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Impact of Extrafloral Nectaries of *Impatiens balsamina* L. on Incidence of *Myrmicaria brunae* and *Camponotus rufoglaucus*

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***Abstract*:** Morphotypes of extrafloral nectarines were elevated at petioles, basal portion of leaf margins; paired, stalked button shaped in petioles, basal portion of leaf margins, smooth surfaced and bigger on petioles, smaller on leaf margins on *Impatiens balsamina* L. The number of EFNs were maximum and minimum at 36th and 42nd standard week in all the three varieties respectively. Population of *Myrmicaria brunae* was higher at 35th, 34th, 36th standard week. Population of *Camponotus rufoglaucus* J. were maximum at 36th standard week in variety 1 -purple and variety 2 – white and 35th standard week in variety 3 -purple with white. Population of *M. brunae* and *C. rufoglaucus* were lowest at 42nd standard week in all the three varieties. In *I. balsamina*, significantly positive correlation was observed between *M. brunae*, *C. rufoglaucus* and EFN numbers. Significantly positive correlation was also observed between *M. brunae* and *C. rufoglaucus* in variety 1,3 but were non-significant in variety.

Keywords: Extrafloral nectaries, *Impatiens balsamina*, *Myrmicaria*, *Camponotus*

Balsam (*Impatiens balsamina* L.) also known as jewel weed, snap weed or touch-me-not belongs to the family Balsaminaceae. It is an annual plant growing up to 20-75cm tall with a thick, but succulent stem, native to India and Myanmar (USDA-ARS 2018). Balsam is mainly cultivated for its medicinal properties and also grown as ornamental. From roots to seeds, all parts of balsam have medicinal properties (Meenu et al., 2015). Balsaminaceae family which includes genus Impatiens contains extrafloral nectaries glands at petioles as stalk shaped (Nalini et al., 2019). Extrafloral nectar (EFN) is a liquid material rich in carbohydrates with a minimum amount of amino acids, lipids, phenols, alkaloids and volatile organic compounds (Nalini and Sneka 2021). The presence of EFN glands in *I. balsamina* were identified by many authors (Nalini et al., 2019) but the morphology of EFN glands differ from one another. Studies are scanty on the evaluation of the impact of EFNs on ant population in *I. balsamina*. So present study was initiated to throw light on it.

MATERIAL AND METHODS

Experiments were conducted in balsam plants during *Kharif* 2021 and *Rabi* 2021-2022 in the pots at the pot culture yard of Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu. No pesticides were sprayed on the crops throughout the study period. The balsam plant was selected for the presence of more extrafloral nectaries (EFNs). The balsam seeds [variety 1 (purple), variety 2 (white), variety 3 (purple with white)] were procured from Tamilachi ornamental garden located at Kanyakumari district.

Morphology and distribution of extrafloral nectaries: Plant parts like stem, petiole, leaves, calyx, buds, bract, bracteoles, on parts of inflorescence, flower stalks and fruits were checked thoroughly for the presence of extrafloral nectaries (EFNs) structures in balsam of age 30 DAS under stereo zoom microscope (Stemi DV4, Zeiss) at 10x magnification (Nalini *et al*., 2019). EFNs were identified by their conspicuous raised glands or recessed basins and are sometimes coloured differently than the surrounding plant material. Morphotype, location, distribution, shape and surface of EFNs on different plant parts were recorded and photographed (Mortazavi et al., 2017).

Effect of EFNs on ants: In the three varieties of balsam, the number of EFNs (visible as a glossy surface) were counted at petiole and basal portion of leaf margin. The number of ants were recorded at 7-11 am (lasting five minutes in each plant) from one month after sowing at weekly intervals (12 counts). Each treatment was replicated ten times. The ants collected with aspirator were stored in 75% ethyl alcohol (Nalini and Sneka, 2021). Identification of preserved ants to species level was done in the Department of Entomology, Faculty of Agriculture, Annamalai University following the taxonomic keys of Bolton (1994); Tiwari (1999) and Hashimoto (2003) using Stemi DV4 Stereo (Zeiss) microscope.

RESULTS AND DISCUSSION

Morphology and distribution of extrafloral nectaries in *I.*

***balsamina*:** Morphotypes of extrafloral nectaries (EFNs) on *I. balsamina* were elevated at petioles and basal portion of leaf margins. EFNs were paired, stalked button shaped on petioles and basal portion of leaf margins. EFNs in *I. balsamina* are smooth surfaced. EFNs sizes were bigger on petioles and smaller on leaf margins. On *I. balsamina* morphotypes of extrafloral nectaries (EFNs) were elevated at petioles and basal portion of leaf margins. EFNs were paired, stalked button shaped in petioles and basal portion of leaf margins. EFNs in *I. balsamina* was smooth surfaced. EFNs sizes were bigger on petioles and smaller on leaf margins. EFNs colours were reddish brown, dark green and pale green in variety 1, 2, 3 respectively. Based on the colour of the flowers, *I. balsamina* was treated as variety 1 (purple), variety 2 (double coloured), variety 3 (white).

Extrafloral nectaries (EFN), nectar-producing glands can be found on leaf laminae, petioles, rachids, bracts, stipules, pedicels, fruit, and other parts of the plant, and their size, form, and secretions differ depending on the taxon (Mizell 2001). The *I. balsamina* has extrafloral nectaries on its leaves and petioles. Extrafloral nectaries in plants of Hong Kong were examined by So (2004). Button-shaped, cup-shaped, stalk-shaped, pit-shaped, and pore shaped are the five major varieties reported on them. The Euphorbiaceae family contains the most extrafloral nectaries, which are always visible structures that attract ants. The extrafloral nectaries of *I. balsamina* are located as paired and stalk shaped on petioles. These observations confirmed the findings of the present study. Nalini et al. (2019) conducted a survey at Annamalai Nagar and Sivapuri. Out of 162 plant species EFNs were identified in 62 plants and listed the morphology and distribution of EFNs for 62 plants for the first time in India and explained that extrafloral nectaries of *I. balsamina* are present on the abaxial surface of the leaf on the lamina and stalked button form at petioles.

Effect of extrafloral nectaries on ants: The number of

Crop name	Morphotype	Location	Distribution	Size	Shape	Surface
Balsam	Elevated nectaries	Petioles and basal portion of leaf margins	Paired	Big (petioles) and small (leaf margin)	Stalked and button shaped	Smooth

Table 1. Effect of EFNs on incidence of *Myrmicaria brunae* and *Camponotus rufoglaucus* in *Impatiens balsamina*

Standard week	Mean number of EFNs/plant*			Mean number of *M. brunae* /plant*			Mean number of *C. rufoglaucus*/plant*		
	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3
31	118.96 (10.82)	81.12 (8.94)	75.84 (8.64)	7.43 (2.71)	6.29 (2.49)	2.94 (1.70)	0.63 (0.79)	0.43 (0.65)	0.29 (0.53)
32	123.20 (11.01)	84.44 (9.12)	78.72 (8.80)	8.83 (2.95)	7.43 (2.71)	4.29 (2.06)	1.14 (1.06)	0.37 (0.60)	0.63 (0.79)
33	128.38 (11.24)	85.84 (9.19)	80.84 (8.92)	18.57 (4.28)	14.43 (3.77)	4.49 (2.10)	1.26 (1.11)	0.43 (0.65)	0.80 (0.89)
34	132.16 (11.41)	89.36 (9.38)	84.00 (9.09)	18.63 (4.28)	15.31 (3.88)	4.66 (2.14)	1.86 (1.35)	0.71 (0.84)	0.57 (0.75)
35	137.28 (11.63)	94.40 (9.64)	89.44 (9.38)	19.29 (4.36)	13.06 (3.59)	7.06 (2.64)	1.34 (1.15)	1.49 (1.21)	0.83 (0.90)
36	144.52 (11.93)	98.48 (9.85)	94.32 (9.64)	16.43 (4.02)	12.11 (3.45)	7.43 (2.71)	2.14 (1.45)	1.77 (1.32)	0.51 (0.71)
37	136.64 (11.60)	88.00 (9.31)	84.32 (9.11)	17.54 (4.16)	11.80 (3.41)	6.11 (2.45)	1.37 (1.16)	0.92 (0.95)	0.69 (0.82)
38	131.92 (11.40)	75.84 (8.64)	68.96 (8.24)	15.97 (3.97)	11.34 (3.34)	5.69 (2.37)	1.21 (1.09)	0.74 (0.85)	0.55 (0.74)
39	126.04 (11.14)	71.60 (8.40)	64.84 (7.99)	14.83 (3.82)	10.06 (3.15)	3.88 (1.95)	1.10 (1.04)	0.52 (0.72)	0.39 (0.62)
40	119.12 (10.83)	69.76 (8.29)	60.08 (7.69)	11.54 (3.37)	6.66 (2.56)	1.83 (1.34)	0.99 (0.99)	0.34 (0.58)	0.29 (0.53)
41	97.2 (9.78)	59.4 (7.65)	51.28 (7.11)	10.06 (3.15)	7.11 (2.65)	0.54 (0.73)	0.78 (0.88)	0.32 (0.56)	0.25 (0.50)
42	84.4 (9.12)	55.2 (7.37)	47.12 (6.81)	7.31 (2.68)	5.49 (2.33)	0.48 (0.69)	0.63 (0.79)	0.26 (0.51)	0.23 (0.48)
Mean	10.99	8.81	8.45	3.64	3.11	1.91	1.07	0.79	0.69
CD (p=0.05)		(0.05)			(0.03)			(0.01)	

*Mean of 10 replications, Values in parentheses are transformed values $\sqrt{x+0.5}$
Variety 1 -purple, Variety 2 - white, Variety 3 -purple with white

EFNs were significantly highest (144.52, 98.48, 94.32) at 36th standard week followed by 35th standard week in variety 1, 2, 3 respectively. The number of EFNs were significantly lowest at 42nd standard week followed by 41st week. Number of *M. brunae* were significantly highest at 35th standard week followed by 34th standard week. Number of *M. brunae* were significantly lowest at 42nd standard week. Numbers of *C. rufoglaucus* were significantly highest at 36th standard week in variety 1 and 2 and 35th standard week in variety 3 respectively followed by 34th, 35th, 33rd standard weeks. Number of *C. rufoglaucus* were significantly lowest at 42nd standard week (Table 1).

The number of EFNs were highest and lowest at 36th and 42nd standard week in all the three varieties. The number of *M. brunae* were highest at 35th standard week in variety 1, 34th standard week in variety 2 and 36th standard week in variety 3 respectively. The number of *C. rufoglaucus* were highest at 36th standard week in variety 1, 2 and 35th standard week in variety 3. The number of *M. brunae* and *C. rufoglaucus* were lowest at 42nd standard week in all the three varieties (Table 1).

Among the three varieties, variety 1 showed highest EFN numbers than variety 2 and 3. The number of EFNs in *I. balsamina* showed increasing pattern from 31st to 36th standard week but from 37th standard week it showed decreasing pattern up to 42nd standard week. As the EFNs of *I. balsamina* were present on petiole and leaf margins their number were more during peak of vegetative stage (34th to 36th standard weeks) in all the three varieties. Also, both ant species (*M. brunae* and *C. rufoglaucus*) were found highest in number on plants during the same period (34th to 36th standard weeks) which shows *I. balsamina* attracted them to safeguard their vegetative parts which are crucial for further flower production. Significantly positive correlation was observed between *M. brunae* (0.764, 0.713, 0.903), *C. rufoglaucus* (0.779, 0.747, 0.769) and EFN numbers in all the three varieties. *M. brunae* and *C. rufoglaucus* were significantly positively correlated in variety 1 and 3 (0.745, 0.787) but were non-significant in variety 2 (0.560) (Table 2).

Nougueira et al. (2012) investigated and found that the proportion of plants tended by ants in each plot was higher in drier not wetter months and at the plant level, ant attendance increased strongly with the number of recently formed shoot nodes, and ants almost never attended plants with limited or no young tissue. Some plant species secrete large amounts of EF nectar from young or middle-aged leaves and less nectar from old leaves (Yamawo et al., 2012), attracting numerous ants to important parts for their growth. This is in accordance to the present study results. Extrafloral nectar is a nutritive resource rich in sugar and amino acids which has a direct impact on ant activity on plants and may improve ant colony fitness (Byk and Del-Claro, 2011). Mohankumar and Nalini (2016) identified ant species such as *Camponotus rufoglaucus* and *Myrmicaria brunae*. For *Mallotus japonicus* plants in the field, most mutualistic ant species such as *Pheidolenoda* and *Crematogaster teranishii* are more frequently observed on EF nectar-rich plants than on other plants, and non-aggressive ant species are observed to visit the plants having less EF-nectar (Yamawo et al., 2014, 2017). These literatures are in line with the present findings.

Several studies narrated the importance of EFNs in sustaining the multitrophic interactions which stated that: EFNs are produced by at least 4,000 plant species throughout the world (Weber and Keeler, 2013). Their nectar functions to attract ants which then repel or kill insect herbivores (Del-Claro et al., 2016, Heil, 2015) and increasing plant growth and reproductive success (Marazzi et al., 2013). Extrafloral nectar implicates the structure and composition of ant communities and for multitrophic ant-plant-herbivore interactions (Dattilo et al., 2015, Lange et al., 2017; Lange et al., 2013). Increased EF nectar production increases ant activity and aggressiveness and is considered to Favor mutualistic ants rather than parasitic ones (Heil, 2013). This partner choice by plants has been considered to be an effective mechanism for stabilizing ant–plant mutualisms (Grasso et al., 2015). Daily variation ant foraging occurred only on plants bearing EFNs. The recruitment of ants to plants with EFNs was five times higher compared to plants without EFNs, with which several species did not interact. Several studies show reduced numbers of ants interacting with plants naturally lacking EFNs compared to plants bearing EFNs in the Brazilian savanna (Maravalhas and Vasconcelos 2014).

Table 2. Simple correlation matrix between EFNs number and different ant species in *Impatiens balsamina*

Parameters	EFNs no.			*M. brunae*			*C. rufoglaucus*		
	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3
EFNs no.	1	1	1	-	-	-	-	-	-
M. brunae	0.764**	0.713**	0.903**	1	1	1	-	-	-
C. rufoglaucus	0.779**	0.747**	0.769**	0.745**	0.560	0.787**	1	1	1

**Significant at 0.01 probability level, variety 1 (purple), variety 2 (white), variety 3 (purple with white)]

CONCLUSION

Morphotypes of extrafloral nectaries (EFNs) on *I. balsamina* were elevated at petioles and basal portion of leaf margins. EFNs were paired, stalked button shaped on petioles and basal portion of leaf margins and smooth surfaced. EFNs sizes were bigger on petioles and smaller on leaf margins. Both ant species (*M. brunae* and *C. rufoglaucus*) were highest number on plants during the same period (34th to 36th standard weeks) which shows *I. balsamina* attracted them to safeguard their vegetative parts which are crucial for further flower production.

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