133.00 a	0.00 b	13.37 ab
BGAbo-05	400.00 a	343.00 a	16.04 a	9.33 b
BGAbo-06	0.00 b	173.00 a	0.00 b	10.88 ab
BGAbo-12	377.00 a	120.00 a	17.59 a	5.07 b
BGAbo-14	363.00 ab	133.00 a	16.01 a	10.88 ab
Tetsukabuto	650.00 a	243.00 a	20.12 a	21.68 a
Mean	298.33	190.83	13.95	11.87
Accession	0.84 ns		0.79 ns	
Environment	1.87 ns		5.16 **	
Ac x E	2.82 **		3.00 *	

[1]Means followed by the same lowercase letters do not differ statistically from each other using the Tukey' test at 5% significance level; **, * significant at 1% and 5% level, respectively.

In the joint analysis for nectar volume, the effects of accessions were not significant, as were the effects of sugar concentration (Table 4). For the environment, the volume of nectar was not significant, but it was significant at the 1% level for sugar concentration. The Accession x Environment (Ac x E) interaction was significant at 1% level for nectar volume and only significant at 5% level for sugar concentration (Table 4). According to the means of accessions for floral types, both for nectar volume and for sugar concentration, it appears that the mean values were lower in the second experiment for almost all accessions (Table 4) with much higher values, accentuated for sugar concentration.

Determining Sugars in the Nectar

In the analyses performed to determine sugars present in the nectar, it was observed that it was comprised of sucrose, glucose, and fructose, and no accessions had significant differences in male flowers. However, sucrose was nearly twice as high as the volume of either glucose or fructose, with a mean value of 1,278.72 g/mL. There were also no differences in sucrose for the female flower. However, a range from 1,036.04 to 1,447.41 g/mL was noted. To indicate whether a plant is melliferous, it is necessary to know the types of sugar, and sucrose was the predominant sugar in the present study, and more importantly, all pumpkin accessions evaluated had very similar values (more than 1,000 g/L) both in male and female flowers. Works demonstrate that the increase in the amount of sucrose improves the food source of honey bees (EDGE et al., 2011), allowing them to collect nectar from pumpkin flowers more frequently and thus enabling good honey production

Sugar content in female flowers was similar to that in male flowers. Therefore, sucrose content was the highest and showed no significant difference among accessions, with a mean value of 1,307.91 g/ mL, although fructose and glucose significantly differed among accessions (Table 5).

However, sucrose content was threefold higher than the contents of the other two sugars (Table 4). According to Edge et al. (2011), bees prefer nectar rich in sucrose instead of nectar rich in glucose and fructose and, in the present study, both floral types had equally high amounts of sucrose. Therefore, it can be assumed that both pumpkin floral types were visited by a similar number of floral visitors, as both floral types are preferred by the bees.

Regarding glucose and fructose, the values of both sugars were higher in the A14RN accession, and glucose and sucrose have very similar mean values, with a difference of 5.26 g/mL. Thus, it can be seen that these sugars are present in the nectar of flowers in similar amounts.

Table 5. Mean sucrose, glucose, and fructose in female flowers of pumpkin, derived from different *C. moschata* accessions and from Tetsukabuto hybrid, in the second experiment in 2017, Juazeiro, BA, Brazil.

Female Flower			
Accession	Sucrose g/mL	Glucose g/mL	Fructose g/mL
BGAbo-06 [2]	1,036.04 a	460.05 ab [1]	464.64 ab
BGAbo-12	1,193.04 a	332.93 ab	258.65 c
A01RN [3]	1,262.23 a	290.79 b	275.13 c
A12RN	1,262.23 a	261.62 b	272.77 c
A14RN	1,341.59 a	561.86 a	492.59 a
A16RN	1,401.11 a	350.36 ab	351.25 abc
A04MP [4]	1,447.41 a	357.75 ab	411.72 abc
A16MP	1,447.41 a	330.41 ab	296.45 bc
Tetsukabuto	1,364.08 a	268.47 b	343.73 abc
Mean	1,306.13	357.14	351.88
Amplitude	411.37	300.24	233.94
F	0.07 [ns]	4.38**	6.18**

[1]Means followed by the same lowercase letters do not differ statistically from each other using the Tukey's test at 5% significance level. Not significant = ns and significant at 1% **. [2]Accession used in the first experiment, derived from the State of Pernambuco; [3]RN= Rio Grande do Norte;[4]MP= Farmers' Market, Juazeiro, BA.

The data of the present study are similar to those found by Ashworth and Galetto (2002), who studied *C. maxima* ssp. *andreana*, showing that female flowers have higher values of sucrose (0.708 g/mL) than male flowers (0.639 g/mL). The same occurred in studies analyzing sugars in male and female flowers of *C. maxima* cv. Big Max (CHATT et al., 2018). However, the flowers of *C. moschata* show a large variation in the amount of sucrose for both floral types, when compared to the other species in the aforementioned studies. There are still few studies that seek to identify the reason for the greater amount of sucrose present in female pumpkin flowers. Thus, the present work provides a basis for future studies, so that it is possible to examine the high presence of sucrose in female flowers and determine whether it is a genetic, physiological or environmental factor.

According to Nepi and Pacini (1993), female flowers are more visited by pollinators as they have a higher amount of sucrose in their nectar. According to Edge et al. (2011), honey bees prefer sucrose-rich nectar instead of glucose- and fructose-rich nectar, and in the present study, both floral types had similarly high amounts of sucrose. Therefore, it can be assumed that both floral types of *C. moschata* were visited by a similar number of floral visitors, as both floral types are preferred by honey bees. The results of glucose and fructose obtained with *C. moschata* accessions showed that, in absolute values, they were quite higher than those found by Ashworth and Galetto (2002) in *C. maxima* ssp. *andreana*. However, the amount of sucrose in the present study was more than twice the amount of each of the other sugars, and this was true in generally all accessions studied and in both floral types. Hence, with regards to the types of sugars, the species *C. moschata* is adequate as melliferous plant.

On the other hand, *C. moschata* has high volumes of nectar and soluble solids compared to other species of great nectariferous importance for honey bees, as observed in studies on nectar conducted by Salomé (2014). In apple trees, nectar volume varied from 1.40 µL to 2.64 µL. Lima et al. (2014), who studied nectar availability in *Luffa cylindrica*, observed a variation in nectar volume ranging from 1.22 µL to 2.94 µL, which was thus similar to that found in apple. The same authors found sugar concentrations from 19.30 (°Brix) to 22.00 (°Brix), which are similar to the concentrations found in the present study. Antunes (2003), studying nectar traits of eucalyptus as a melliferous plant, observed that nectar volume ranged from 34.5 µL to 36.0 µL.

However, the number of flowers present in apple and eucalyptus trees is quite higher than those

present in pumpkin, and the number of male flowers is quite higher than the number of female pumpkin flowers. Mélo et al. (2010), studying floral biology of *C. moschata*, found a proportion between flowers of 3.4 male flowers for 1 female flower and this disadvantage compared to melliferous plants such as apple and eucalyptus might slightly minimize the importance of this species as a melliferous plant, although honey bees need a high amount of energy to collect small amount of nectar from each flower, as Salomé (2014) highlighted.

On the other hand, it is worth noting that aside from having flowers with high amounts of nectar, which makes them quite conspicuous and attractive to honey bees, pumpkin can be used as oleraceous plant for the production of fruits for cooked consumption as well as melliferous plants, and thus, one single plant might have a double function. Moreover, it is a crop with good adaptation to the climatic conditions of the Semiarid Northeastern region of Brazil, enabling a production in two cycles, thus ensuring fruits and bee pasture throughout the year.

In order to maximize the performance of pumpkin plants, considering their double function, both oleraceous and melliferous, the results of the present experiments indicate a high variation in the phenotypical response of accessions regarding nectar production and sugar content, and in accession performance concerning nectar volume and sugar content in both seasons during which the accessions were evaluated.

Thus, it is very important to obtain progeny of the most promising accessions for the different characters of interest so as to: (1) evaluate the genetic influence on the expression of these characters, and (2) to select genotypes that are suitable for a good nectar production in both floral types associated with high sugar content, mostly sucrose. Additionally, these progenies must have fruits with good-quality pulp for cooked consumption, must be fruitful, and have a high beta-carotene content. Therefore, the breeding program might develop suitable genotypes for this double function, of producing good-quality fruits with good traits for nectar production.

Associating honey-producing traits with a good production of fruits for commercial purposes in *C. moschata* will allow an additional income for the rural producer. Aside from obtaining melliferous plants, which are fruitful and have high beta-carotene contents, the farmers can also become beekeepers and diversify their income, with beehive renting for increased yield with pollination. Pollination and honey production might ultimately favor both honey bees and farmers (FREITAS; IMPERATRIZ-FONSECA, 2005).

CONCLUSIONS

This study shows that both floral types of *C. moschata* have melliferous potential considering nectar volume, sugar content, and pH. There is predominance of sucrose in both floral types of the species. There is phenotypical variability in quantitative and qualitative floral nectar traits of pumpkin accessions.

The contribution of this study confirms that the species *C. moschata* can be considered as a melliferous plant, notably when honey production is associated with the exploitation of fruits for cooked consumption.

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