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# Quantifying the Floral Nectar Production of the Threatened Aloe *Benishangulana* (Aloaceae) Herb to Uplift the Existing Conservation Approach

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## ABSTRACT

*Aloe benishangulana Sebsebe & Tesfaye is a threatened member of geophytes under the family Aloaceae represented by a single population restricted to a narrow zone (1000m$^2$) with three mature individuals in Benishangul Gumuz Regional State, Assosa area, Village-11. To quantify the floral nectar production 1014 flowers were sampled from six openhandedly watered open-grown plots from April to June 2022 and October to April 2023. The collected data was analyzed by using Prism 8.4.3 (Graph Pad Software). The statistical analysis for investigating the differences among insect types in flower visitation rate, which was the main study question, was conducted using R 3.0.2. The lmer function from the lme4 package was utilized for all analyses. A. benishangulana showed 16 mean visitations per 1m period by honeybees (23%). During stage II of floral development, the average cumulative sugar content per flower in floral nectar was 0.9 mg. However, during stage III, it increased significantly to 1.25 mg. Similarly, the average cumulative nectar volume was 0.99 µL during stage III. Although it was higher (1.36 µL) during stage II, this difference was not statistically significant. The mean sugar concentration (°Brix) was 67.8 during stage II, and it was slightly higher (70.3) during stage III, but again, not significantly so.*

***Keywords:*** *Aloaceae, Aloe benishangulana, geophyte, nectar, threatened*

## 1. Introduction

The Aloaceae family has been used as a source of medicine for both humans and animals since ancient times. In the present day, there is a growing global demand for food and raw materials that contribute to human health, nutrition, and disease prevention [10]. Nectar secretion in plantations increases the frequency of cross-pollination, which ultimately leads to higher yields and better-quality of fruits [4, 5, 13, and 14]. Aloe species including *Aloe greatheadii* are the major indigenous beeplant in Africa [9].


***Figure1. Flowers of Aloe benishangulana, with three bud development phases***

Phenotypic floral traits, such as the color of the corolla, the shape and size of the flower, the volume and concentration of nectar, the scent, and the timing of anthesis, are often developed to attract potential pollinators [8] and [19].

Aloe benishangulana is a threatened member of the geophytes in the family Aloeceae from Benishangul Gumuz Regional State, Ethiopia [20]. These authors also provide an account of the reproductive biology of other A. benishangulana synonyms. It is known that the flowers of this species have three developmental stages: stage I (immature flower shorter than 2cm); stage II (mature, but flower bud still closed and flower length between 2-4cm); and stage III (mature and flower bud is open and with >4cm length) (Figure. 1). The nectar sampling was only conducted during the Stage II phase, in which the corolla is fully open but the anthers have not yet dehisced. Nectar plays a crucial role in attracting animal pollinators [6] [7], and therefore, our objectives were to expand the sample size for studying nectar production and to collect data from both floral phases, thus addressing a gap in our current understanding.

## 2. Materials and Methods

### 2.1. Insects' census data

Different accessions of *A. benishangulana* plants were conserved at the Bambasi Medicinal Plant Field Gene Bank using bulbs collected from the Village-11 locality outcrop [20] approximately eight years ago. To determine the number of bees visiting the flowers of the aloe under investigation, we counted a total of 1,014 flowers with open florets on a strip of aloe at the Bambasi Medicinal Plant Field Gene Bank sites. The strips were marked, and we recorded the number of bees visiting these flowers hourly between 4:00 am and 10:00 pm local time. From April 15th to May 3rd, 2023, we collected 1,014 flowers from four open-grown plant plots that were generously watered. When we observed an insect, we counted the number of "flowers" it probed in one minute, starting the stopwatch at the first probing.

After counting for one insect, we would move further down the patch or to another plant to observe a different insect, avoiding pseudo-replication. On average, it took nearly a minute to complete a count for one insect.

### 2.2. Nectar sugar production data

For each recorded nectar volume and refractometer reading, we pooled the nectar from approximately 1014 flowers. Subsequently, the total nectar volumes and amount of sugar were divided by the number of flowers to create a per-flower basis graph (refer to Fig. 2). The nectar extraction process involved the use of micro-capillary tubes, and the procedure was observed through a dissecting microscope. This extraction was conducted immediately after removing the flowers from the plant. Cumulative nectar volume, sugar concentration, and total sugar content were calculated for each sample using the methods described by [16] and [22]. It's worth noting that a very thin film of nectar remained at the base of the corolla after removal, suggesting that the reported volumes and amount of sugar are likely underestimated. The nectar sugar production measurement was done for 1014 flowers of *A. benishangulana* species planted in Bambasi Medicinal Plants Field Gen Bank the field from April–June 2022 and October–April 2023 using the same methods as [15], [11] To ensure comparability with values obtained from published literature, sampling locations were restricted to the Bambasi Medicinal Plants Field Gen Bank due to limitations in the natural population of the species.

The total volume of sugar produced in two floral development stages (s; µg of sugars per 24 hr) was calculated using the formula:

$$s = 10dvC$$

Where: - $v$ is the volume collected (µl)
- $d$ is the density of a sucrose solution at concentration $C$
- $C$ is the concentration of the sucrose solution

The density ($d$) of the sucrose solution was obtained using the formula: $$d = 0.0037921C + 0.0000178C^2 + 0.9988603$$

### 2.3. Data analysis

To demonstrate that every plant attracted a variety of insects and to offer a comprehensive overview of the diversity of insect species per plant type, we present only descriptive statistics for the census data. For examining the variations in insect visitation rates to flowers, which was the primary focus of the study, all statistical analyses were conducted in R 3.0.2 utilizing the lmer function from the lme4 package [2].

When both groups passed the Shapiro-Wilk normality test and had similar variances [17], T-tests (two-tailed) was utilized to assess significant differences between the two stages of floral development. In cases where one or both groups did not pass the normality test and/or had dissimilar variances [24], the Mann-Whitney test (two-tailed) was employed.

## 3. Results

### 3.1. Census of Insect Present

Seven out of eight insect functional groups, including honey bees, butterflies, hoverflies, non-Apidae bees, beetles, flies, and wasps, were found to be abundant in A. benishagulana flowers, with a total of 2296 individuals recorded (see Figure 2).

Some seasonal variation was observed, with wasps (predominantly Vespidae) being most prevalent in summer, coinciding with the bloom period in fall, likely due to the peak population of their colonies during this time [11], [18], [20]. Honey bees were the most common insect observed in the census (23%), followed by butterflies (17%), hoverflies (14%), and wasps (13%). However, flies were not abundant in winter, presumably because their colonies typically decline and die out in late summer.

**Table 1:** Summary of observations, mean visitation rate, and post hoc significance of floral visitation. Rows are ordered based on visitation rate. Data on the rate of floral visitation (flowers probed/minute) were collected from a total of 2296 insects.

| Insect group | Observation (n) | % | Mean rate/minute | Post hoc significance |
|---|---|---|---|---|
| Honey bee | 520 | 23 | 10.6 | 0.0003[a] |
| Butterflies | 398 | 17 | 4.9 | 0.0010[b] |
| Hoverflies | 321 | 14 | 5.3 | 0.0300[c] |
| Wasps | 307 | 13 | 2.8 | 0.0080[d] |
| Non-Apidae bees | 286 | 13 | 2.1 | 0.0040[e] |
| Beetles | 209 | 9 | 3 | 0.060[ef] |
| Moths | 179 | 8 | 2.4 | 0.0007[g] |
| Flies | 76 | 3 | 3 | 0.050[gh] |

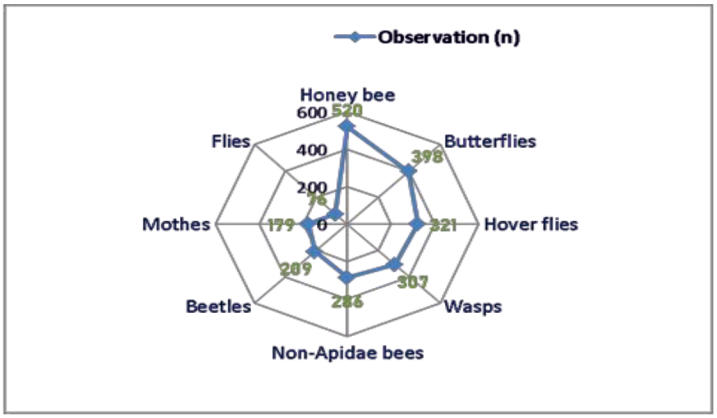


**Figure 2: Radar describing the insect groups' visitation over Aloe benishangulana**

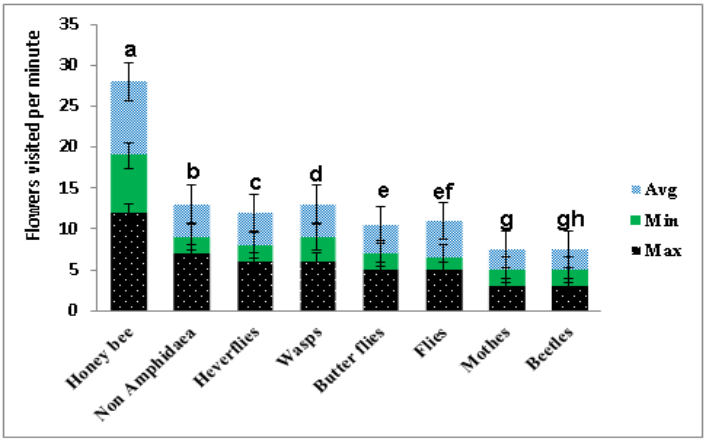


**Figure 3: Insects group with flower visitation analysis**

None-Apidae bees visit flowers at the slowest rate (2.1 flowers/minute), while honey bees visit flowers at the fastest rate (10.6 flowers/minute) and hoverflies visit flowers at nearly the fastest rate (5.3 flowers/minute) following honey bees. All pairwise comparisons between insect groups showed significant differences, except for flies and butterflies, as well as flies and moths. Post hoc results are indicated by letters, where groups sharing letters do not significantly differ. Box plots depict medians, lower and upper quartiles, with whiskers extending to either the maximum or minimum data points, or up to 1.5 times the interquartile range. Insect groups are ranked according to their flower probing rate.

### 3.2. Quantity of nectar

The nectar volume measured was 1.87 µL (range 0.3–2.1 µL, SD 0.346) in stage II of development, and 0.45 µL (range 0.46–2.22 µL, SD 0.24) in stage III. In stage II of floral development, the sugar concentration (oBrix) averaged 71.8 (range 38–81, SD 6.78), while during the hermaphroditic phase, it averaged 70.3 (range 35–79, SD 12.24). The sugar content per flower was 0.9 mg (range 0.4–1.6 mg, SD 0.37) in the pistillate phase and 1.25 mg (range 0.9–1.8 mg, SD 0.25) in the hermaphroditic phase (Figure 3).

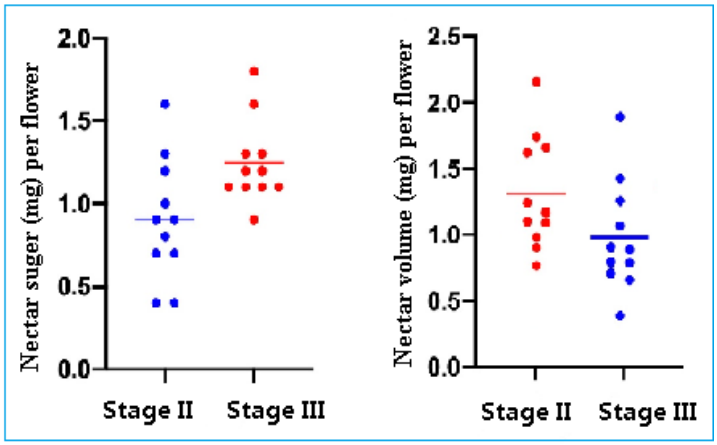


**Figure 3.** The total sugar content per flower is notably higher in stage III of the development phase compared to stage II. However, the overall nectar volume is generally greater during stage II than in stage III, although not significantly so.

There was a significant difference in sugar content between stage II and stage III of the development phases (*P = 0.004, T = 2.43, df = 14*). While more nectar was found in stage II of the development phase, the difference in nectar volume between the two floral development stages was close to being significant (*P = 0.005, T = 1.044, df = 14*). Sugar concentration (°Brix) was higher in stage II of the development phase, although not significantly so (P = 0.096, Mann Whitney U = 29.6, n = 12 in each group).

## 4. Discussion

The frequent floral visitation revealed that more than five insects and some birds love to visit the flower of *A. benishangulana* with néctar and pollen as a reward. Moreover, honey bees are the most abundantly visiting insects of all groups. This result is different from the other studies stating that sunbirds (Nectarinia souimanga; Nectariniidae) therefore appear to be the primary pollinators [6], [18]. The birds are specialized pollinators that invert the stiff tubular flowers of *Aloe divaricala* and *A. benishangulana* with their beaks and collect the nectar produced without damaging the flowers [3].

[7]. Moreover, it was observed that diverse groups of insects visitation of the plant's flower is an indication of better production and suitability of sugar as a food reward. This idea is in agreement with the study [21] pointing that specialized pollination by birds, chafer beetles, pompilid wasps, vespid wasps, and possibly bee species was because of strong attraction by the flower.

*A. benishangulana* has three flower stages (Figure 1). This discrepancy may be attributed to differences in floral morphology, physiology, and ecological context between the two species. Factors such as flower size, shape, and age, as well as environmental conditions and pollinator behavior, can influence nectar production and availability. Further research is needed to elucidate the underlying mechanisms driving variations in nectar production among different plant species and their implications for plant-pollinator interactions and ecosystem dynamics. Given that the amount of sugar per flower is much larger in stage III than in stage II floral development phase and that nectar volume is often higher during stage II than stage III development phase, it was discovered that nectar is produced during both floral development phases (II & III). This species has higher volumes of sugar and nectar than the most popular species of Allium [1]. Nectar's water component evaporates while its sugar component stays in the flower if it is not extracted by hand or by a pollinator [23]. In contrast, Human H's study [12]. These discrepancies highlight the importance of considering species-specific characteristics and ecological factors when interpreting nectar production and composition data. Further investigation into the underlying mechanisms driving nectar variation within and among plant species is warranted to enhance our understanding of plant-pollinator interactions and ecosystem dynamics. and therefore, the existente of low volume in stage III was due to evaporation of wáter through flower opening at this stage, and somehow higher average concentration sugar during stage II was because the flower bud at this stage was mature but was still closed so that evaporation and disturbance by insects were minimal. To the contrary the study by Human H, [12]. The study showed minor variations in nectar volume and concentration between the floral tube and the bulb. In contrast, research by Human H. (12) revealed no significant differences in nectar volume and concentration between the bulb and the floral tube of Aloe greatheadii. However, significant variations were observed between different flower stages of the same species.There are few field gen bank collections of *A. benishangulana* and little is known about this threathened species and therefore, the study was aimed at filling the information gap on reproductive biolodg of the targated species and hence uplift the existing conservation approach stipulated by Ethiopian Biodiversity Institute [16, 20, 23].

## 5. Conclusion

Our study result revealed that like many other known plants with melliferous flowers *A. benishangulana* plant was visited by significant number of insects most notably by honeybees. Moreover, the quantity of nectar and sugar determined intermes of volume for *A. benishangulana* falls in a tolerable range which enables the species to have a significant value not only as a medicine, a value that could be gained from its endemicity, ornamental values but also food for honeybees. Therefore, recommending this evergreen and continuously flowering succulent endemic geophyte for outdoor and backyard plantation has a manifold benefits. On one hand, the plant has an

attractive flower that could be used as an ornament. But for most, since this species is melliferouse it is an input to intensify urban agriculture especially in the apiculture sector where nowadays the government of Ethiopia is taking a tremendous initiative, and on the other hand, it will be an important strategy and approach to conserving this threatened species with a limited natural population.

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