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ORIGINAL ARTICLE

Extrafloral nectaries on leaf margins and their ant visitors in bitter gourd, *Momordica charantia* (Cucurbitaceae): A case of facultative mutualism

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Abstract. Extrafloral nectaries (EFNs) are nectar-secreting structures found on various plants that attract insects, especially ants, which may in turn provide indirect defense against herbivores. This study investigates the presence, morphology, and ecological role of EFNs in *Momordica charantia* (bitter gourd) and documents the diversity and spatial behavior of its ant visitors. Field observations and scanning electron microscopy revealed button-shaped EFNs located along the adaxial leaf margin, which first appeared at the flowering stage and peaked during the early fruiting stage before declining. Six facultative ant species – *Tapinoma melanocephalum*, *Crematogaster subnuda*, *Camponotus compressus*, *Camponotus sericeus*, *Tetraponera rufonigra*, and *Leptogenys* sp. – were recorded visiting the EFNs. All species spent significantly more time on the leaf margins than on other plant parts, with *T. melanocephalum* being the only species to show a strong preference for flowers. The findings highlight a stage-dependent EFN production in *M. charantia* and a preferential ant visitation pattern centered around EFNs, suggesting a potential role for these glands in ant-mediated plant defense. This study underscores the need for further experimental research to assess the defensive benefits of EFN-visiting ants in crop protection.

INTRODUCTION

The Extrafloral nectaries (EFNs) are nectar-secreting glands known to be present on a wide diversity of plants, particularly of tropical and sub-tropical habitats (Díaz-Castelazo et al., 2004). They are reported to occur in at least 100 plant families of angiosperms (Schupp & Feener, 1991; da Costa Barbosa et al., 2025). The morphology of EFNs on EFN-bearing plant taxa has been documented to vary extensively, being scale-like, stalk-shaped, pit shaped, cup-shaped or button-shaped (Díaz-Castelazo et al., 2005). Extrafloral nectar contains mainly sugar (15–75% by weight) along with small amounts of amino acids and other organic compounds (Lanza, 1988; Galetto & Bernardello, 1992; Balduino et al., 2022). EFNs are not directly involved with pollination but have been extensively documented to be visited by ants (Heil et al., 2001; Heil & McKey, 2003) along with a few other insect taxa like flies and wasps (Cuautle & Rico-Gray, 2003; Balduino et al., 2022).

Ant-plant mutualism, especially in species bearing extrafloral nectaries (EFNs), is a well-known interaction where plants offer nectar as a reward to ants in exchange for protection from herbivores. This interaction can significantly reduce herbivory but may also disrupt pollination, leading to complex ecological trade-offs (Calixto et al., 2024). EFNs attract aggressive ants that deter herbivorous insects, reducing leaf and floral damage. For example, *Vicia faba* plants experienced lower herbivory in the presence of ants (Gish et al., 2016), and *Macaranga* species showed over 60% reduction in damage due to ant defense (Heil et al., 2001). Similar outcomes have been reported across multiple taxa (Del-Claro et al., 2016), demonstrating that EFN-mediated ant activity enhances plant fitness by promoting growth and survival.

Thus, while EFNs confer anti-herbivore benefits, they also present reproductive and energetic costs. The mutualism is best understood as facultative, with outcomes dependent on ecological context.

Numerous studies support the protection hypothesis, which suggests ants attracted to EFNs defend the host plant by reducing herbivory and enhancing reproductive success (Janzen, 1977; Tilman, 1978; Koptur, 1984; Oliveira, 1997; Oliveira et al., 1999; Oliveira & Del-Claro, 2005,

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Costa-Silva et al., 2025). However, limited research has examined the full diversity of arthropods visiting EFNs, especially on crop plants (Rudgers, 2004).

The present investigation was carried out to study the EFNs and EFN-visiting ant species of bitter gourd plant, *Momordica charantia* (Linnaeus). *M. charantia* (Family: Cucurbitaceae) is known variously as bitter gourd, balsam pear, bitter melon, bitter cucumber, and African cucumber (Heiser, 1979), widely cultivated in India, Vietnam, China, and other African and American Countries. In the plains of India, the summer crop is sown from January to February, whereas the rainy season crop is sown in the month of May. *M. charantia* is a climber having weak stems and long, flexible tendrils that help it grow upwards and attach to available support. Bitter gourd fruits are a good source of carbohydrates, proteins, vitamins and minerals have the highest nutritive value among cucurbits (Miniraj et al., 1993; Desai & Musmade, 1998). The extract of bitter gourd fruit possess antioxidant, antimicrobial, antiviral, antihepatotoxic, and antiulcerogenic properties while also having the ability to lower blood sugar (Gayathry & John, 2022).

Although *M. charantia* has been reported to bear EFNs on the leaf surface (Jayanti et al., 2019), their presence and morphology remain poorly documented. In contrast, a study on cucurbits in Nigeria even reported the absence of EFNs in this species (Okoli & Onofeghara, 1984). Understanding the diversity of insect visitors to EFNs is crucial, especially as tritrophic interactions involving natural enemies are gaining importance in eco-friendly pest control strategies (Agarwal & Rastogi, 2008; Heil, 2008). Given that bitter gourd is susceptible to numerous pests, a clearer understanding of EFN-mediated indirect plant defense could inform better pest management practices.

Our preliminary field observations revealed that EFNs first appear on *M. charantia* leaves at the onset of flowering stage. Coinciding to their emergence, the plants were visited by several species of ants. This temporal overlap suggests a potential ecological link between EFN production and ant activity, warranting a detailed investigation into the morphology, abundance, and seasonal variation of EFNs, as well as their role in attracting and structuring ant visitor communities. To date, no studies have been conducted on the nature of ant association with this plant. Therefore, we hypothesize that EFN abundance in *M. charantia* peaks at specific phenological (early fruiting) stage, attracting a variety of visiting ant species and leading to their increased activity along the leaf margins. This study investigates the following questions (1) Which plant stages of *M. charantia* bear EFNs? (2) Which ant species visit the EFNs of *M. charantia* (3) What is the spatial visitation pattern of different EFN-visiting ant species on *M. charantia* plants?

2. MATERIAL AND METHODS

This study pertaining to presence, abundance and morphology of extrafloral nectary as well as the visiting ant species along with their visiting pattern on *M. charantia* plants was carried out at St. Andrew`s College Campus, Gorakhpur (26.7606°N, 83.3732°E), India in 2024. The study area falls within the humid subtropical climate zone (Cwa) of the Köppen-Geiger classification, characterized by hot summers, a pronounced monsoon season, and mild, dry winters. Fieldwork was conducted from May to October, encompassing the region's transition from hot-dry (May to June; mean temperature: 31 ± 2°C; relative humidity: 47–64%; average rainfall: 126 ± 11.5 mm) to hot-humid monsoonal conditions (July–September; mean temperature: 28 ± 2°C; relative humidity: 83–84%; average rainfall: 277 ± 12.6 mm), followed by the comparatively pleasant post-monsoon period in October (mean temperature: 25 ± 2°C; relative humidity: ~74%; average rainfall: 43 ± 4.7 mm).

2.1. Plant cultivation

Bitter gourd seeds were sown in 30 medium-sized polybags filled with a 1:1:1 mixture of soil, sand, and compost (mature organic compost prepared from a mixture of vegetable kitchen waste, dry leaves, and cow dung in a 2:1:1 ratio) in the month of May and were kept out doors under natural conditions in the botanical garden of St. Andrew's College with a minimum distance of three feet between each of them. Proper support was provided for the plants to climb and grow. Plants were maintained throughout the fruiting season.

2.2. EFN morphology and abundance

The presence of active EFNs was confirmed with the help of Brix refractometer during all the plant stages of *M. charantia* (Fig. S1). To study EFN presence and abundance, 15 leaves were randomly collected from each growth stage – pre-flowering, flowering, early-fruiting, and late-fruiting plants. EFNs were examined individually under a hand lens, and their number and position on each leaf were recorded. For detailed morphological analysis, dried leaf samples from the early- and late-fruiting stages were sent to Cytogene, Lucknow, for scanning electron microscopy (SEM).

2.3. Ant presence and collection

Ten plants were randomly selected and examined daily from the seedling stage onward for the presence of ants. Ant specimens were collected and preserved for identification in the laboratory at St. Andrew's College.

2.4. Ant spatial visiting patterns

To investigate the spatial visiting pattern of plant-visiting ant species, a mature plant in early-fruiting stage (at this stage leaf's margins bear significantly high number of EFNs as compared to flowering and late fruiting stage at the same time the plants have open flowers as well) was randomly selected and all plant visiting ant species were separately observed for their spatial plant-visiting pattern as each ant visits various plant parts in a sequence and spend time over each plant part. To record the visiting activity of ants individually, an ant present on the main stem was located and time spent by it on each plant part (stem, leaf margin, leaf surface and flower) was recorded carefully with help of a stop watch. The time spent on each plant part by the ant was precisely recorded and each observation was limited to a maximum of 10 min per ant. This process was repeated for 30 ants, covering all ant species observed on the plant during this stage.

2.5. Statistical analysis

The mean number of EFNs across different plant stages was analyzed using one-way ANOVA, followed by Tukey's post hoc test. The mean time spent by six ant species on different plant parts was analyzed using the Kruskal-Wallis test, also followed by Tukey's post hoc test.

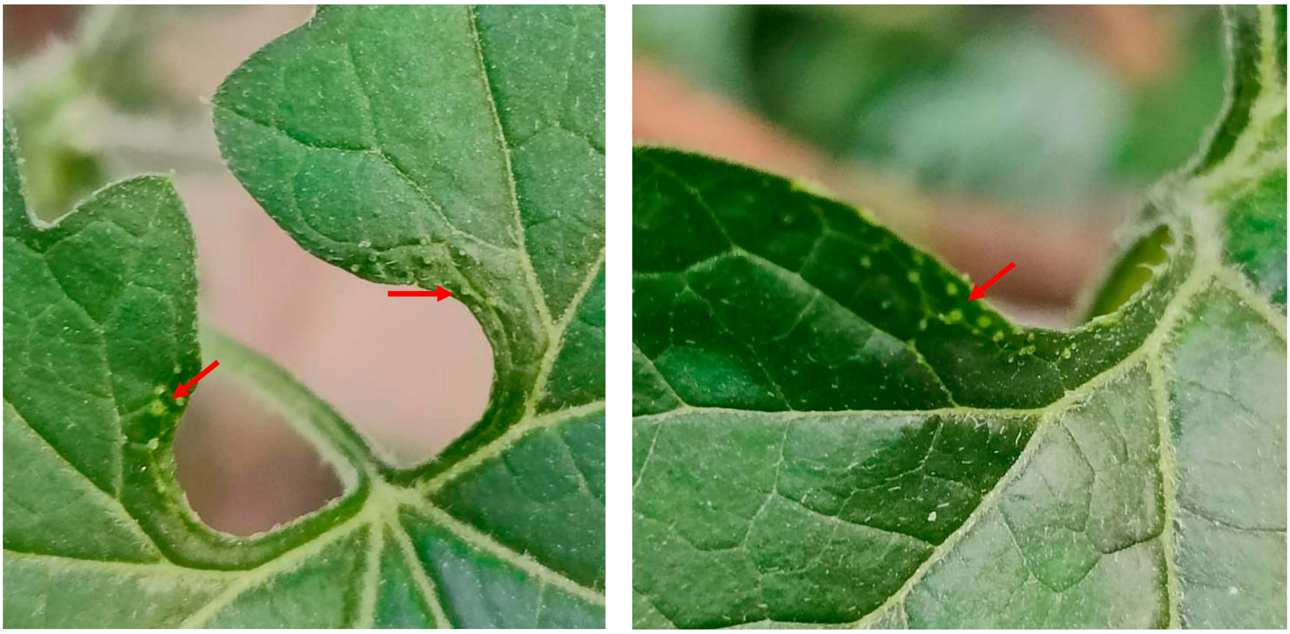

Fig. 1. Extrafloral nectaries on leaf margin of adaxial surface of *Momordica charantia* during flowering period.

3. RESULTS

Leaves of *M. charantia* exhibited numerous button-shaped extrafloral nectaries (EFNs) on the adaxial surface of the leaf margin during flowering, early-fruiting, and late-fruiting stages (Fig. 1). EFNs were absent in pre-flowering stage leaves.

Statistical analysis revealed a significant variation in EFN abundance across the four plant stages ($F_{3,56} = 95.990$, $P < 0.001$). The early-fruiting stage showed a significantly higher mean number of EFNs compared to flowering and late-fruiting stages ($P < 0.05$), with none observed in the pre-flowering stage (Fig. 2).

Scanning electron microscopy (SEM) images (Fig. 3) showed EFNs arranged both in clusters and scattered along the leaf margin. In early-fruiting stages, the EFNs appeared as open, cup-like structures with raised rims. These button-shaped EFNs, under higher magnification, displayed a central concavity with densely packed secretory cells (Fig. 3A), classifying them as "viscosum" type – raised above the leaf surface as per Rao & Ramayya (1992). In the late-fruiting stage, EFNs were elliptical, thick-margined, with narrow openings and shallower, shrunken cavities (Fig. 3 B).

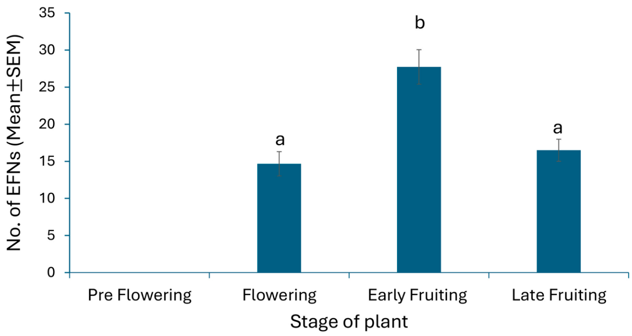

Fig. 2. Number of Extrafloral nectaries (Mean ± SE) on leaves of *Momordica charantia* during pre-flowering, flowering, early-fruiting and late-fruiting stages. Different letters indicate statistically significant differences. (One-way ANOVA followed by Tukeys post hoc test: P < 0.05).

Six facultative ant species (Hymenoptera: Formicidae) were observed visiting the plants: *Tapinoma melanocephalum* Fabricius, *Crematogaster subnuda* Mayr, *Camponotus compressus* Fabricius, *Tetraponera rufonigra* Jerdon, *Camponotus sericeus* Fabricius, and *Leptogenys* sp. (Fig. 4)

The six ant species displayed different spatial visiting patterns on the plant as per the hypothesis that EFN abundance in *M. charantia* plants varies with plant phenology which in turn shapes the diversity of visiting ant species and their spatial visiting pattern as well. The statistical analyses showed that there was significant difference in the mean time spent on four plant parts by ant species (Kruskal Wallis, H: *C. compressus* = 85.52, *T. rufonigra* = 102.589, *Leptogenys* sp. = 80.038, *Cr. subnuda* = 103.141, *Ta. melanocephalum* = 72.644, *C. sericeus* = 104.309, P < 0.001 and df = 3 in each case). Tukeys post hoc test revealed that mean time spent on leaf margin of all the six ant species was significantly higher (P < 0.05) as compared to stem, leaf surface and flower. *C. compressus*, *Leptogenys* sp., and *Cr. subnuda* spent the least time on flowers and leaf surfaces ($P < 0.05$). *T. rufonigra* and *C. sericeus* did not visit flowers, while *Ta. melanocephalum* spent a significantly longer duration on flowers than the other flower-visiting species (Fig. 5).

4. DISCUSSION

The idea of mutualism between plants and defensive ants was first introduced by Thomas Belt (1874), and has since been supported by numerous studies highlighting ants as effective biotic defenders of plants (Janzen, 1966; Koptur, 1984; Fonseca, 1994; Rosumek et al., 2009). A recent

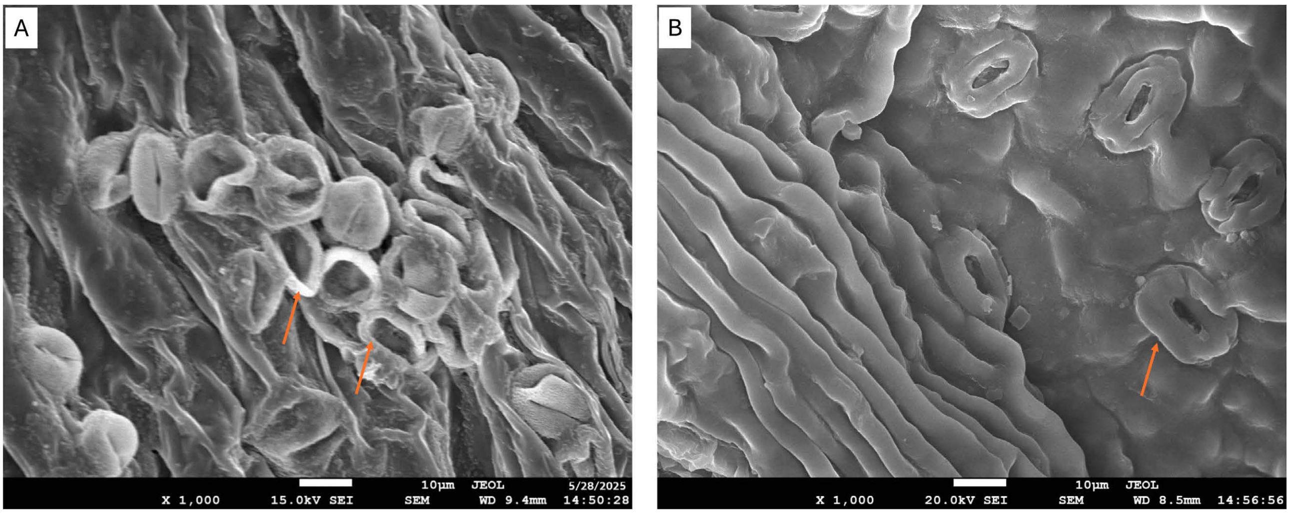

Fig. 3. Scanning electron micrograph of extrafloral nectaries present on adaxial leaf surface of *Momordica charantia* during: A – early fruiting stage; B – late fruiting stage.

study based on phylogenetic signal analyses suggests that ecological interactions between plants and ants might be recently established and rely on the generalist and opportunistic nature of the ant species (Gómez-Lazaga et al., 2025). Moreover, factors like ecological traits, anthropogenic transformation and abiotic elements may also influence the ant-plant interactions (Gómez-Lazaga et al., 2025). A recent study showed that ant-plant mutualism reduces leaf herbivory in a tropical shrub, *Banisteriopsis malifolia* by hosting trophobionts, which indirectly reduced pollinator visit duration and frequency highlighting a nuanced and context-dependent outcomes, rather than a general absence of trade-offs (Costa-Silva et al., 2025). Studies show that there is a context-dependent indirect cost of the facultative ant-plant interaction. Ants visiting the extrafloral nectaries deter pollinators in *Heteropterys pteropetala* (Assunção et al., 2014) and *B. malifolia* (Nogueira et al., 2021) lowering fruit set and revealing indirect costs of this interaction. Ant–plant interactions are broadly categorized into myrmecophytes, which provide nesting sites and form obligate mutualisms with ants, and myrmecophiles, which offer food rewards such as extrafloral nectar (EFN) or honeydew, resulting in facultative associations (Heil, 2010). *M. charantia* fits within the myrmecophilic category, producing EFNs that attract sugar-loving ant species.

Seasonal variation in plant phenological patterns modifies the activity of EFNs and the abundance of ants and herbivores (Calixto et al., 2021). The findings of this piece of investigation clearly show that EFNs in *M. charantia* are absent during the vegetative stage and begin developing at the onset of flowering. Initially appearing near the petiole on the leaf's posterior margin, they gradually spread along the entire margin. Their abundance varies across developmental stages, peaking during early fruiting and declining during late fruiting.

The EFNs of *M. charantia* in early-fruiting stage are rounded, cup-shaped structures with a concavity lined by nectariferous cells, the cup margin is wide and elevated while the EFNs of late-fruiting stage leaves are shrunken with a shallow depression, elliptical in shape having thick margin and narrow opening. The elevated EFNs have been known to produce large volume of nectar rich in sugar content (Díaz-Castelazo et al., 2005) and the change in shape of EFNs from early-fruiting to late-fruiting stage is possibly corelated to the secretory activity of the EFNs which may be at decline with the senescence of plant.

Six ant species were recorded on *M. charantia*: *Tapinoma melanocephalum*, *Crematogaster subnuda*, *Camponotus sericeus*, *Camponotus compressus*, *Leptogenys* sp., and *Tetraponera rufonigra*. All are arboreal and sugar-loving;

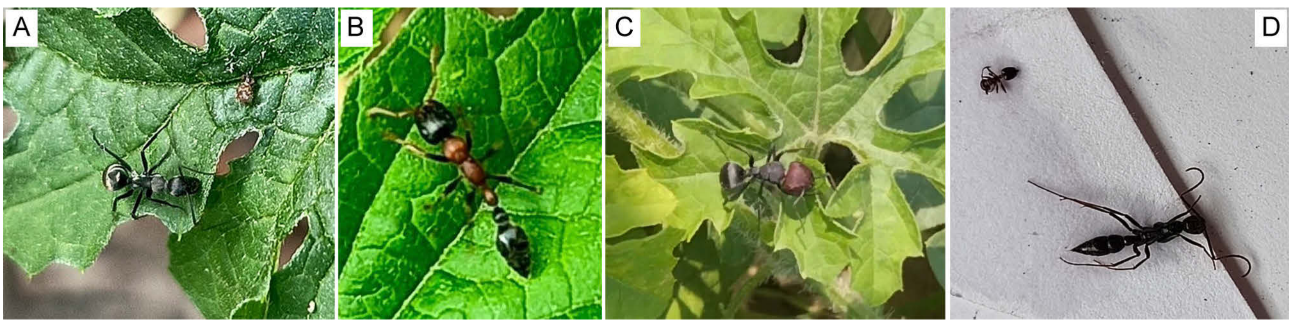

Fig. 4. Ant species visiting *Momordica charantia*. A – *Camponotus compressus*; B – *Tetraponera rufonigra*; C – *Camponotus sericeus*; D – *Crematogaster subnuda* and *Leptogenys* sp.

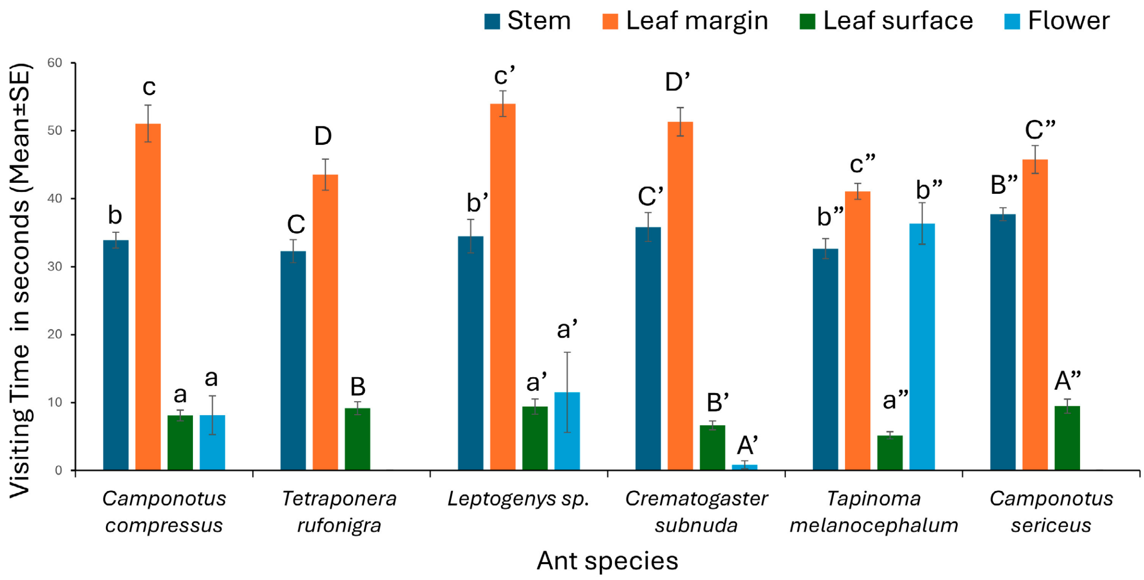

Fig. 5. Spatial visiting pattern of six plant visiting ant species on *Momordica charantia* plants. Means within a panel capped with different letters show significant differences. (Kruskal Wallis test followed by Tukeys post hoc test: P < 0.05.)

among them, *T. rufonigra* and *Leptogenys* sp. are primarily predatory. All species visited stems, leaf surfaces, and especially leaf margins. However, flower visitation varied; only *Ta. melanocephalum*, *C. compressus*, *Cr. subnuda*, and *Leptogenys* sp. visited flowers, with *Ta. melanocephalum* spending the longest time there. In contrast, *C. sericeus* and *T. rufonigra* avoided flowers altogether (fruit surfaces were completely ignored by all ant species during observations).

The most preferred plant region was the leaf margin, where ants spent the majority of their time, likely due to the abundance of EFNs. This preference aligns with the concept that EFN production is a defensive strategy to recruit ants against herbivores (Samuel & Rastogi, 2022). Statistical analyses confirmed that while time spent on stems did not vary significantly among species but time spent on EFN-bearing margins was significantly higher than time spent on leaf surfaces or flowers.

In myrmecophilous interactions, many plants adopt the strategy of distributing food rewards widely across leaves, petioles, and stems, thereby increasing their accessibility to foraging insects, particularly ants (Fiala & Maschwitz, 1991; Heil & McKey, 2003). In *Macaranga tanarius*, for example, this strategy promotes frequent and widespread ant visitation, enhancing plant defense against herbivores (Wong et al., 2025). A comparable condition occurs in *M. charantia*, where extrafloral nectaries (EFNs) are abundantly positioned along the leaf margins. This spatial arrangement makes nectar resources available to a broad range of visitors, including ants, thereby fostering facultative ant–plant mutualism in which ants opportunistically exploit nectar while simultaneously providing variable protective benefits to the plant. Furthermore, the presence of *Crematogaster subnuda, Camponotus sericeus,* and *Camponotus compressus* on *M. charantia* suggests a defensive strategy, as species of *Camponotus* and *Crematogaster* are recognized as effective plant protectors, frequently attacking herbivorous insects on extrafloral-nectar–bearing plants (Souza et al., 2024).

Our findings support the notion that EFN-mediated ant attraction in *M. charantia* represents a facultative mutualism. However, unlike well-documented obligate ant–plant systems, the defensive efficacy of these facultative ant visitors remains unverified. Further experimental studies are necessary to confirm whether these ants offer protection against herbivores. Additionally, the effect of plant developmental stage on EFN production and ant visitation patterns warrants closer investigation.

The presence of *Leptogenys* sp. on *M. charantia* is remarkable as this ant genus is known to be ground-dwelling, nocturnal, and predatory (Wachkoo et al., 2018) but also accepts honey-soaked swabs when provided under experimental conditions (Hashimato et al., 1997). Thus, it can be concluded that arboreal activity of *Leptogenys* sp. observed on these plants suggests an opportunistic shift from predation to sugar foraging when sugar source is easily available.

5. CONCLUSION

This study underscores the role of EFN-mediated ant visitation in *M. charantia* as a facultative ant-plant mutualism linked to its reproductive phenology. EFNs develop only during the flowering and fruiting stages, with their morphology and abundance changing across developmental phases – suggesting a correlation with secretory activity and potential defense needs. Six ant species were documented exploiting nectar resources, showing strong preference for EFN-bearing leaf margins. The diversity of visitors, their differential plant-region use, and the peak EFN activity during early fruiting collectively indicate a strategy to attract opportunistic ants. While this interaction appears to facilitate ant recruitment, its actual protective benefit against herbivores requires further investigation. Future manipulative experiments are essential to validate the defensive function of visiting ants and to unravel how plant ontogeny modulates EFN production, ant attraction, and overall effectiveness of this facultative mutualism.

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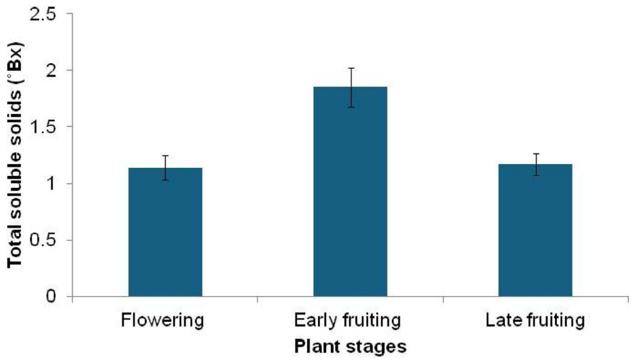

Fig. S1. Total soluble solids (degrees Brix) of extrafloral nectar of *Momordica charantia* during flowering, early-fruiting and late-fruiting stages.