



STUDIES ON MORPHOLOGY, DISTRIBUTION OF EFNS AND THE ASSOCIATION OF ANTS WITH EXTRA-FLORAL NECTARIES BEARING PLANTS

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Abstract

Morphology (Location, shape) and distribution of EFNs for 62 plants were listed for the first time in India. Fabaceae possessed nectaries on peduncle, on petiole, on Interpetiolar region, on last pair of paripinnately compound leaflets and on lower portion of petiolule. Among Cucurbitaceae EFN location was on abaxial surface of leaf, bract, bracteoles, flower bud, calyx. Amongst Malvaceae family mostly nectaries present on abaxial surface of leaf and one species have on digitate leaf base and bottom of petiole, stem (*Ceiba pentandra*). In Euphorbiaceae nectaries were present on different sites like, abaxial surface of leaf, petiole, stem and calyx. Convolvulaceae mostly own nectaries on abaxial surface of leaf on midrib and base of leaf. Six morphotypes of EFNs were found in 62 plants. Distribution of EFNs on different plant parts falls within the three categories like, single, paired, uniform and scattered. The study on association of ants with 56 EFN-bearing plant species was done for the first time in India. Fabaceae found to be associated with all the three subfamilies of ant. Plant species under family Cucurbitaceae were linked with six species of ants. In Malvaceae, only two ant subfamilies are associated. With Euphorbiaceae, four ant species were associated all falling under the subfamily Myrmicinae. With Convolvulaceae five ant species were related.

Key Words : Morphology, distribution, EFNs, association of ants, EFN-bearing plants.

Introduction

Floral nectar has long been discussed in the context of pollination and thus is widely thought to promote beneficial plant-insect interactions. By contrast, EFN is secreted on the vegetative and less commonly the reproductive parts of a plant and does not contribute to its pollination. It was not until about a half-century later that the protectionists hypothesis became widely accepted (Delpino, 1874). Most, if not all, extrafloral nectary-bearing plants secrete EFN to attract ants, wasps, and parasitoids as the enemies of their enemies to enhance top-down control of the herbivore and thereby their defense against herbivores (Heil, 2008; Heil and McKey, 2003).

The commonest resource plants offer to ants is EFN,

a liquid substance rich in carbohydrates with dilute concentrates of amino acids, lipids, phenols, alkaloids and volatile organic compounds (Gonzalez-Teuber and Heil, 2009). Carbohydrates have been suggested to be key resources for arboreal ants (Davidson *et al.*, 2003). Elias (1983) explained that EFNs show external morphological diversity, distinct anatomical structures, and different ways of releasing nectar. Some nectaries are formed by secretory parenchyma and nectar release occurs via stomata (Fahn, 1979). In others, the nectar is released by cuticle rupture (Paiva *et al.*, 2007). Furthermore, EFNs may also be non vascularized or vascularised.

Ants does many ecological roles that are directly or indirectly beneficial to humans, including natural pest control (Perfecto, 1991; Philpott and Armbrrecht, 2006), soil aeration (Gabet *et al.*, 2003) and nutrient cycling

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(Wagner *et al.*, 2004). Also various ant species, particularly army ants, can be considered top predators because they exert a significant impact on other arthropod populations (O'Donnell *et al.*, 2007). In view of their abundance, their stability as populations, and their feeding habits, ants have a major influence in many habitats (Carroll and Janzen, 1973).

The majority of studies upon ant-plant mutualism have attempted to show that ants defend their host plants against herbivores in exchange for a secure and complete food source which can be presented as extrafloral nectar (EFN). Very few studies have focussed on arthropod diversity at the EFNs (Rudgers, 2004), particularly of crop plants (Agarwal and Rastogi, 2010). With this idea the present study was initiated to know the morphology, distribution of EFNs and ants associated with EFN-bearing plants.

Materials and methods

Survey for EFN-bearing plants and ants

Survey was conducted at Annamalainagar (11°24'N 79°44'E, +5.79 m) and Sivapuri (11°24'N 79°41'E, 5.79m) from September, 2014 to March, 2016. The climate is sub-humid tropical with three distinct seasons occurring at the study area: summer between April and mid of June, winter from November to mid of March (winter), and monsoon season from October to December. Random survey was made at weekly intervals along 50 arbitrarily selected sites in the study area. Totally 162 plants were examined. From EFN-bearing plants, branches were collected with flowers or inflorescence, fruits and put inside individual polythene covers and secured with rubber bands. Details of location of collection, plant name and date were written on the cover and taken to the laboratory.

Ant species present on EFN-bearing plants were collected by hand collection. Collected ant samples were stored in 75 per cent ethyl alcohol. Sorted specimens were placed in glass vials with proper labels having the location of collection, plant name and date. When more than ten individuals were present, seven were removed and point mounted on triangle "points" between procoxa and metacoxa, pinned by entomological pin (size "3") and labelled. All excess ants were stored in 90 per cent ethyl alcohol. Before the ants dried, their legs were pushed ventrally and away from the body, and mandibles of some specimens opened, to facilitate identification (Musthak Ali, 1981).

Identification

Identification of plants

EFN-bearing plants were observed through Stemi

DV4 Stereo (Zeiss) microscope for the confirmation of EFN structures. Identification of plants were done up to species level at IEBL (Insect Ecology and Behavioural Lab), Department of Entomology, Faculty of Agriculture, Annamalai University and confirmation was made with specialists in the Department of Botany, Faculty of Science and Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University.

Morphology and distribution of EFNs

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 EFN structures under stereo zoom microscope (Stemi DV4, Zeiss) at 10x magnification. EFNs were identified by their conspicuous raised glands or recessed basins and are sometimes coloured differently than the surrounding plant material. Location, distribution and morphotype of EFNs on different plant parts were recorded following Diaz-Castelazo *et al.*, (2005) and May Ling (2004) respectively.

Identification of ants and their associations with EFN-bearing plants

Identification of preserved ants to species level were done at IEBL (Insect Ecology and Behavioural Lab), 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. The ant- plant associations were identified and noted for the EFN-bearing plants alone.

Results and Discussion

Morphology and distribution of EFNs

From the survey during September, 2014 to March, 2016, totally 162 plants were examined from the study area. Of which 62 plants were found to have EFN. Morphology and distribution of EFNs for 62 plants were listed for the first time in India Table 1. The families with the more species of EFN- bearing plants were Fabaceae (22 species), followed by Cucurbitaceae (6 species), Malvaceae (6 species), Euphorbiaceae (5 species) and Convolvulaceae (4 species). Basically, six morphotypes of EFNs were found in the present study. EFNs found in the 62 plants vary considerably in size, shape, position and distribution on the leaves and other plant parts. In the present study, it was found that in the family Fabaceae, genus like *Vigna*, *Dolichos* and *Abrus* possessed nectaries on peduncle; *Albizia amara*, *Acacia*, *Desmanthus* and *Samanea* have nectaries on petiole; *Cassia occidentalis* and *Leucana* on Interpetiolar

Table 1: Shape and distribution of EFNs present in different plant species.

S.No	Plant family	Plant species	Site (Location) of EFNs	Shape of EFNs	Distribution of EFNs
1.	Apocynaceae	<i>Vinca rosea</i> L.	Peduncle	Pore -shaped	Uniform
2.	Araceae	<i>Anthurim plowmanii</i> Croat.	Petiole	Pore -shaped	Paired
3.	Balsaminaceae	<i>Impatiens balsamina</i> L.	Abaxial surface of leaf on lamina	Stalked and button-shaped	Paired
4.	Bignoniaceae	<i>Kigelia africana</i> (Lam.) Benth.	Fruit	Button-shaped	Uniform
5.	Bignoniaceae	<i>Tecoma stans</i> (L.) Juss. ex Kunth	Calyx	Pit -shaped	Uniform
6.	Cactaceae	<i>Opuntia littoralis</i> (Engelm.) Cockerell	Areole	Pore -shaped	Scattered
7.	Convolvulaceae	<i>Ipomoea carnea</i> Jacq.	Abaxial surface of leaf on midrib, base of calyx	Pore -shaped	paired/ Uniform
8.	Convolvulaceae	<i>Ipomoea cairica</i> Sweet.	Base of leaf, calyx	Pore -shaped	Paired/ Uniform
9.	Convolvulaceae	<i>Ipomoea aquatica</i> Forsk.	Abaxial surface of leaf near lower portion of midrib, calyx	Pore -shaped	Paired/ uniform
10.	Convolvulaceae	<i>Ipomoea batatas</i> (L.) Lam.	Base of the leaf	Pore -shaped	Paired
11.	Cucurbitaceae	<i>Momordica charantia</i> L.	Abaxial surface of leaf	Pore -shaped	Uniform
12.	Cucurbitaceae	<i>Lagenaria siceraria</i> (Molina) Standl.	Near leaf base	Button-shaped	Paired
13.	Cucurbitaceae	<i>Coccinia grandis</i> (L.) Voigt	Abaxial surface of leaf	Pore -shaped	Uniform
14.	Cucurbitaceae	<i>Cucurbita pepo</i> L.	Abaxial surface of leaf	Stalk- shaped	Uniform
15.	Cucurbitaceae	<i>Luffa acutangula</i> (L.) Roxb.	Abaxial surface of leaf, bract, bracteoles, flower bud, calyx	Pore- shaped	Uniform/scattered
16.	Cucurbitaceae	<i>Luffa aegyptiaca</i> Mill.	Abaxial surface of leaf, bract, bracteoles, flower bud, calyx	Pit and pore-shaped	Uniform /Scattered
17.	Combretaceae	<i>Terminalia catappa</i> L.	Adaxial surface of leaf on midrib and secondary vein	Pore -shaped	Paired/Scattered
18.	Euphorbiaceae	<i>Ricinus communis</i> L.	Abaxial surface of leaf base, inflorescence of peduncle, petiole	Button shaped	Paired/Scattered
19.	Euphorbiaceae	<i>Chrozophora rotleri</i> (Geiseler) A. Juss. ex Spreng.	Abaxial surface of leaf	Pit -shaped	Paired
20.	Euphorbiaceae	<i>Croton</i> sp.	Petiole	Pore -shaped	Uniform
21.	Euphorbiaceae	<i>Euphorbia heterophylla</i> L.	Stem, calyx	Pit and Pore - shaped	Uniform/Scattered
22.	Euphorbiaceae	<i>Croton bonplandianus</i> Baill.	Abaxial surface of leaf on midrib	Pit -shaped	Paired
23.	Fabaceae	<i>Cassia occidentalis</i> L.	Interpetiolar region	Pit -shaped	Single
24.	Fabaceae	<i>Cassia hirsuta</i> L.	Near axillary bud	Pit and button shaped	Single
25.	Fabaceae	<i>Albizia amara</i> (Roxb.) Boiv.	Petiole	Pore -shaped	Single
26.	Fabaceae	<i>Albizia lebeck</i> (L.) Benth.	Petiole, last pair of paripinnately compound leaflets.	Pit -shaped	Single /scattered
27.	Fabaceae	<i>Vigna mungo</i> (L.) Hepper	Peduncle	Pore -shaped	Scattered
28.	Fabaceae	<i>Vigna radiata</i> (L.) R. Wilczek	Peduncle	Pore -shaped	Uniform

Table 1 cont.

Table 1 cont.

29.	Fabaceae	<i>Vigna unguiculata</i> (L.) Walp.	Peduncle		Pore -shaped	Scattered
30.	Fabaceae	<i>Vigna trilobata</i> (L.) Verdc	Peduncle		Pore -shaped	Scattered
31.	Fabaceae	<i>Vigna</i> sp.	Peduncle		Pore -shaped	Scattered
32.	Fabaceae	<i>Dolichos lablab</i> L.	Peduncle		Pore -shaped	Scattered
33.	Fabaceae	<i>Dolichos lablab</i> var. <i>typicus</i> L.	Peduncle		Pore -shaped	Scattered
34.	Fabaceae	<i>Delonix regia</i> (Boj. ex Hook.) Raf.	Near last pair of paripinnately compound leaflets, on petiole		Button- shaped	Scattered
35.	Fabaceae	<i>Samanea saman</i> (Jacq.) Merr.	Lower portion of petiolule		Pit -shaped	Uniform
36.	Fabaceae	<i>Samanea</i> sp. 1	Petiole		Pit -shaped	Single
37.	Fabaceae	<i>Samanea</i> sp. 2	Petiole, last pair of paripinnately compound leaflets.		Pit- shaped	Scattered
38.	Fabaceae	<i>Acacia baileyana</i> F. uell	Petiole		Pit and pore - shaped	Single
39.	Fabaceae	<i>Acacia auriculiformis</i> A.Cunn.ex Benth.	Petiole		Pore -shaped	Single
40.	Fabaceae	<i>Prosopis juliflora</i> (Sw.) DC.	Lower portion of petiolule		Button-shaped	Uniform
41.	Fabaceae	<i>Abrus precatorius</i> L.	Peduncle		Pore -shaped	Scattered
42.	Fabaceae	<i>Tamarindus indica</i> L.	Abaxial surface of leaflets		Pore -shaped	Scattered
43.	Fabaceae	<i>Leucaena leucocephala</i> (Lam.) de Wit	Interpetiolar region		Pit -shaped	Single
44.	Fabaceae	<i>Desmanthus virgatus</i> (L.) Willd.	Petiole		Pit -shaped	Single
45.	Lamiaceae	<i>Ocimum gratissimum</i> L.	Stem		Pore -shaped	Single
46.	Lamiaceae	<i>Clerodendrum bungei</i> Stud.	Abaxial surface of leaf near midrib		Pore -shaped	Uniform
47.	Lamiaceae	<i>Gmelina asiatica</i> L.	Calyx		Pore- shaped	Uniform
48.	Liliaceae	<i>Lilium longiflorum</i> Thunb.	Calyx		Pore -shaped	Scattered
49.	Malvaceae	<i>Hibiscus cannabinus</i> L.	Abaxial surface of leaf on midvein, petiole, calyx, flower bud		Pore- shaped	Uniform/Single
50.	Malvaceae	<i>Hibiscus rosa-sinensis</i> L.	Abaxial surface of leaf on veins		Pore- shaped	Uniform
51.	Malvaceae	<i>Gossypium hirsutum</i> L.	Abaxial surface of leaf on midrib, on epicalyx		Pit and Pore - shaped	Single
52.	Malvaceae	<i>Thespesia</i> sp. 1	Abaxial surface of leaf, calyx		Slit- shaped	Scattered
53.	Malvaceae	<i>Thespesia</i> sp. 2	Abaxial surface of leaf		Slit- shaped	Uniform
54.	Malvaceae	<i>Ceiba pentandra</i> (L.) Gaertn.	Digitate leaf base and bottom of petiole, stem		Pit shaped	Single/ scattered
55.	Malpighiaceae	<i>Malpighia emarginata</i> (D.C)	Calyx		Button shaped	Uniform
56.	Moraceae	<i>Ficus hispida</i> L.f.	Abaxial surface of the leaf on veins		Pore- shaped	Scattered
57.	Moringaceae	<i>Moringa oleifera</i> Lam.	Petiolate		Stalk -shaped	Uniform/Scattered
58.	Pedaliaceae	<i>Sesamum indicum</i> L.	Peduncle		Pit-shaped	Single
59.	Pedaliaceae	<i>Sesamum allatum</i> Thomn.	Peduncle		Pit-shaped	Single
60.	Passifloraceae	<i>Turnera ulmifolia</i> L.	Base of the leaf		Pit-shaped on short stalk	Paired
61.	Rubiaceae	<i>Morinda citrifolia</i> L.	Capitulum		Pore- shaped	Uniform
62.		Unidentified sp.	Peduncle		Pore- shaped	Single

region; *Delonix regia*, *Samanea* sp. 1 and *Albizia lebbbeck* on last pair of paripinnately compound leaflets; *Prosopis julifera* and *Samanea saman* on lower portion of petiolule Table 1.

Among Cucurbitaceae in *Momordica charantia*, *Coccinia grandis* and *Cucurbita pepo* EFN location was on abaxial surface of leaf; others like *Luffa acutangula*, *Luffa aegyptiaca* possess on abaxial surface of leaf, bract, bracteoles, flower bud, calyx (Table 1). Amongst Malvaceae family mostly nectaries present on abaxial surface of leaf and one species have on digitate leaf base and bottom of petiole, stem (*Ceiba pentandra*). In Euphorbiaceae nectaries were present on different sites like, abaxial surface of leaf, petiole, stem and calyx. Convolvulaceae mostly own nectaries on abaxial surface of leaf on midrib and base of leaf. In other families, Pedaliaceae on peduncle, Rubiaceae on capitulum, Cactaceae on areole, Bignoniaceae on fruit and calyx.

May Ling (2004) pointed out that from the types of EFNs observed, the species specificity seems rather weak which is similar to the present findings. According to his observations in a study with EFNs in Hong Kong plants, members of the family Caesalpiniaceae have stalk-shaped EFNs between the leaflets while members of the Convolvulaceae have pore-shaped EFNs on the petiole just below the lamina. Members of Mimosaceae have pit-shaped EFNs. However, members of Euphorbiaceae have varied EFNs, from the cup-shaped in *Vernicia montana* to four maculate glands in *Alchornea trewioides*.

In accordance to the present observations Ghosh *et al.*, (2015) also stated that extrafloral nectaries are nectar-secreting structures that are especially common in the family Fabaceae. Light and electron microscopic structure reveals that the morphologically differentiated petiolar extrafloral nectaries of *Acacia auriculiformis*, *A. catechu*, *A. mangium* have an complex anatomical structures consisting of an epidermis, central secretory region and vascular region. Four species possess more than one morphotypes of gland structure.

Extrafloral nectaries (EFNs) occur in 93 flowering plants and five fern families and can be abundant among the flora of many habitats worldwide, especially in the tropics (Bentley, 1977; Koptur, 1992; Oliveira and Freitas, 2004). The nectar glands are secreting glands which are structurally diverse and occur on virtually all above ground plant organs; they are especially common on the leaf blade, petiole, young stems, stipules and on reproductive structures such as buds, calyx, inflorescence axis, lower peduncles and fruits (Koptur, 1992; Elias, 1983). Similar studies were also conducted by Diaz-Castelazo *et al.*,

(2005) whom reported that for 13 of the species studied (65%), the EFNs were associated with vegetative tissues (leaves, stems, meristems, etc.). Roughly one-third (35%) of the species (seven of 20) had EFNs associated with both vegetative and reproductive structures (buds, bracts, inflorescence stems, etc.); no plants with EFNs associated exclusively with reproductive structures were found for the selected species. Ten species distributed among five plant families presented EFNs in more than one location on the plant body. EFNs were observed on leaf blades of five of the 20 species, (25%) on young stems (25%), growing meristems (20%), stipules (20%), calyx/ fruits/ bracts (20%), inflorescence stems (15%), leaf petioles (15%) and leaf rachis (15%).

It is understood from the available reports that the location of EFN glands vary conspicuously among the members of different families such as Cythraceae where the EFNs present in the leaf blade, in Vochysiaceae EFNs seen in the stem, peduncle, petiole and in Chrysobalanaceae and Malpighiaceae the EFNs located in leaf blade. In families like Ochnaceae, Fabaceae, Rosaceae, Malvaceae, Myrsinaceae, Bignoniaceae, Verbanaceae the EFNs are present in the stipules, rachis, petiole, leaf blade, stem (Machado, 2008). Besides in members belong to families Apocynaceae, Boraginaceae, Cactaceae, Compositae, Convolvulaceae, Meliaceae, Tureraceae the EFNs are located in nodes, bracts and phyllaries also (Diaz-Castelazo *et al.*, 2005). The shape of such glands varies very conspicuously in many other species. *Chamaecrista fasciculata* has cup shaped while *Senna marilandica* and *S. hepecarpa* are clavate (Durkeel *et al.*, 1999); as hair like structures (trichomes) in *Hibiscus pernambucensis* (Rocha, 2009). Elevated EFN in plants such as *Terminalia argentea*, *T. brasiliun*, *Lafoensia pacari* and *Enterolobium gummiferum*. Flattened type of glands are present in *Licania humilis*, *O. urateaspectabilis*, and in *O. castanaefolia* whereas in *Bauhinia rufa*, *Rapanea guianensis*, and *R. lancifolia*, it is has glandular trichomes (Machado *et al.*, 2008). The macromorphological features viz., shape, size and colour revealed that the EFN of *Cassia hirsuta* is spherical in shape which is attached in the basal portion of the petiole through a very short stalk. The number of glands in each plant differ from one another as glands are present at the base of each petiole evenly. The number of glands is depending on the number of compound leaf in a plant which in turn vary according to the age of the plant (Tamby and Yogamoorthi, 2015).

In the present study Fabaceae had single morphotype of EFN in 24 species of which pore-shaped EFN in 12 species; pit- shaped EFN in *Cassia occidentalis*, *Albizia*

Table 2: Association of ant species with EFN-bearing plant species.

S. No.	Plant family	Plant species	Ant subfamily	Ant species
1.	Apocynaceae	<i>Vinca rosea</i> L.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
			Pseudomyrmecinae	<i>Tetraponera nigra</i> (Jerdon)
2.	Arecaceae	<i>Anthurim plowmanii</i> Croat.	-	-
3.	Balsaminaceae	<i>Impatiens balsamina</i> L.	-	-
4.	Bignoniaceae	<i>Kigelia Africana</i> (Lam.) Benth.	Pseudomyrmecinae	<i>Tetraponera nigra</i> (Jerdon)
5.	Bignoniaceae	<i>Tecoma stans</i> (L.) Juss. ex Kunth	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
			Pseudomyrmecinae	<i>Tetraponera nigra</i> (Jerdon)
6.	Cactaceae	<i>Opuntia littoralis</i> (Engelm.) Cockerell	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
7.	Convolvulaceae	<i>Ipomoea carnea</i> Jace.	Myrmicinae	<i>Crematogaster</i> sp.
				<i>Pheidole</i> sp.
8.	Convolvulaceae	<i>Ipomoea aquatic</i> Forssk	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
9.	Convolvulaceae	<i>Ipomoea cairica</i> Sweet.	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
			Pseudomyrmecinae	<i>Tetraponera nigra</i> (Jerdon)
10.	Convolvulaceae	<i>Ipomoea batatas</i> (L.) Lam.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
11.	Cucurbitaceae	<i>Momordica charantia</i> L.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
12.	Cucurbitaceae	<i>Lagenaria siceraria</i> (Molina) Standl.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
13.	Cucurbitaceae	<i>Coccinia grandis</i> (L.) Voigt	Myrmicinae	<i>Crematogaster</i> sp.
14.	Cucurbitaceae	<i>Cucurbita pepo</i> L.	Myrmicinae	<i>Meranoplus bicolor</i> (Guerin-Meneville)
				<i>Solenopsis geminata</i> (Fabricius)
15.	Cucurbitaceae	<i>Luffa acutangula</i> (L.) Roxb.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
			Myrmicinae	<i>Solenopsis geminata</i> (Fabricius)
16.	Cucurbitaceae	<i>Luffa aegyptiaca</i> Mill.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
			Myrmicinae	<i>Pheidole</i> sp.
17.	Combretaceae	<i>Terminalia catappa</i> L.	Myrmicinae	<i>Solenopsis geminata</i> (Fabricius)
18.	Euphorbiaceae	<i>Ricinus communis</i> L.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
				<i>Monomorium scabriceps</i> (Mayr)
				<i>Solenopsis geminata</i> (Fabricius)
19.	Euphorbiaceae	<i>Chrozophora rottileri</i> (Geiseler) A. Juss. ex Spreng	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
20.	Euphorbiaceae	<i>Croton</i> sp.	-	-
21.	Euphorbiaceae	<i>Euphorbia heterophylla</i> L.	Myrmicinae	<i>Solenopsis geminata</i> (Fabricius)
22.	Euphorbiaceae	<i>Croton bonplandianus</i> Baill.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
				<i>Pheidole</i> sp.

Table 2 cont.

Table 2 cont.

23.	Fabaceae	<i>Cassia occidentalis</i> L.	Myrmicinae	<i>Meranoplus bicolor</i> (Guerin-Meneville)
24.	Fabaceae	<i>Cassia hirsuta</i> L.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
25.	Fabaceae	<i>Albizia amara</i> (Roxb.) Boiv.	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
26.	Fabaceae	<i>Albizia lebbek</i> (L.) Benth.	Pseudomyrmecinae	<i>Tetraponera rufonigra</i> (Jerdon)
27.	Fabaceae	<i>Vigna unguiculata</i> (L.) Walp.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus irritans</i> (Smith, F)
28.	Fabaceae	<i>Vigna mungo</i> (L.) Hepper	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
				<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus irritans</i> (Smith, F)
			Myrmicinae	<i>Camponotus sericeus</i> (Fabricius)
			Pseudomyrmecinae	<i>Solenopsis geminata</i> (Fabricius)
			Formicinae	<i>Tetraponera nigra</i> (Jerdon)
29.	Fabaceae	<i>Vigna radiata</i> (L.) R. Wilczek	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
				<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus irritans</i> (Smith, F)
				<i>Camponotus sericeus</i> (Fabricius)
30.	Fabaceae	<i>Vigna trilobata</i> (L.) Verdc	Pseudomyrmecinae	<i>Tetraponera nigra</i> (Jerdon)
			Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
31.	Fabaceae	<i>Vigna</i> sp.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
32.	Fabaceae	<i>Dolichos lablab</i> L.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
			Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
33.	Fabaceae	<i>Dolichos lablab</i> var. <i>typicus</i> L	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
				<i>Camponotus sericeus</i> (Fabricius)
			Pseudomyrmecinae	<i>Tetraponera nigra</i>
34.	Fabaceae	<i>Delonix regia</i> (Boj. ex Hook.) Raf.	Myrmicinae	<i>Phidole</i> sp.
				<i>Crematogaster</i> sp.
35.	Fabaceae	<i>Samanea saman</i> (Jacq.) Merr.	Pseudomyrmecinae	<i>Tetraponera rufonigra</i> (Jerdon)
36.	Fabaceae	<i>Samanea</i> sp. 1	Formicinae	<i>Oecophylla smaragdina</i> (Fabricius)
37.	Fabaceae	<i>Samanea</i> sp. 2	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
38.	Fabaceae	<i>Acacia baileyana</i> F. Muell.	Myrmicinae	<i>Phidole</i> sp.
				<i>Myrmecaria brunnea</i> Saunders
39.	Fabaceae	<i>Acacia auriculiformis</i> A.Cunn. ex Benth.	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
40.	Fabaceae	<i>Prosopis juliflora</i> (Sw.) DC.	Formicinae	<i>Camponotus compressus</i> (Fabricius)

Table 2 cont.

Table 2 cont.

41.	Fabaceae	<i>Abrus precatorius</i>	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
42.	Fabaceae	<i>Tamarindus indica</i> L.	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
43.	Fabaceae	<i>Leucaena leucocephala</i> (Lam.) de Wit[1]	Myrmicinae	<i>Crematogaster</i> sp.
44.	Fabaceae	<i>Desmanthus virgatus</i> (L.) Willd.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
45.	Lamiaceae	<i>Ocimum gratissimum</i> L.	-	-
46.	Lamiaceae	<i>Clerodendrum bungei</i> Steud.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				Myrmicinae <i>Myrmecaria brunnea</i> Saunders
47.	Lamiaceae	<i>Gmelina asiatica</i> L.	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
48.	Liliaceae	<i>Lilium longiflorum</i> Thunb.	-	-
49.	Malvaceae	<i>Hibiscus camabinus</i> L.	Myrmicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
			Formicinae	<i>Camponotus compressus</i> (Fabricius)
50.	Malvaceae	<i>Hibiscus rosasinensis</i> L.	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
51.	Malvaceae	<i>Gossypium hirsutum</i> L.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
				<i>Camponotus sericeus</i> (Fabricius)
				<i>Camponotus irritans</i> (Smith, F)
			Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
52.	Malvaceae	<i>Thespesia</i> sp. 1	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
53.	Malvaceae	<i>Thespesia</i> sp. 2	Myrmicinae	<i>Crematogaster</i> sp.
54.	Malvaceae	<i>Ceiba pentandra</i> (L.) Gaertn.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
55.	Malpighiaceae	<i>Malpighia emarginata</i> (DC)	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
56.	Moraceae	<i>Ficus hispida</i> L.f	Myrmicinae	<i>Myrmecaria brunnea</i> Saunders
57.	Moringaceae	<i>Moringa oleifera</i> Lam.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
58.	Pedaliaceae	<i>Sesamum indicum</i> L.	Formicinae	<i>Camponotus rufoglaucus</i> (Jerdon)
			Myrmicinae	<i>Solenopsis geminata</i> (Fabricius)
				<i>Monomorium scabriceps</i> (Mayr)
59.	Pedaliaceae	<i>Sesamum allatum</i> Thonn.	Formicinae	<i>Camponotus compressus</i> (Fabricius)
				<i>Camponotus rufoglaucus</i> (Jerdon)
60.	Passifloraceae	<i>Turnera ulmifolia</i> L.	Myrmicinae	<i>Solenopsis geminata</i> (Fabricius)
61.	Rubiaceae	<i>Morinda citrifolia</i> L.	Formicinae	<i>Polyrhachis rastellata</i> (Latreille)
62.		Unidentified sp.	-	-

lebbeck, *Samanea saman*, *Samanea* sp. 1, *Samanea* sp. 2, *Leucaena leucocephala* *Desmanthus virgatus* (Table 1). Also it bore two morphotypes of EFN in single plant in two species namely, *Cassia hirsuta* and *Acacia bellayana* (pit and button-shaped EFN, pit and pore-shaped EFN respectively).

Cucurbitaceae had three morphotypes of EFNs viz., pore (*Momordica charantia*), button (*Lagenaria sineraria*) and stalk (*Cucurbita pepo*) shaped EFN Table 1. Also only *Luffa aegyptica* had two morphotypes (pit and pore-shaped EFN). Similarly Euphorbiaceae possess pore (*Croton* sp.), button (*Ricinus communis*), and pit (*Chrozophora rottleri*) shaped EFN. Only *Euphorbia heterophylla* had two morphotypes (pit and pore-shaped EFN). Malvaceae bore pore (*Hibiscus cannabinus*, *Hibiscus rosa-sinensis*), pit (*Ceiba pentandra*) shaped EFN and also peculiarly bore slit shaped in *Thespesia* sp. 1 and sp. 2. In *Gossypium hirsutum* two morphotypes of EFN (pore and pit-shaped EFN) were present. Convolvulaceae (*Ipomoea carnea*, *Ipomoea cairica*, *Ipomoea aquatica*, *Ipomoea batatas*) and Lamiaceae (*Ocimum gratissimum*, *Clerodendrum bungei*, *Gmelina asiatica*) both had pore-shaped EFN in all their recorded species. Passifloraceae *Turnera ulmifolia* had pit-shaped EFN on short stalk.

May Ling (2004) elaborately explained on five morphotypes of EFNs. In that he reported that button-shaped EFNs occur as a pair of round structures at the base of the lamina, alongside the midvein or slightly below. When the leaf is expanding these EFNs are green and often covered with a web of light brown scales during their development. When the leaf is fully expanded, the scales fall off, and the EFNs are green, dark green, or brown. These button-shaped EFNs may be round, as in *Aleurites moluccana*, or oval, as in *Sapium discolor*. In some species, button-shaped EFNs are rather thin and are called maculate glands, usually found at the base of lamina. *Macaranga tanarius* with its very large peltate leaves has several such minute glands bordering the apical margin. They number from 5 to 7 in total and are oval in shape with a flat or concave surface. Maculate glands are more conspicuous in *Alchornea trewioides*. Cup-shaped EFNs are uncommon locally, occurring in six species only. One example is found in *Passiflora suberosa*, a herbaceous climber. These EFNs arise on both sides of the petiole as two small pubescent knobs. Sections of these glands during development show that these knobs gradually become cup-shaped, with a stalk at the base. The rim of the cup is thin and secretory cells occur at the centre of the cup. Stalk-shaped EFNs are also common locally, found in several genera belonging

to different families. Among the 13 species of *Cassia* in Hong Kong, *C. surattensis* is the most common roadside tree. Stalk-shaped EFNs occur as thin stalks on the leaf axis between the lowest 2nd to 4th pairs of leaflets. The apex of the EFN is round and remains so as long as it is secretory. Similar stalk-shaped EFNs are also seen in *Moringa oleifera*, but they are found between every pair of leaflets. Species of *Impatiens* have slender, fleshy, inconspicuous, and almost ephemeral EFNs at a distance from the base of the lamina. Pit-shaped EFNs are not obvious externally and can only be found by careful examination. A typical pit is often seen in *Leucaena leucocephala*. *Acacia auriculiformis*, an introduced species from New Zealand, has a much larger phyllode (up to 15 cm long, 5 cm wide), but the pit-shaped EFNs are similar in position and almost in size to *A. confusa*. These EFNs are closely appressed to the surface and barely visible, and these cryptic structures often elude the naked eye. Species in *Ipomoea* and *Pharbitis* have such obscure pore-shaped embedded-type EFNs at the base of lamina. They are so minute that they are almost invisible externally. The above observations are coinciding with the present findings.

Both vascularized and non-vascularized nectaries were found. Among the first, elevated, flattened, hollow, pit (Zimmerman, 1932; Elias, 1983) could be recognized and what are described as transformed nectaries that have common morphological attributes i.e. abscission scars (Bluthgen and Reifenrath, 2003). Among the non-vascularized EFNs, scale-like nectaries (Zimmerman, 1932; Elias, 1983), capitate, peltate and unicellular secretory trichomes were found. Among five plant species, four belonging to the Papilionoid legume subfamily, displayed more than one morphological type of EFN (Diaz-Castelazo *et al.*, 2005).

EFNs occur on both vegetative (e.g., young stem, leaves, petioles, stipules) and reproductive structures (e.g., buds, calyx inflorescence axis, flower peduncles, fruit) and are structurally diverse (Diaz-Castelazo *et al.*, 2004). 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 (So, 2004; Diaz-Castelazo *et al.*, 2005). These literatures supports the present findings.

Distribution of EFNs on plant parts falls within the three categories like, single, paired, uniform and scattered Table 1. Among the major families Fabaceae (*Albizia*, *Vigna*, *Samanea*, *Acacia*), Euphorbiaceae (*Croton*) and Malvaceae (*Thespesia*) even within same genus distribution of EFNs varies among the parts. But

in Convolvulaceae it was mostly same among the genus *Ipomoea* (paired and uniform) and in Cucurbitaceae even in different genus it was similar (*Cucurbita pepo*, *Coccinia grandis*, *Momordica charantia*- uniform). Also two types of distribution was found in single plant itself in the species like, (*Ipomoea carnea*, *Ipomoea cairica*, *Ipomoea aquatica*, *Euphorbia heterophylla*, *Albizia lebbeck*, *Hibiscus cannabinus*, *Cela pentandra*, *Moringa oleifera*, *Luffa acutangula*, *Luffa aegyptiaca*, *Terminalia catappa*, *Ricinus communis*). Similar findings were also reported by Diaz-Castelazo *et al.*, (2005). From the above investigations it was obvious that the location as well as morphotypes and distribution of EFNs were more diverse among the EFN-bearing plant species studied.

Identification of ants and their associations with EFN-bearing plants

With sixty two EFN-bearing plants 14 species of ants were found to be associated falling under three subfamilies (Formicinae, Myrmicinae and Pseudomyrmecinae). The study on association of ants with 56 EFN-bearing plant species was done for the first time in India. In the present study Fabaceae found to be associated with all the three subfamilies including the species like, *Camponotus compressus*, *Camponotus irritans*, *Camponotus rufoglaucus*, *Camponotus sericeus*, *Oecophylla smaragdina*, *Myrmecaria brunnea*, *Solenopsis geminata*, *Pheidole* sp. *Monomorium scabriceps*, *Meranoplus bicolor*, *Tetraponera rufonigra*, *Tetraponera nigra* except *Polyrhachis rastellata* and *Crematogaster* sp. Also all plant species falling under Fabaceae were found to be associated with more than one ant species except very few Table 2.

EFNs were not directly involved with pollination but have been extensively documented to be visited by ants (Heil *et al.*, 2001; Heil and McKey, 2003) along with a few other insect taxa (e.g., flies, Hesperheide, 1985; wasps, Cuautle and Rico-Gray, 2003). Support for the protection hypothesis or the positive impact of ants on the plants comes from many experimental studies showing increased herbivory and/or lower seed production when ants are excluded from plants (Janzen, 1977; Tilman, 1978; Schemske, 1980; Koptur, 1984; Oliveira, 1997; Oliveira *et al.*, 1999; Oliveira and Del-Claro, 2005).

Plant species under family Cucurbitaceae were linked with six species of ants viz., *Camponotus compressus*, *Myrmecaria brunnea*, *Crematogaster* sp., *Pheidole* sp., *Meranoplus bicolor* and *Solenopsis*

geminata. In Malvaceae, only two subfamilies were associated and most commonly *Camponotus compressus* and *Camponotus rufoglaucus* were found in all the plant species studied except *Thespesia* sp. 2. The following ant species found to be associated with Malvaceae: *Myrmecaria brunnea*, *Camponotus sericeus*, *Camponotus irritans*, *Myrmecaria brunnea* and *Crematogaster* sp. With Euphorbiaceae, ant species associated were *Myrmecaria brunnea*, *Monomorium scabriceps*, *Solenopsis geminata* and *Pheidole* sp. all falling under the subfamily Myrmecinae. With Convolvulaceae (*Ipomoea*) ant species related were *Crematogaster* sp., *Pheidole* sp., *Camponotus compressus* (Fabricius), *Camponotus rufoglaucus* and *Tetraponera nigra*. On plant species of Bignoniaceae family, *Myrmecaria brunnea*, *Tetraponera nigra* were found. With Pedaliaceae, *Camponotus rufoglaucus*, *Camponotus compressus*, *Solenopsis geminata* and *Monomorium scabriceps* were associated. On few plant species viz., *Anthurim plowmanii*, *Impatiens balsamina*, *Croton* sp., *Ocimum gratissimum*, *Lilium longiflorum*, unidentified sp. ants could not be found during the survey Table 2.

According to Lokeshwari *et al.*, (2015) ants of sub-family Dolichoderinae, viz., *Tapinoma indicum* (Forel), *Tapinoma melanocephalum* (Fabricius) and *Technomyrmex albipes* (Fr. Smith) were observed to be associated on Malvaceous and Cucurbitaceous host plant as those of Sub-family; Formicinae viz., *Camponotus compressus* (Fabricius) and *Paratrechina longicornis* (Latreille) tending melon aphids on Malvaceous, Cucurbitaceous and Solanaceous host plants. On the contrary, *C. parvus* Emery was observed on cucurbitaceous host plant and *Prenolepis* sp. was observed on solanaceous host plant. The ant species of sub-family; Myrmecinae viz., *Solenopsis geminata* (Fabricius), was associated on host plants of Malvaceae, Cucurbitaceae and Solanaceae while *Crematogaster* sp. was associated on Malvaceous and Asteraceous hosts. Plant while *Myrmecaria brunnea* Saunders was recognized on cucurbitaceous host plant. *C. compressus* is the most encountered dominant ant species associated with many aphid species on variety of host plants (Ozdemir *et al.*, 2008, Kataria and Kumar, 2013) suggesting its widespread distribution and ability to adapt to various environmental conditions of the study area. This is in accordance with the present findings.

Rudgers (2004) found that visits of ants were naturally variable (in morphology and occurrence). EFNs affect plant fitness correlates, suggesting that the associated ant community (which defends the plant

against herbivores) is influencing the evolution of EFN traits in wild cotton. In this context it cannot be overlooked that the morphological and secretory features of the EFNs of the plants influence attractiveness to ants and that, presumably this can affect the evolution of EFN traits.

As the study area is dominated by arboreal, grassland and agroecosystems the dominant ant species of all these ecosystems like *Camponotus compressus*, *Camponotus rufoglaucus* and *Myrmecaria brunnea* were recorded in many EFN species. This is in line with the studies conducted by Mohankumar and Nalini (2016).

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