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FLORAL BIOLOGY AND BEEKEEPING POTENTIAL OF *Croton heliotropiifolius* KUNTH (EUPHORBIACEAE) AT SEMI ARID REGION, BRAZIL[1]

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ABSTRACT - The aim of the present study was to determine the beekeeping potential of *Croton heliotropiifolius* Kunth (Euphorbiaceae) based on flowering strategy, floral characteristics, available floral resources, and the individuals frequencies and foraging activities of *Apis mellifera* L. on its flowers in Fazenda Ingazeira, Brazil. Each *C. heliotropiifolius* plant possessed inconspicuous diclinous flowers clustered into 560±60 dense terminal inflorescences containing 59±12 pistillate and 160±26 staminate flowers. Although both types of flowers produced nectar (pistillate: 2.8±0.8 µL; staminate: 0.3±0.06 µL), *A. mellifera* workers mostly visited staminate flowers (98%) during the peak visitation period (08:00–11:00 h). Moreover, during the foraging period, each plant produced approximately 5.376×10^8 pollen grains, ensuring approximately 33 visitation cycles from a colony of *A. mellifera* workers that picked up an average of 405 pollen grains per visit per flower. Further, *C. heliotropiifolius* showed an annual cornucopia flowering strategy with high flower production for five months, which is an important characteristic that ensures floral resource availability in the environment. These findings suggest that *C. heliotropiifolius* has a great potential for apiculture in this region.

Keywords: Apiculture. *Apis mellifera* L.. Caatinga. Floral resources.

BIOLOGIA FLORAL E POTENCIAL APÍCOLA DE *Croton heliotropiifolius* KUNTH (EUPHORBIACEAE) NA REGIÃO DO SEMI-ÁRIDO, BRASIL

RESUMO - O presente estudo teve por objetivo determinar o potencial apícola de *Croton heliotropiifolius* Kunth, baseado na sua estratégia de floração, características florais, recursos florais ofertados, frequência e atividade de forrageamento de indivíduos de *Apis mellifera* L. às flores na Fazenda Ingazeira, Brasil. *C. heliotropiifolius* possui flores díclinas e inconspícuas agrupadas em densas inflorescências terminais, em que cada indivíduo produz 560±60 inflorescências, com 59±12 flores pistiladas e 160±26 flores estaminadas, tendo cada flor estaminada a capacidade de produzir aproximadamente 6×10^3 grãos de pólen. Embora ambas as flores produzam néctar (2,8±0,8 e 0,3±0,06 µL, nas flores pistiladas e estaminadas, respectivamente), os espécimes de *A. mellifera* concentraram suas visitas nas flores estaminadas (98%), com pico das visitações registrados entre 08:00-11:00 h. Foi estimado que nas atividades de forrageamentos, essa abelhas removem em média 405 grãos de pólen por visita em cada flor, desta forma, como um indivíduo da espécie estudada pode produzir aproximadamente $5{,}376 \times 10^8$ grãos de pólen, tal quantidade é suficiente para suportar até 33 ciclos de visitações de uma colônia de *A. mellifera*. Além disso, *C. heliotropiifolius* apresentou um padrão de floração anual, com altas produções de flores por cinco meses, manifestando estratégia de floração cornucópia, representando uma característica importante por favorecer na maior disponibilização de recursos florais nos ambientes. Portanto, esses dados demostram o grande potencial de *C. heliotropiifolius* para a apicultura na região.

Palavras-chave: Apicultura. *Apis mellifera*. Caatinga. Recursos florais.

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INTRODUCTION

Members of Euphorbiaceae, an angiosperm family with 300 genera and more than 8,000 species, occur in tropical regions that harbor different types of habitats and vegetation (GBIF SECRETERIAT, 2019). A total of 66 genera and 973 species of this family have been documented in Brazil, and approximately 24% of these species are found in the Caatinga phytogeographical domain (SILVA et al., 2020). Among the 66 genera, *Croton* is the most abundant, with more than 300 species identified from different vegetation domains in the northeastern region of Brazil, where they occur in dry to humid regions and even on rocky outcrops (SILVA et al., 2010; CARUZO et al., 2020).

The shrub species *Croton heliotropiifolius* Kunth, is widely distributed in the northeastern region and less frequently in the central-western and southeastern regions of Brazil (HURBATH; CARNEIRO-TORRES; ROQUE, 2016). Plants of this species are well-adapted to desert environments with high anthropization, forming dense populations (SILVA et al., 2010). The species is ecologically significant because it contributes to the reestablishment of the edaphic balance by supplying mineral nutrients and organic matter to the soil (GOREVITCH; SCHEINER; FOX, 2009; MORIMITSU; VERGENE; TOMAZ, 2014), as well as food resources for the fauna (BLUTHGEN, 2012).

Previous studies on floral biology have reported high frequencies of bees exploiting the floral resources of *Croton* species, primarily the *Apis mellifera* L. workers (REDDI; SUBBA-REDDI, 1985; PASSOS, 1995; PIRES; SOUSA; TERADA, 2004), and 19 *Croton* species have already been identified as important resources for apiculture in semi-arid regions (SANTOS et al., 2006). As most plants of apicultural importance are herbs or shrubs considered as weeds, they have been extensively subjected to deforestation (SANTOS et al., 2006). Thus, it is necessary to study their floral biology, with an emphasis on the bees that visit and exploit these resources, for the sustainable use of plant species and the diverse bee species that exploit their floral resources (ABOU-SHAARA, 2015).

Therefore, the aim of this study was to assess the potential of *C. heliotropiifolius* for apiculture in semi-arid regions. Furthermore, we determined the floral biology and floral resources (pollen grains and nectar) of this species, which are explored by *A. mellifera* individuals, to assess the ability of the plants to provide these resources.

MATERIALS AND METHODS

Study site

We investigated the flowering phenology and floral biology of *C. heliotropiifolius* in Fazenda Ingazeira (13°54′48.1″S, 42°23′6.8″W) in the municipality of Caetité, southwestern Bahia, Brazil. Fazenda Ingazeira is located 20 km away from the municipal center of Caetité, with an annual average temperature ranging between 15 and 27 °C and an average annual rainfall of approximately 743 mm (INMET, 2018). The region is characterized by a dry season from May to October and a rainy season from November to April. *C. heliotropiifolius* plants were sampled from natural clusters composed of a few, sparsely distributed plants or from groups of more than 20 individuals spread across a 2 ha grassland currently under regeneration.

Floral biology

To estimate the number of inflorescences per individual and flowers per inflorescence, we first determined the mean number of inflorescences produced by mature individuals (height: 1.5 m) (n = 10) and then estimated the mean number of pistillate and staminate flowers borne on each inflorescence. Additionally, we monitored the development of staminate and pistillate flowers daily in five marked inflorescences of four individuals from the bud stage through senescence, and recorded the hour of anthesis and the period of pollen release (PASSOS, 1995; DAFNI; KEVAN; HUSBAND, 2005). Lengths of stylets and stigmas (n = 40) were also measured using a digital caliper. For olfaction testing, fresh flowers were collected at 08:00 h in a sealed glass container and the released odor was analyzed after 3 h (DAFNI; KEVAN; HUSBAND, 2005).

To estimate the quantity of nectar produced, 40 pre-anthesis bud-stage inflorescences from randomly selected, staminate and pistillate flowers were isolated for 48 and 24 h, respectively, using organza bags after bud opening. Nectar from the 20 pistillate flowers (one flower per inflorescence) was withdrawn using a 10 µL micro syringe, and nectar sugar concentration was estimated using a refractometer (scale: 0–90%). However, owing to insufficient nectar production, nectar measurements in the 20 staminate flowers were made using Whatman filter paper No. 1, following the protocol described by Dafni, Kevan and Husband (2005).

Additionally, we determined the visitation patterns of bees including, collected resources, bee behavior, frequency per inflorescence per hour, and visiting time, all by direct, continuous visual observations between 05:00–18:00 h over 16 days, for a total of 192 h of observation. Plants with the largest number of pistillate flowers were selected for observation during the first eight days (96 h), while individuals with maximum abundance of staminate flowers were observed during the next eight days (96 h). We followed the protocol proposed by Dafni, Kevan and Husband (2005) to estimate the amount of pollen grains in the 40 pre-anthesis buds, which was used to determine pollen grain availability in flowers exposed to 5 and 10 visitations from *A. mellifera* individuals. The presence of starch and lipids in the pollen grains was estimated colorimetrically using Lugol and Sudan IV reagents, respectively.

Flowering phenology

We recorded the phenology of 30 marked individuals semi-quantitatively on a biweekly basis from September 2017 to August 2018. We assessed flowering intensity by assigning individual plants to one of the following five categories (FOURNIER, 1974): 0 = absence; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100%. Moreover, the flowering index was evaluated following the methodology of Bencker and Morellato (2002), which involves qualitative estimation of the presence or absence of flowering individuals in the population.

Subsequently, the number of staminate and pistillate flowers was quantified at the population level using five inflorescences randomly selected in 20 individuals on a weekly basis throughout the flowering period. The pattern of sexual expression (PS) of the species was determined by dividing the number of pistillate flowers by the number of staminate flowers; where, PS=0 indicated only staminate flowers, PS=E indicated only pistillate flowers, and PS>0 indicated overlap with presence of staminate and pistillate flowers (DOMÍNGUEZ; BULLOCK, 1989).

Statistical analysis

Spearman correlation index was used to assess the effects of the climatic variables (temperature and rainfall) on flowering, while flowering synchrony in the population was measured using the synchrony index (Z); where, Z=0 indicated no synchrony, Z>0.5 indicated high synchrony, and Z=1 indicated perfect synchrony (AUGSPURGER, 1983; PEDRONI; SANCHEZ; SANTOS, 2002).

RESULTS AND DISCUSSION

Floral biology

The monoecious shrub *C. heliotropiifolius* was found to bear 560±60 inflorescences per plant (Figure 1a), with diclinous flowers borne on erect racemes (Figures 1b-c). There were 59±12 and 160±26 pistillate and staminate flowers per inflorescence, respectively; thus, approximately 73% of the flowers borne on an inflorescence were staminate, which is a characteristic feature of the species (REDDI; SUBBA-REDDI, 1985; DOMÍNGUEZ; BULLOCK, 1989; PASSOS, 1995; PIRES; SOUSA; TERADA, 2004). Pistillate and staminate flowers lasted on the inflorescences for three and two days, respectively.

Flowers blossomed acropetally, with pistillate flowers always maturing earlier than staminate flowers. Thus, in a selected inflorescence, the female phenophase (i.e., anthesis of pistillate flowers), which lasted for six days, occurred in two consecutive 3 day periods (Figure 1b). Five days after the female phenophase, the male phenophase (i.e., anthesis of the staminate flowers) began in the inflorescences, with 10 flowers blossoming every day for the next 16 days (Figure 1c).

Pistillate flowers were pedicellate, with five light-green sepals and three styles, each measuring 1 mm, with 3.3±0.2 mm-long bifid stigmas (n = 40) (Figure 1d). The ovaries were brown and superior with three uniovulate locules and axial placentation, and orange nectar glands were located between the sepals and the ovary (Figure 1d). The pistillate flowers matured slowly, and the maturation process extended from the early hours of the day up to 12:00 h, during which complete flower anthesis occurred, which was indicated by the complete horizontal extension of the styles to expose the bifid stigmas with their ends facing upwards. The mean volume of nectar produced per pistillate flower was 2.8±0.8 µL, with a sugar concentration of 13.4±4.1%; the flower odor was characterized as sweet.

Pedicellate staminate flowers were also pentamerous, with green sepals and inconspicuous white petals, and had 22±2 stamens per flower (Figure 1e). Filaments were showy, with basifixed anthers and longitudinal dehiscence, and the nectar glands were located at the attachment points of the filaments (Figure 1e). Complete anthesis in staminate flowers occurred at 08:00 h, exposing the pollen grains for release at 09:00 h. The amount of nectar recorded in staminate flowers was 0.3±0.06 µL, and the sugar concentration was too low to be measured. However, similar to the pistillate flowers, the odor released by the staminate flowers was characterized as sweet.

Although both pistillate and staminate *C. heliotropiifolius* flowers produced nectar, *A. mellifera* individuals visited staminate flowers more frequently for pollen grains (Figure 1f).

Plants with dense terminal inflorescences, such as *C. heliotropiifolius*, are showy and thus attract a great diversity of visitors (GORAIN; CHARAN; AHMED, 2012). Moreover, the reproductive morphological characteristics of *C. heliotropiifolius* reported in the present study, including inconspicuous radial flowers with sweet odor, are consistent with reports on species with greater potential for apiculture in the northeast region of Brazil, such as *C. blanchetianus* Baill, *C. grewioides* Baill. and *C. sonderianus* Müll. Arg. (SANTOS et al., 2018).

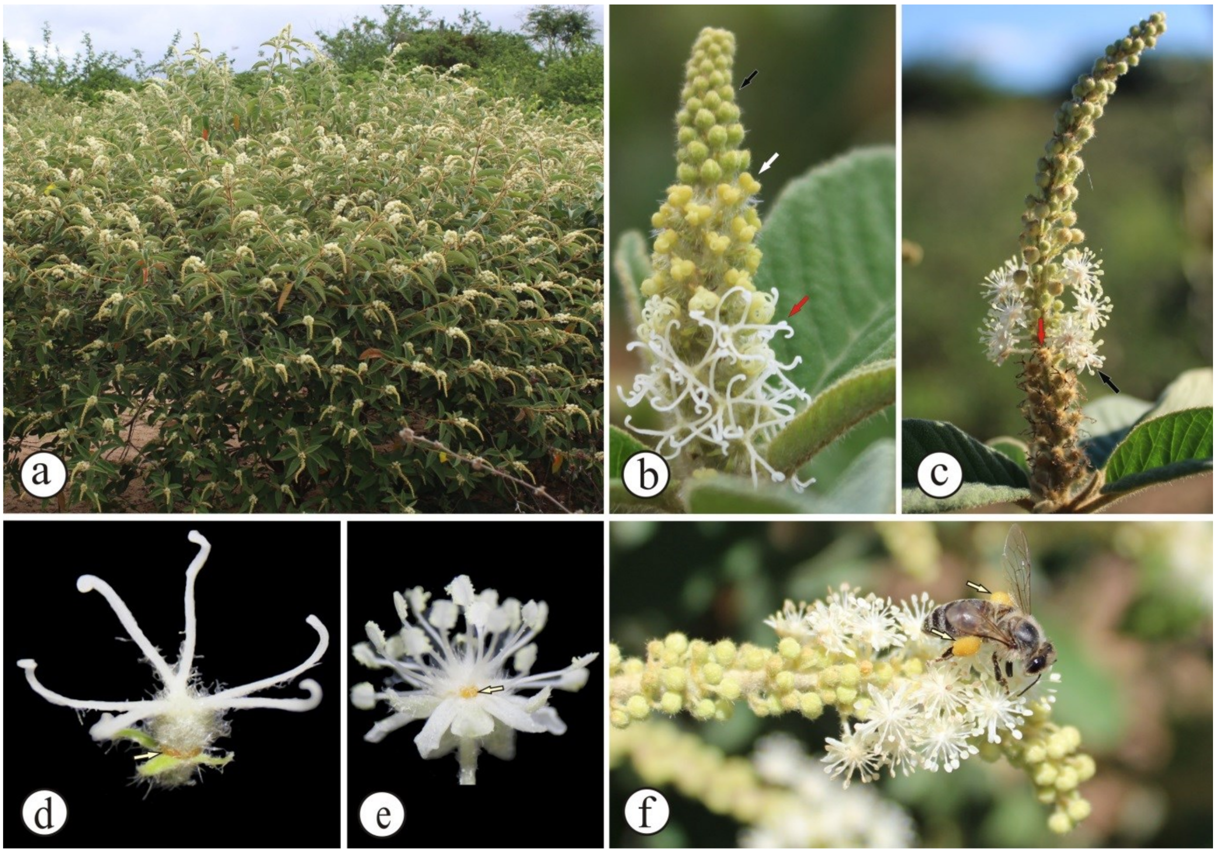

Figure 1. a. Individuals of *Croton heliotropiifolius* Kunth (Euphorbiaceae) showing inflorescences exposed above the foliage; b. An inflorescence during anthesis in pistillate flowers (red arrows) showing buds of pistillate (white arrows) and staminate flowers (black arrows); c. An inflorescence during anthesis in staminate flowers showing senescence of pistillate flowers (red arrows) and the beginning of anthesis in staminate flowers (black arrows); d. Nectar glands in a pistillate flower (arrow); e. Nectar glands in a staminate flower (arrow); f. Workers of *Apis mellifera* L., 1978, foraging on staminate flowers at Fazenda Ingazeira, Caetité, Bahia, Brazil.

The visiting frequency by *A. mellifera* individuals on pistillate flowers was 2%, with the peak visitation period between 06:00 h and 09:00 h (Figure 2a), while 98% of the visits were recorded on staminate flowers, with the peak visitation period from 08:00 h to 11:00 h (Figure 2b). Moreover, *A. mellifera* workers visited staminate flowers until 12:00 h daily, indicating that the primary resource of interest was the pollen grains, as the highest number of visits were recorded between 06:00 and 12:00 h, which included the period during which anthesis occurred (08:00–09:00 h). Reddi and Subba-Reddi (1985) reported similar observations in *C. bonplandianus* Baill, wherein *Apis* workers concentrated exclusively on staminate flowers for foraging pollen grains.

The pollen grains of *C. priscus* Croizat and *C. floribundus* Spreng are the main floral resource foraged by workers of *A. mellifera* (PASSOS, 1995). However, these bees forage other species for nectar (PIRES; SOUSA; TERADA, 2004), for example, in *C. urucurana* Baill, bees forage for pollen grains in the morning (09:00–11:00 h) and for nectar produced in pistillate flowers in the afternoon (15:00–17:00 h). Thus, the foraging behavior of bees on *Croton* species warrants further investigation. Moreover, in this study, *C. heliotropiifolius* was an important source of pollen grains for the worker bees of *A. mellifera*, while other species might exhibit different behaviors, foraging for nectar or both nectar and pollen, as reported in *C. urucurana* (PIRES; SOUSA; TERADA, 2004).

When mixed with regurgitated nectar and glandular secretions, pollen grains containing starch, such as those of *C. heliotropiifolius*, comprise a highly nutritious diet for bees (GHOSH; JUNG, 2017). Additionally, lipids, which increase the attractiveness of pollen grains, are essential for bees to synthesize royal jelly and accumulate energy reserves (DOBSON, 1988). Hence, large amounts of pollen grains are necessary for the development of healthy bee colonies (HUANG, 2012).

The data regarding to pollen grain available in the pre-anthesis buds, as well as on flowers exposed to five and 10 visitation of *Apis mellifera* specimens are be shown at Table 1.

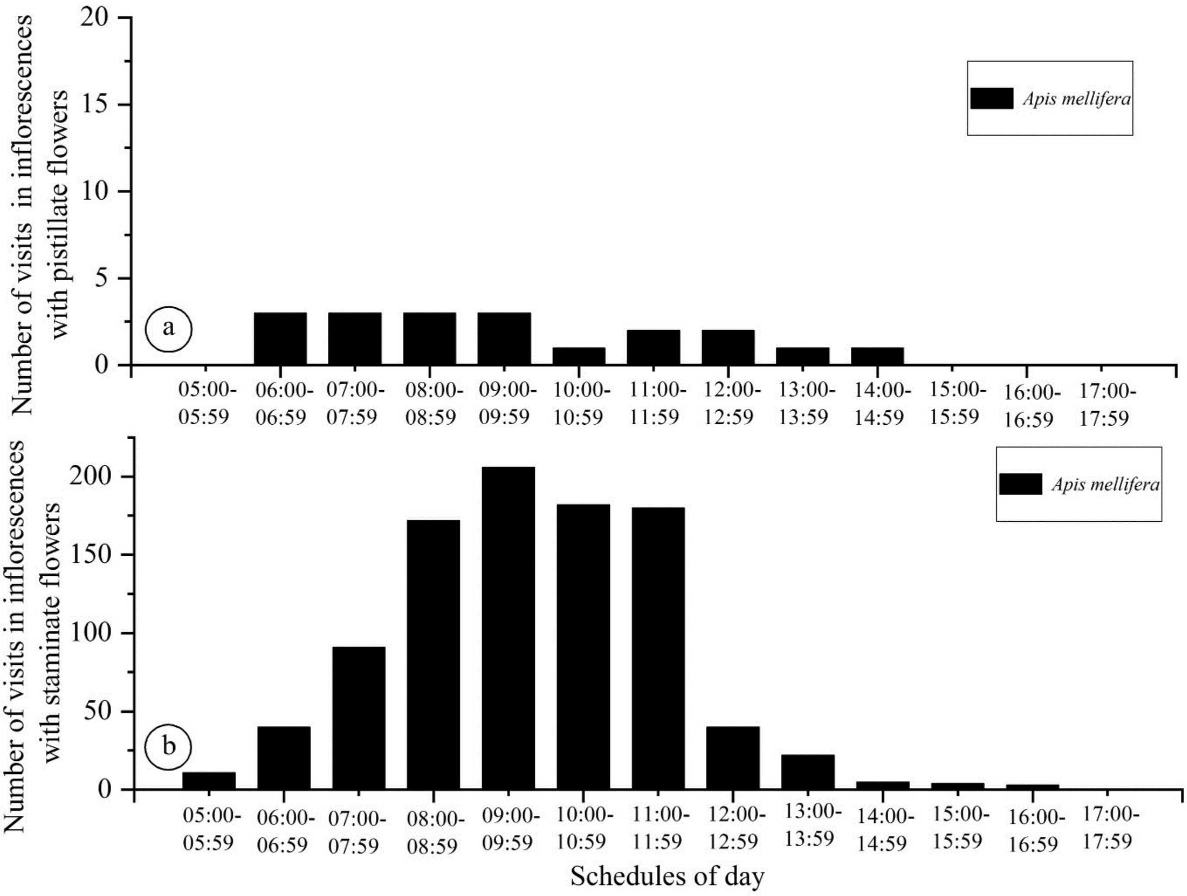

Figure 2. Daily visitation pattern of *Apis mellifera* L. individuals recorded during an observation period of 192 h, between 05:00 h and 18:00 h in the inflorescences of *Croton heliotropiifolius* Kunth (Euphorbiaceae) at Fazenda Ingazeira, Caetité, Bahia, Brazil. a. Daily pattern of visits to pistillate flowers; b. Daily pattern of visits to staminate flowers.

Table 1. Availability of pollen grains in pre-anthesis buds and on flowers of *Croton heliotropiifolius* Kunth (Euphorbiaceae) exposed to 5 and 10 visitations of *Apis mellifera* L. workers at Fazenda Ingazeira, Caetité, Bahia, Brazil.

Analyzed parameters (n=40)	Quantity of available pollen grains
Buds at pre-anthesis	6000±1250
Flowers after 5 visits	2500±1250
Flowers after 10 visits	1950±1590

Based on the quantitative analysis of pollen grains available per flower (Table 1) and the number of flowers produced per individual of *C. heliotropiifolius*, the number of pollen grains produced by an individual with a canopy at chest height was estimated to be 5.376×10^8. As each individual bee was capable of removing c.a. 405 pollen grains per visit (Table 1), we estimated that each individual of *C. heliotropiifolius* had the capacity to support approximately 33 visitation cycles from a large colony of *A. mellifera* workers, which includes approximately 4×10^4 worker bees (ROUBIK, 1980). This indicates the potential of this *Croton* species to produce and provide pollen grains to the bees of *A. mellifera*.

Although the northeastern region of Brazil does not have commercial producers of bee pollen, the national and international demand for apicultural pollen for human consumption is growing (SANCHEZ et al., 2012). For example, in the state of Bahia, apicultural pollen production undertaken along the southern and extreme southern coast caters only the local demand (MEIRA et al., 2017), underlining the importance of the contribution of apiculture to the developing economy of this state. Therefore, further studies are required to investigate the potential of apicultural pollen-producing plants, such as *C. heliotropiifolius*, to meet the floral resources demand for human consumption.

Flowering Phenology

Flowering in *C. heliotropiifolius* occurred during the rainy season from the end of November to the beginning of April (Figure 3), and was significantly correlated with rainfall (r=0.66, p=0.0043, n = 30) and temperature (r=0.62, p=0.001, n = 30). However, the peak flowering phenophase ranged from December to January, during which, the indices were > 50% (Figure 3b). Moreover, the high activity indices recorded in the months of December (94%), January (100%), and the second-half of February (94%), and a synchrony index of 0.77 illustrated the annual flowering pattern of *C. heliotropiifolius*, wherein all individuals of the population flowered at the same time during the reproductive cycle (Figure 3b).

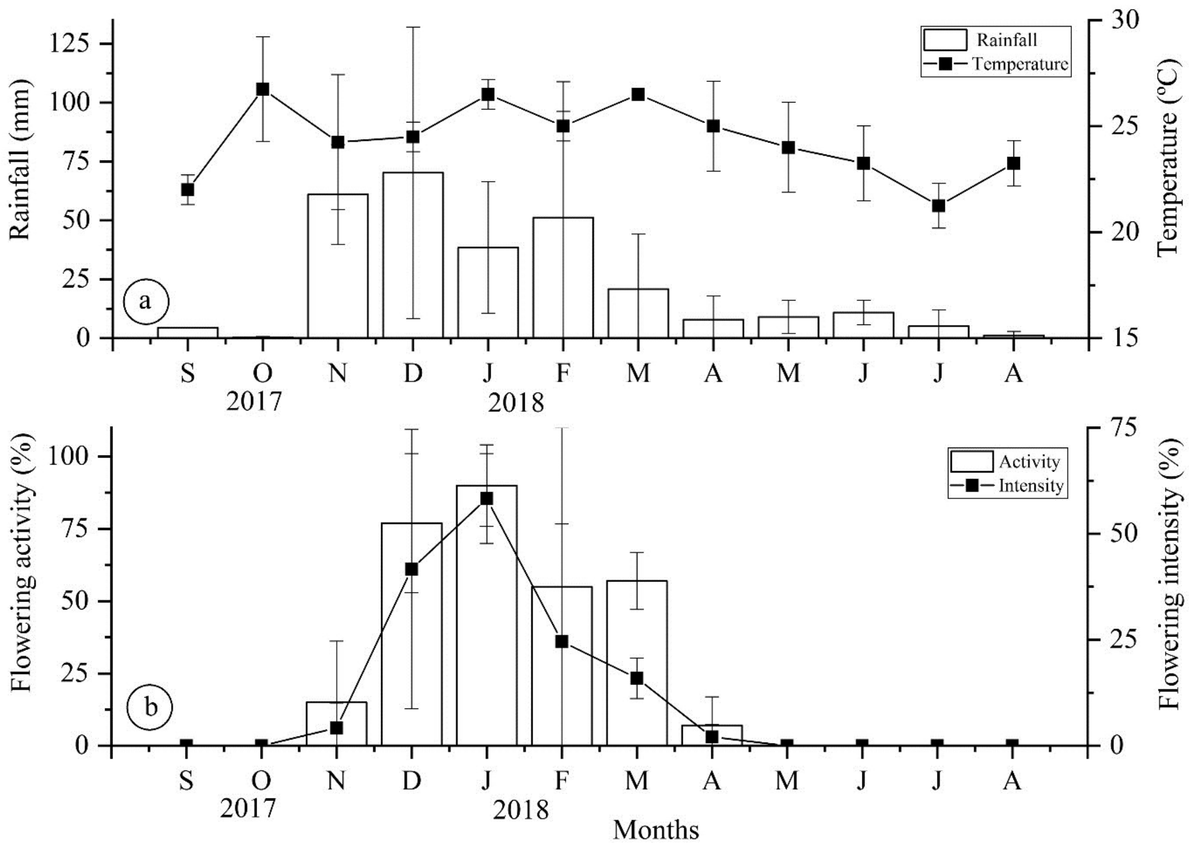

Figure 3. a. Mean monthly rainfall and temperature data (with standard deviations) of Caetité, Bahia, Brazil, from September 2017 to August 2018, based on the data collected at station 83339 of the Instituto Nacional de Meteorologia (INMET); b. Mean monthly flowering activity and flowering intensity (with standard deviations), in *Croton heliotropiifolius* Kunth (Euphorbiaceae) at Fazenda Ingazeira, Caetité, Bahia, Brazil.

The annual flowering pattern in *C. heliotropiifolius* (NEWSTROM; FRANKIE; BAKER, 1994), along with the cornucopia flowering strategy (GENTRY, 1974), is a phenological characteristic that ensures greater number of flowers and floral resources in their environments for several days (FERNANDES; VENTURIERI; JARDIM, 2012), thereby supporting foraging by several non-specialized visitor guilds.

The weekly quantitative phenological survey of available flowers (open flowers) on the five inflorescences of randomly selected individuals revealed three peaks of staminate flower production, namely, December (81.7%), January (54.5%), and February (95.7%). However, the peak in pistillate flower production was observed only in January (45.5%), indicating that staminate flowers were more abundant (Figure 4a). Moreover, the PS in the selected individuals (Figure 4b) revealed that most flowers exhibited exclusively male functions (PS= 0) in December, January, and February, while only a few individuals exhibited exclusively female functions (PS=E), and a considerable number of individuals exhibited overlaps between the two (PS>0).

Therefore, the high frequency of visits from *A. mellifera* workers on staminate flowers can be attributed to the dominance of these flowers in *C. heliotropiifolius* populations during the flowering period.

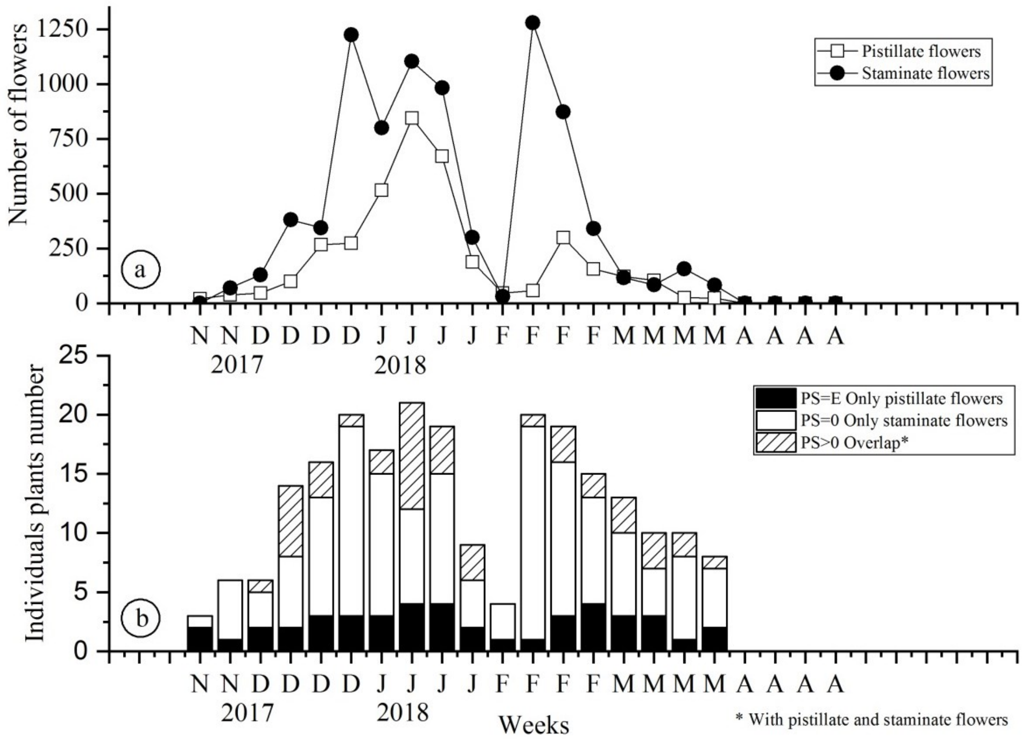

Figure 4. a. Weekly quantitative phenological survey data of available (open) flowers on five inflorescences per individual (n = 20) of *Croton heliotropiifolius* Kunth (Euphorbiaceae) during the flowering period (22 November 2017–28 March 2018); b. Weekly data of patterns of sexual expression in *C. heliotropiifolius* (n = 20) during the flowering period at Fazenda Ingazeira, Caetité, Bahia, Brazil.

CONCLUSION

The beekeeping potential of *C. heliotropiifolius* is clearly highlighted by the high visitation rates of bees to its flowers, its intense and synchronous flowering, and its capacity to produce abundant pollen grains, which serve as an important nutrition source for *A. mellifera*. These characteristics reveal the importance of the utilization and management of *C. heliotropiifolius* in beekeeping activities.

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