Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Bee pollination effects on yield and chemical composition of West Indian gherkin fruits (*Cucumis anguria* L., Cucurbitaceae) in the Brazilian semi-arid region

Efeitos da polinização por abelhas na produtividade e composição química de frutos de maxixe (Cucumis anguria L., Cucurbitaceae) no semiárido brasileiro

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Abstract

Animal pollination plays a key role in global agricultural production and especially of monoecious crops, which are essentially dependent on pollinators. The West Indian gherkin fruit (*Cucumis anguria* L., Cucurbitaceae) is a monoecious vegetable adaptable to adverse abiotic conditions, resistant to diseases, and rich in minerals and vitamins, thus being a relevant alternative for improving nutritional security of socioeconomically vulnerable populations. The knowledge on the influence of pollination and of specific pollinators on chemical characteristics of fruits would help pollinators' management, but it is still poorly understood. In this study we investigated the influence of pollination on quantitative and qualitative aspects of fruits fruits of West Indian gherkin fruits (*Cucumis anguria* L., Cucurbitaceae) in the Brazilian semi-arid region. Data on pollination biology and on fruits resulted from controlled crosses (open-OP, cross-CP and *Apis mellifera* Linnaeus, 1758 pollinations) were compared among crosses: number, length, weight, number of seeds, firmness and chemical traits related to flavor and shelf life. Flowers were pollinated by four bee species, and *Apis mellifera* was the most frequent. followed by two native bee species. OP and *A. mellifera* resulted in more fruits than CP. Fruits resulting from OP were heavier than CP and had similar weight when compared to *A. mellifera*. The other variables did not differ between treatments. The better performance of OP and *A. mellifera* when compared to CP is probably related to the xenia, *i.e.*, the influence of tissues bearing paternal genes (pollen and pollen tube) in maternal tissues. OP and *A. mellifera* experiments apparently resulted in the deposition of a greater genotypic diversity of the pollen loads when compared to CP. This result is also explained by the higher functional diversity of pollinators related to OP when compared to CP. This study not only elucidates immediate impacts on yield but also emphasizes the deeper connections between floral biology, pollinator diversity, and sustainable crop production, once West Indian gherkin profit was enhanced by bee pollination.

Keywords: apidae, crop pollination, ecosystem services.

Resumo

A polinização animal desempenha um papel fundamental na produção agrícola global e especialmente nas culturas monóicas, que são essencialmente dependentes de polinizadores. O maxixe das Índias Ocidentais (*Cucumis anguria* L., Cucurbitaceae) é uma hortaliça monóica adaptável a condições abióticas adversas, resistente a doenças e rica em minerais e vitaminas, sendo assim uma alternativa relevante para melhorar a segurança nutricional de populações socioeconomicamente vulneráveis. O conhecimento sobre a influência da polinização e de polinizadores específicos nas características químicas dos frutos ajudaria no manejo dos polinizadores, mas é pouco compreendido. Neste estudo investigamos a influência da polinização nos aspectos quantitativos e qualitativos de frutos do semiárido brasileiro. Dados de biologia da polinização e de frutos resultantes de cruzamentos controlados (OP-polinização aberta, CP-cruzada e *Apis mellifera* Linnaeus, 1758) foram comparados entre cruzamentos: número, comprimento, peso, número de sementes, firmeza e características químicas relacionadas ao sabor e prazo de validade. As flores foram polinizadas por quatro espécies de abelhas, sendo *Apis mellifera* a mais frequente. OP e *A. mellifera* resultaram em mais frutos que CP. Os frutos resultantes de OP foram mais pesados que os de CP e apresentaram peso semelhante quando comparados aos de *A. mellifera*. As demais variáveis não diferiram entre os tratamentos. O melhor desempenho de OP e *A. mellifera* quando comparado ao CP provavelmente está relacionado à xenia, ou seja, à influência dos tecidos portadores de genes paternos (pólen e tubo polínico) nos tecidos maternos.

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Received: March 6, 2024 – Accepted: July 4, 2024

Experimentos com OP e *A. mellifera* aparentemente resultaram na deposição de maior diversidade genotípica das cargas polínicas quando comparados com CP. Este resultado também é explicado pela maior diversidade funcional dos polinizadores relacionados ao OP quando comparado ao CP. Este estudo não apenas elucida os impactos imediatos no rendimento, mas também enfatiza as conexões mais profundas entre a biologia floral, a diversidade de polinizadores e a produção agrícola sustentável, uma vez que o lucro do maxixe das Índias Ocidentais foi aumentado mesmo quando polinizado por uma espécie de abelha exótica.

Palavras-chave: apidae, polinização de culturas, serviços ecossistêmicos.

1. Introduction

The production of Cucurbitaceae crops is essentially dependent on pollinators (Klein et al., 2018), as they have unisexual flowers (monoecy, *i.e.*, they have male and female flowers on the same individual; Chomicki et al., 2020). Monoecious crops are more strongly affected by the global decline of pollinators (Potts et al., 2010; IPBES, 2016) when compared to hermaphrodite ones, which may set fruits through self-pollination (Klein et al., 2007; IPBES, 2016). The West Indian gherkin fruit (*Cucumis anguria* L., Cucurbitaceae) is a fruit vegetable originated in Africa and nowadays cultivated in world tropical and subtropical regions (Schaefer and Renner, 2011). It has extensive adaptability to adverse conditions and reduced water needs (Filgueira, 2003), is resistant to various pests, not requiring agricultural pesticides (Duarte et al., 2015). Also, fruits are rich in minerals and vitamins (Thiruvengadam and Chung, 2014) and can be consumed in different ways (fresh, cooked and preserved; Nascimento et al., 2011). Thus, West Indian gherkin can be considered an interesting crop for improving nutritional security of socioeconomically vulnerable populations.

Although it is known that West Indian gherkin production is essentially dependent on pollination (Sousa et al., 2013; Knapp and Osborne, 2019), the influence of pollination and of specific pollinators on chemical characteristics of fruits is poorly unknown. As is the case with more than 70% of agricultural crops, the animal pollination improves not only the quantity but also the quality of Cucurbitaceae production (Azmi et al., 2019; Donoso and Murúa, 2021; Khalifa et al., 2021). In this study we aimed to answer the following question: Does bee pollination influence quantitative and qualitative aspects of West Indian gherkins produced in a semi-arid region in Brazil? We hypothesize that bee pollination improve both quantitative and qualitative aspects of production.

2. Materials and Methods

2.1. Study area and studied species

The study was carried out in an open field in the city of Garanhuns, Pernambuco State (8°54’05.8”S 36°27’51.2”W; altitude of 823m), semi-arid region of NE Brazil. Before planting, the soil was prepared with organic fertilization (bovine manure) according to technical recommendations (IPA, 2008). Irrigation was carried out using a localized drip system, consisting of fixed hoses and drippers spaced approximately 20 cm apart.

Data on floral biology and floral visitors were obtained from a plantation established in November 2022 (dry season) using 135 certified West Indian gherkin seedlings (variety “maxixe do norte”, Feltrin Sementes Company, 21 days after sowing) in an area of 128 m^2 (16m long by 8m wide), with 1m distance between lines and between plants. Data on the pollination treatments were collected in March 2023 (dry season), when 65 seedlings (21 days after sowing) were planted in an area of 20m^2 (14m long by 10m wide) with a spacing of 2m between rows and 1m between plants. Maintenance of the cultivated areas was carried out every 15 days, with manual cleaning of weeds and thinning of senescent leaves to reduce the rate of disease spread.

2.2. Floral biology and floral visitors

Although the floral biology of the crop is already known, data checking is recommended before conducting the pollination treatments, as variations of reproductive crop traits among regions and varieties may occur. The period of anthesis was checked in 10 male and 10 female flowers marked during the pre-anthesis stage, distributed in 10 individuals. For all flowers, we recorded if the anthers were dehiscent, and the pollen was available at the beginning of anthesis. In 10 female flowers we checked the stigma receptivity by using the peroxidase techique at the beginning of anthesis and other 10 flowers at the end of anthesis. Other 20 recently opened flowers (10 male and 10 female flowers for each test) flowers were collected and the presence of osmophores was checked using the neutral red method for 15min (Dafni et al., 2005).

Floral visitors were recorded through 26 h and 30 min of focal observations in the field, homogeneously distributed from 7:00 am to 2:00 pm, during three days with similar climatic conditions (favorable to insect visitation), when 20 plants were observed in a rotation system. Each plant was sampled for 15 min, followed by a five-minutes break. At each visit, the visiting species, number of flowers visited, their sex (male or female) and contact of the floral visitor with stigma or anthers were recorded. Visitors who contacted the reproductive structures of flowers were considered as pollinators and others were considered as flower robbers.

2.3. Pollination experiment

To evaluate the influence of pollination on production, 38 plants were randomly selected in the central portion of the plantation and each plant individual were subjected to the following pollination treatments (one pollination treatment for each flower): open pollination (OP hereafter; the flower was marked and maintained available for floral visitors; n=38), cross-pollination (CP hereafter; the flower was manually pollinated with a mixture of pollen from three other plants; n=31; Dafni et al., 2005) and pollination efficiency by *Apis mellifera* Linnaeus, 1758 (the flower received a single visit of *A. mellifera* n=33). We choose this species as it was the most frequent pollinator recorded during focal observations in the plantation. For CP and *A. mellifera* treatments, flowers were bagged since the pre-anthesis stage, and pollination was conducted during the morning of the first day of anthesis. After being pollinated, the bags were put back in until floral senescence.

2.4. Data collection

Thirteen days after the pollination day, we counted the number of fruits and collected them. Each fruit was evaluated in relation to morphometric and chemical traits that are closely related, respectively, to market value and fruit ripeness (Chitarra and Chitarra, 2005), as those aspects influence flavor and shelf life. We recorded for each fruit, the length and width (measured by using a digital caliper), weight (measured by using a semi-analytical digital scale) and firmness (using a digital penetrometer). For chemical analyses we selected soluble solids, by using a pocket refractometer and samples diluted in 10g of fruits and 20ml of distilled water, pH by using a calibrated pHmeter with 5g of sample processed with 50mL of distilled water at room temperature, and acidity titratable by volumetrics with NaOH 0.1N and the phenolphthalein a 1% as the indicator. All chemical analyses were conducted following the protocols of the Association of Official Agricultural Chemist (AOAC, 1992).

2.5. Data analysis

All analysis were performed by linear models comparing each dependent variable mean values among pollination treatments (OP, CP or *Apis mellifera*). Specifically for fruit set, in used the binomial error to estimate the successes once it was measured per flower as success or failure (Zar, 2010). The models' assumptions were checked using *testResiduals* function from *DHARMa* package (Hartig, 2020) and just soluble solids variable was square-root transformed for statement of residuals normality. Accordingly, all models tested for significance using *Anova* function from *car* package (Fox & Weisberg, 2011) followed by marginal means tests with Tukey adjusts from the *emmeans* function and package (Lenth et al., 2018). We conducted all analysis in R environment v. 4.1.2 (R Core Team, 2021) and all graphs are plotted using mean value ± standard error.

3. Results

3.1. Floral biology and flower visitors

The male and female flowers are medium-sized (corolla diameter of 15.7 ± 2.1; 18.5 ± 2.9, respectively), with five green sepals and five yellow petals that turn cream as anthesis progresses. Male flowers have two or three stamens, with yellow anthers of longitudinal dehiscence, offering pollen and nectar as resources. Female flowers have a pistil with an inferior ovary and provide only nectar to floral visitors. Both floral types had an acrid odor. The petals, anthers and stigma showed a positive reaction to the neutral red solution.

The beginning of anthesis of both flower types occurred in the early morning (6:00 and 7:00 a.m.), when pollen was available, and the stigma was receptive. The staminate flowers remained open until 2:00 pm, and the pistillate flowers until 3:00 pm. After this time, flowers closed their petals, opening them again the following day, when the process was repeated, and the flowers finally senesced.

Eight insect species were observed visiting the flowers: five bees of Apidae family [*Apis mellifera*; *Exomalopsis sp.*,; *Trigona spinipes* (Fabricius, 1793) and *Plebeia flavocinta* (Cockerell, 1912)], a wasp (Vespidae family), a fly (Culicidae family) and a beetle (Chrysomelidae, *Diabrotica speciosa* Germar, 1824; Figure 1).

Apis mellifera was the most frequent pollinator (82% of the visits), followed by *Exomalopsis* sp. and *P. flavocinta* (Figure 2). Bees collected pollen and nectar from male flowers, and nectar from female flowers, into which they inserted their heads and contacted the stigma. *P. flavocinta* and *Exomalopsis* sp. spent longer period scratching the center of the flower when compared to other pollinators and, for this reason, had a greater area of contact between their bodies and the stigma. *Trigona spinipes*, the wasp and the beetle behaved as floral robbers, since they only visited male flowers. The beetle only ate parts of the petals, not approaching the anthers.

Visits occurred from 7:00 to 3:00 p.m., with a peak between 9:00 and 10:00 a.m. Floral visitors had a greater number of visits to male flowers (Figure 2).

3.2. Influence of pollination on fruit set and quality traits

Fruit set differed statistically between treatments ($F_{2,99}$= 9.6223, $p < 0.001$; Figure 3). OP (78%) and *A. mellifera* (62%) resulted in more fruits than CP (27%). There were also differences related to fruit weight ($F_{2,55}$ = 3.024, $p < 0.001$, Figure 3), as fruits resulting from OP were heavier than CP and had similar weight when compared to those resulting from *A. mellifera*. The other variables did not differ between treatments: length ($F_{2,57}$ = 1.402, p = 0.255), diameter ($F_{2,54}$ = 1.344, p = 0.270), number of seeds ($F_{2,52}$ = 2.769, p = 0.072), firmness ($F_{2,54}$ = 2.192, p = 0.122), acidity ($F_{2,47}$= 0.697, p = 0.503), soluble solids ($F_{2,51}$ = 0.241, p = 0.786) and pH ($F_{2,47}$ = 1.43, p = 0.250; Figure 3 and 4).

4. Discussion

The floral biology was similar to other studies, except time of anthesis and of flowers senescence, which may differ among regions (Siqueira et al., 2011; Carneiro Neto et al., 2018).

The higher number of visits to male flowers can be explained by the well-known higher proportion of these flowers compared to female ones of some cultivars (1:21, Carneiro Neto et al., 2018) and in Cucurbitaceae in general (Siqueira et al., 2011). The lower proportion of female flowers makes the crop even more dependent on pollinators. *A. mellifera* was recorded as the main pollinator in a West Indian gherkin fruit plantation in SE Brazil (72% of all visits), followed by and native

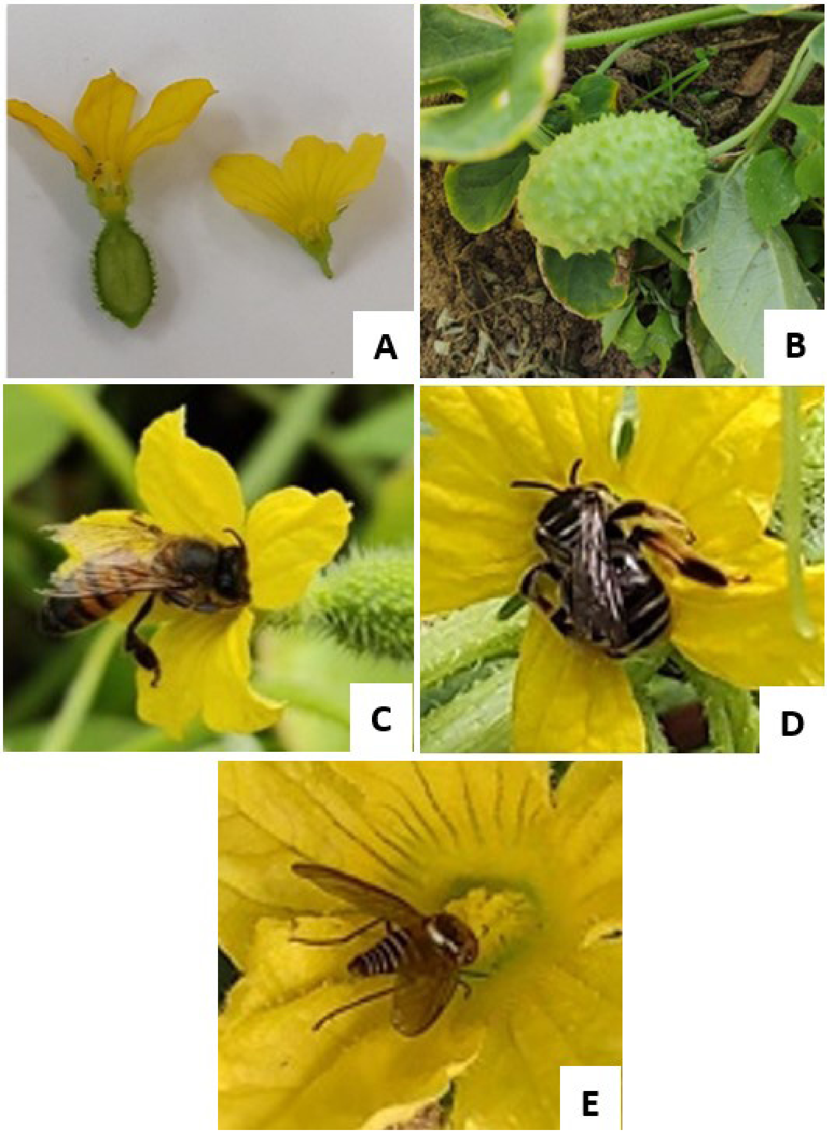

Figure 1. Flowers (A), fruit (B) and pollinators of West Indian gherkin (*Cucumis anguria* L., Cucurbitaceae) in a semiarid area of NE Brazil. C: *Apis mellifera*; D: *Exomalopsis* sp.; E: Diptera of the Culicidae family.

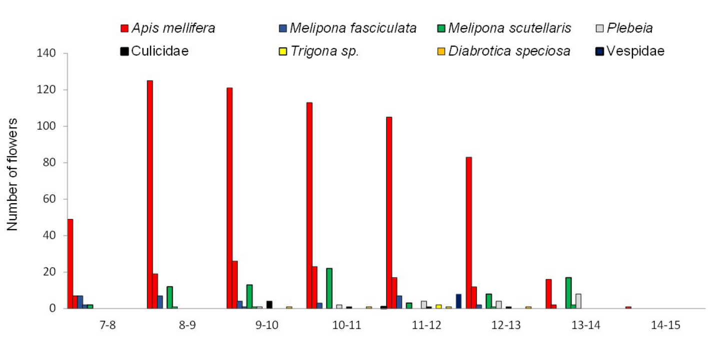

Figure 2. Frequency of floral visitors of West Indian gherkin (*Cucumis anguria* L., Cucurbitaceae) in a semiarid area of NE Brazil. For each floral visitor, the first bar corresponds to male, and the second bar corresponds to female flowers.

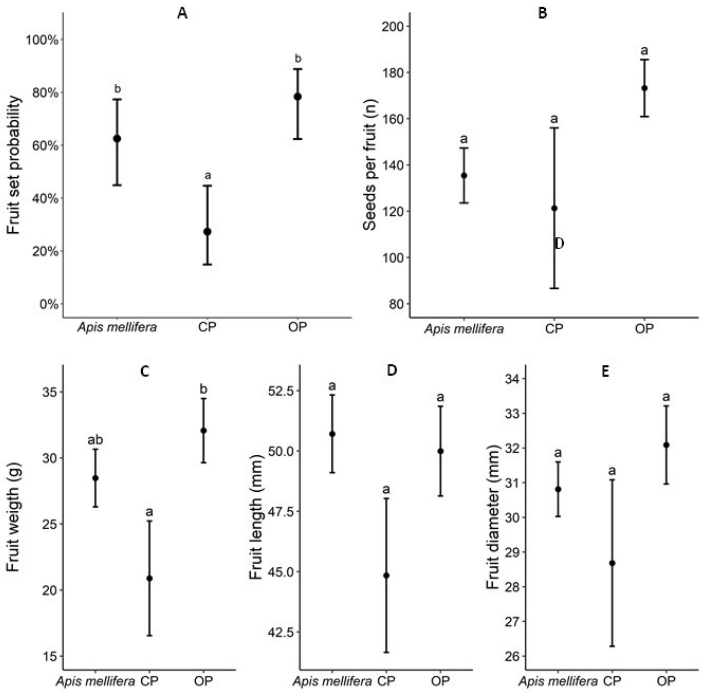

Figure 3. Effects of pollination treatments on fruit set (A), number of seeds (B), weight (C), length (D) and diameter (E) of West Indian gherkin (*Cucumis anguria* L., Cucurbitaceae) in a semiarid area of NE Brazil. CP: cross-pollination; OP: open pollination. Same letters represent effect similarities between treatments.

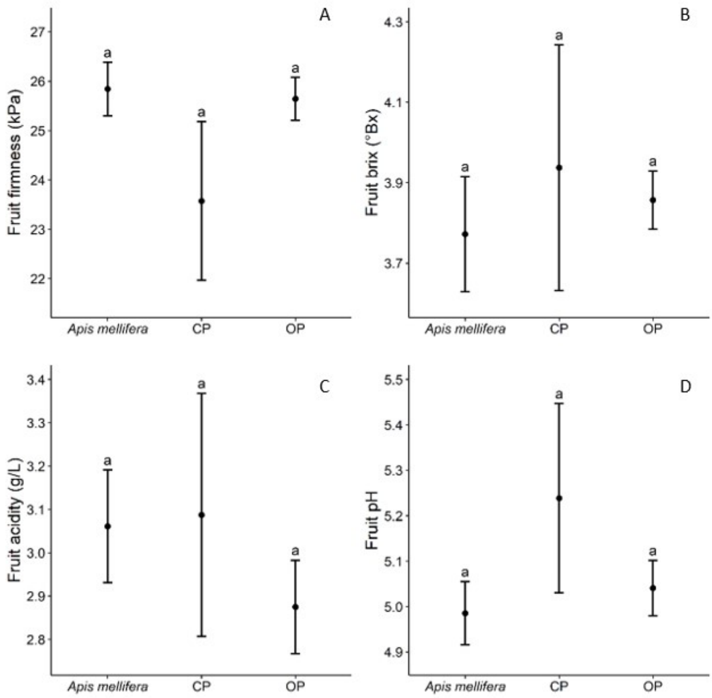

Figure 4. Effects of pollination treatments on fruit firmness (A), brix (B), acidity (C) and pH (D) of West Indian gherkin (*Cucumis anguria* L., Cucurbitaceae) in a semiarid area of NE Brazil. CP: cross-pollination; OP: open pollination. Same letters represent effect similarities between treatments.

bees (27.8%, on average), mainly *P. flavocinta* (16.7%), *Exomalopsis* sp. (8.3%) and *Melissodes* sp. (2.78%; Malerbo-Souza et al., 2019). In other study of NE Brazil, the most common pollinators of the West Indian Gherkin fruit were the native bees *Trigona guianae* Cockerell, 1912 *e Augochlora* sp., with sporadic visits of butterflies (Sousa et al., 2013).

The better performance of OP and *A. mellifera* experiments when compared to CP can be explained by two aspects. First, the greater genotypic diversity of the pollen loads deposited onto stigmas in the formers, which may be a result of the greater number of pollen-donating individuals that bees visit when compared to those used in the CP experiment (three, in the case of this study). Studies show that those pollen loads influence crop production in both quantity and quality (Chai et al., 2023 and references therein). The impact of pollen loads on fruit features seems to be related to the diffusion of one or more signaling substances from the pollen tube and/or the male nuclei across fruit tissues (Perazza et al., 1998). The influence of tissues bearing paternal genes (pollen) in maternal tissues is called xenia (Denney 1992), and is well described for some fruits (e.g., Gaaliche et al., 2011; Sabir, 2015; Gharaghani et al., 2017; Chai et al., 2023), grasses (Pozzi et al., 2018) and vegetables (Piotto et al., 2013). The second aspect regards the functional diversity of pollinators: flowers submitted to OP received visits from a greater diversity of pollinators than *A. mellifera* treatment, what implies in a greater diversity of morphological features (*e.g.*, body size, number of setae and proboscis length), in combination with behavior components (*e.g.*, foraging strategy, visiting time and duration, mode of flower handling; Fründ et al., 2013; Garibaldi et al., 2016), which complement each other. Moreover, OP may had received a higher genotyic diverse of pollen loads when compared to CP, which was performed by using three individuals only. In a study conducted in NE Brazil, Sousa et al. (2013) recorded higher fruit set after OP and CP when compared to *A. mellifera, Augochlora* sp. e *Trigona guianae*, and fruit weight of the formers was twice the weight of the bee experiments.

5. Conclusions

The dominance of *Apis mellifera* in pollination, alongside the nuanced behaviors of other species, highlights the diverse strategies employed by animal pollinators. The disparity in visitation rates between male and female flowers emphasizes the crop's vulnerability and reliance on these crucial agents for reproduction. Furthermore, the differential fruit set and quality traits resulting from varied pollination treatments underscore the significance of genetic and functional diversity in pollen loads and pollinator communities. This study not only elucidates immediate impacts on fruit yield but also emphasizes the deeper connections between floral biology, pollinator diversity, and sustainable crop production once West Indian gherkin profit was enhanced when pollinated by exotic and native bees.

Acknowledgements

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Financing Code 001) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (research grant: 428303/2018-8, fellowship: 312372/2021-3) and FAPESP (research grant: 2021/09247-5 and 2023/04378-0) who provided financial support for the conduct of the research but had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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