



Ants as pollination vectors of ant-adapted *Euphorbia hirta* L. plants

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Abstract

A wide range of ant-plant mutualistic associations are known to occur in terrestrial ecosystems. Although many species of plants recruit ants to obtain protection from herbivores, myrmecophily is rare, since only few plant species are known to be adapted for pollination predominantly by ants. The annual herb, *Euphorbia hirta*, is characterized by the ‘ant-pollination syndrome’ including traits such as unisexual flowers, inconspicuous cyathia clustered in globose cymes, inflorescence-associated tiny nectaries and short, semi-erect stem. In present study, we aimed to examine the pollination ecology of *Euphorbia hirta* and the role of ants in its pollination. Field and laboratory experiments conducted to understand the association between *E. hirta* plants and ants revealed that seven ant species (belonging to five genera and three subfamilies) visited *E. hirta* plants. The visitation patterns of ants revealed that in a single foraging trip the ants visited a number of plants indicating their potential role in cross-pollination. Field-based exclusion experiments revealed production of significantly higher number of fruits in *E. hirta* plants under open pollination treatment as compared to other treatments. Scanning electron microscopy revealed that the adherence of the pollen grains to the bristles located on ant mouth-parts was facilitated by the three vertical, semi-circular grooves present on the grain surface. The number of fruits produced per plant was higher in ant-included plants as compared to only winged insect-pollinated and only self-pollinated plants suggesting that the plant-visiting ants are successful pollination vectors of *E. hirta* and while promoting self-pollination also contribute towards cross-pollination services.

Keywords ‘ant-pollination syndrome’ · Cross-pollination · Ant nests · Inflorescence-associated nectaries

Introduction

Association between insects and plants frequently leads to various types of antagonistic or mutualistic interactions. Entomophily, i.e., pollination by insects, is one of the best studied mutualistic associations in which the plant benefits from its association with the pollinating insect species. Majority of flowering plants are visited by taxonomically diverse insects and pollinator specialization is relatively less common (Ollerton 1996; Waser et al. 1996). Consequently, many insect-pollinated flowers are highly conspicuous with specific shape, size and colour of the corolla; often have adhesive pollen grains and a substantial caloric reward in the form of floral nectar (Newstrom and Robertson 2005; Faegri

and Van Der Pijl 2013). In contrast, wind pollinated plants have small inconspicuous flowers, produce large amounts of powdery pollen and are often lacking the floral nectar (Berry and Calvo 1989; Albuquerque et al. 2013).

Apart from being the most dominant taxon of phylum Arthropoda, ants are also common and abundant in a variety of terrestrial ecosystems. In general, ants are not considered to be good pollinators on account of their smooth body surface (which prevents pollen adherence), their self-grooming behavior and the presence of metapleural glands, the secretion of which inhibits pollen germination (Beattie et al. 1984, 1985). Although ant species having affinity for sugar are reported to be floral visitors of several plant species, they are either found to be nectar thieves (Lach 2005), or disrupt pollination, either directly by damaging flowers (Martins et al. 2020), or by interfering with pollinator vectors (Cembrowski et al. 2014; Sinu et al. 2017; Villamil et al. 2018; Unni et al. 2021) or indirectly by adverse effect on pollen viability caused by metapleural gland secretions (Beattie et al. 1984, 1985; Dutton and Frederickson 2012). Moreover, many species of ants are usually much smaller than the floral

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reproductive structures of the visited plants. Ants are also considered to be poor cross-pollinating agents on account of their crawling habit, while their smooth body surface is not conducive for pollen adherence (Del-Claro et al., 2019 and references therein). Further, pollenivory (feeding on pollen) is also known to occur in some ant species (Urbani and Andrade 1997; Byk and Del-Claro 2010; Czechowski et al. 2011).

Ants as pollination vectors could be evolutionarily favoured in habitats which have a high abundance of different ant species and the plants are short with near-ground-level inflorescences and abundant, small, sessile, open flowers and nectar is the main reward (Hickman 1974; Gómez et al. 1996; de Vega et al. 2009). In fact, these features together characterize the ‘ant-pollination syndrome’ (Hickman 1974). In recent years, many studies have revealed the role of ants as pollinators, leading to an increase in the recognition of ants as possible or even effective pollinators. Consequently, ants have been implicated as the main pollinator in at least 57 plant species (de Vega et al. 2009). Many more plant species have been added to this list in recent times (Claessens and Seifert 2017; Kuriakose et al. 2018; Del-Claro et al. 2019).

Pollination by ants is commonly regarded as a low-energy system, where plant species, while not using energetically costly resources to attract insect pollinators, have evolved certain traits for attracting ants (Ibarra-Isassi and Sendoya 2016). The role of ants as pollinators has been reported (Gómez et al. 1996; de Vega et al. 2009; Del-Claro et al. 2019; Delnevo et al. 2020) in many plant species, which exhibit specific traits (such as small height, prostrate-erect stem, are located close to the soil surface, have a high density of flowers occurring close to the stem and possess very small nectaries). Ants along with one or more species of winged pollinators are documented to provide pollination services to some plant species, particularly of arid and alpine areas (Gómez and Zamora 1992; Gómez et al. 1996; Regupathy and Ayyasamy 2011; Delnevo et al. 2020). The participation of many individuals in the transfer of pollen grains from the anther to the stigma is suggested to compensate for their inefficiency (Gómez and Zamora 1992). Additionally, the worker ants being wingless visit relatively fewer numbers of flowering plants in a single foraging trip. For instance, *Pheidole pallidula* (Formicidae: Myrmicinae) ants were recorded as visitors of only a single inflorescence of *Jatropha curcas* (Euphorbiaceae) in one foraging bout and unlike the co-visiting honeybees, the ants were not involved in cross-pollination activities (Samra et al. 2014). Consequently, ants are known to be effective as pollinators only in those plant species which have adapted in one or more ways to facilitate ant-provided pollination services (Gómez et al. 1996; Ibarra Isassi and Sendoya 2016; Delnevo et al. 2020). Moreover, the floral nectar-collecting ant species have the

potential to be involved in the pollinating activities, particularly in those plant species which cannot attract prominent pollinators. Some plants are known to be specialized for ant pollination, and in such cases, floral volatiles may attract ant species that provide pollination services (de Vega et al. 2014).

The pollination ecology of very few ant-pollinated plant species has been studied in India (Aluri 1990; Kuriakose et al. 2018; Raju and Ramana 2018). The garden spurge, *Euphorbia hirta* L. (Euphorbiaceae), commonly known as the asthma plant, is an annual, monoecious (bears male and female flowers on the same plant) weed plant, which flowers almost throughout the year. Although native to America, it is now widely reported in many tropical and subtropical regions (Huang et al. 2012; Rahman and Akter 2013). It is considered to be one of most common and ubiquitous species of the Euphorbiaceae, which is commonly known as the spurge family (Merrill 1981).

Our preliminary field observations revealed visitation of *E. hirta* plants by several species of ants. Till date, no studies have been conducted on the nature of ant association with this plant. We hypothesize that ants obtain food resources from *E. hirta* plants and in turn facilitate pollination services to the visited plants. Ants along with some species of winged insects have been documented as pollinators of several plants belonging to genus *Euphorbia* (Chandra et al. 2013). Hence, it is important to understand whether ant visits to *E. hirta* plants is related to their role as pollinators. Therefore, the following questions were addressed in the present study: (1) what is the diversity of ants on *E. hirta* plants? (2) Does the presence of *E. hirta* plants influence the location of ant nests? (3) Do the ants play a role as pollination vectors of *E. hirta*?

Materials and methods

Study site and system

The field work was conducted from February to July, during 2 years (2018 and 2019), in the Botanical garden of Banaras Hindu University, in Varanasi, India (25°18' N and 80°1' E, 76 m above mean sea level). In this region, the monsoon rains occur from July to September while the winter season extends from November to January (Srivastava 2001). During the rainy and the winter seasons, foraging activities of ants are infrequent; hence a 6-month study was conducted, each year. The study period encompassed the transient spring (February–March) and summer (April–June) seasons and continued till the onset of monsoon season (i.e., till July, each year).

Euphorbia hirta is an annual herb (height ~ 15–50 cm), with prostrate, semi-erect or ascending stem, blotched

leaves and small inconspicuous inflorescence in the form of cyathia (Rahman and Akter 2013), arranged in globose cymes (Fig. 1). The whole plant is pilose and its sap contains latex. *Euphorbia hirta* plant flowers throughout the year. Flowers and fruits with seeds are usually present on the plant at the same time. Each inflorescence consists of very small, reduced unisexual flowers, in which a single female flower is surrounded by few male flowers in an involucre which bears minute nectaries on its periphery. *Euphorbia hirta* is an early colonizer of bare land and can often be found on the weed strips of agricultural fields, roadsides, gardens, fallow lands and waste places. The plant is traditionally used in many parts of Asia for treatment of various diseases, including asthma (Ekpo and Pretorius 2007).

Arthropod diversity and abundance on *E. hirta* plants

To record the diversity and abundance of *E. hirta* plant-visiting arthropods, weekly observations were taken on randomly selected, mature *E. hirta* plants ($n = 20$) from 08:00

to 10:00 h (as the preliminary studies showed the peak foraging activity of the various ant species during this time), from February to July, each year. The number of ant visitors (belonging to each of the plant-visiting ant species) per plant was recorded (observation time spent per plant = 5 min) by the visual scan method (Ekka and Rastogi 2019). The ant specimens were collected and preserved in 75% alcohol and identified till species level with the help of experts from Zoological Society of India, Kolkata, India.

Spatial patrolling patterns of ants

The spatial patrolling pattern is the time spent by an individual worker ant on each plant part during a single patrolling episode (total time taken in ascending and descending from the plant) (Ekka and Rastogi 2019). To determine the spatial patrolling pattern of each plant-visiting ant species, only a single worker ant was allowed to ascend the plant ($n = 15$, each species) during each observation, and the time spent by an individual ant on the inflorescence, leaves and stem, during a single patrolling episode was recorded. All

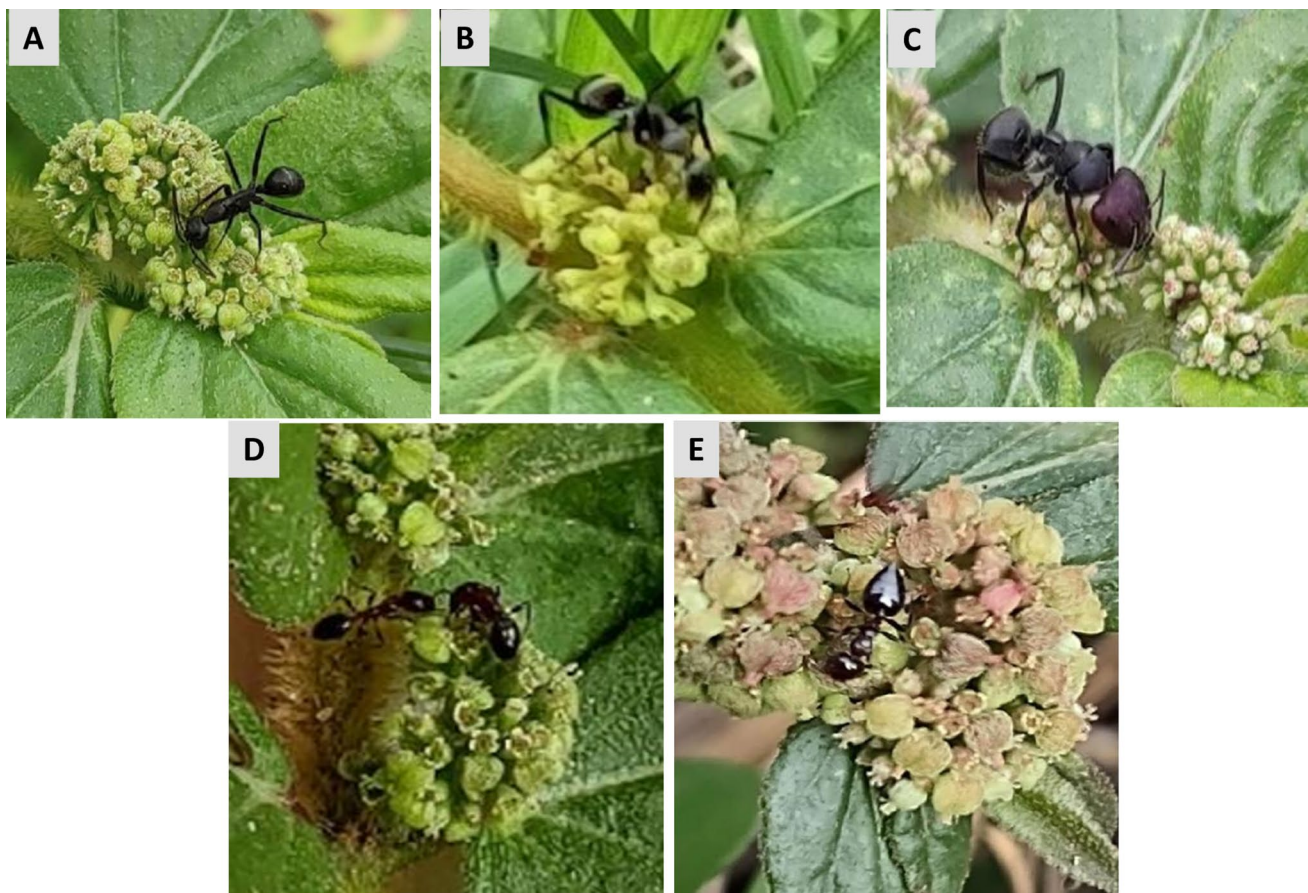


Fig. 1 Five species of ant visitors on inflorescence-associated nectaries of *Euphorbia hirta*: **A** *Camponotus compressus* **B** *Ca. parius* **C** *Ca. seri-ceus* **D** *Trichomyrmex scabriceps* and **E** *Crematogaster subnuda*

observations were taken on mature *E. hirta* plants between 08:00 and 10:00 h.

Spatial distribution of ant nests with reference to *E. hirta* plant clusters

Preliminary field observations revealed that *E. hirta* plants grew in clusters with the mean number of individual plants being 12.34 ± 0.36 in each cluster. The clusters ($n = 30$) of *E. hirta* plants were located in the open field. Nests of different ant species were located by visually scanning the area in the vicinity of the plants or by following the ant trails by attracting the worker ants by means of baits (honey/boiled egg pieces/dead insect) which were placed in that area and then following the returning ants to their nests. The number of nests of each of the seven ant species located at various circular distances (1, 3, 5, 7 and 9 m) from the centre of *E. hirta* plant clusters was recorded.

Plant visitation patterns of ants

Number of *E. hirta* plants visited by a single ant of a specific species during a single foraging trip was recorded to find whether the ants have the potential to play any role in the cross-pollination activity. Plant visitation pattern of members of each ant species was recorded on *E. hirta* plants by the quadrat sampling method ($n = 15$, each ant species; area of each quadrat = 3 m^2), between 08:00 and 10:00 h. A single worker ant of each species was carefully marked (after it exited the nest) with a tiny drop of the quick-drying paint (Testors Aircraft Finishing Set), as per the method used by Rastogi (2007) and then followed to examine its plant visitation pattern. Number of *E. hirta* plants per quadrat visited by the marked ants was recorded.

Exclusion experiments to investigate the role of ants as pollinators of *E. hirta* plants

To investigate the role of ants in pollination of *E. hirta*, field-based exclusion experiment was conducted from Jan to May, 2021. Mature *E. hirta* plants were randomly selected and then placed into four groups ($n = 30$, in each group) each provided with different treatment: (i) Open pollination: the inflorescences of *E. hirta* were open for winged and crawling insects (including ants) as well as for wind pollination, (ii) Winged insect pollination and anemophily: to examine the role of wind and/or other flying insect visitors in *E. hirta* pollination, the entry of ant visitors was prevented by the application of a sticky barrier (tangle trap) at the base of the stem and renewed every alternate day throughout the experimental period, (iii) Ant pollination: butter paper was tied loosely on the whole plant to prevent winged insects and to permit the entry of only the crawling ant-visitors,

(iv) Self-pollination: this involved the exclusion of insects (including ants) and wind, which were restricted by the application of tangle trap and by wrapping butter paper around the stem (Samra et al. 2014), respectively. Weekly data of the number of fruits produced by each plant, of each category were collected during the entire experimental period.

Scanning electron microscopy (SEM)

To examine the floral biology of *E. hirta*, the cyathia with male and female parts were carefully dissected out from *E. hirta* inflorescence and fixed in 2.5% glutaraldehyde and 2% formaldehyde (in the ratio of 1:9) in 0.1 M phosphate buffer (at pH of 7.4) for 12 h at 4°C. The samples were next washed in phosphate buffer three times for 15 min each at 4°C. Dehydration was done in an acetone dilution series (30%, 50%, 70%, 90%, and twice in 100% followed by dry acetone). After critical drying of the samples (at 37°C for 48 h), they were mounted on double-sided carbon tape on stubs. For the visualization and identification of pollen grains, the inflorescence with flowers was directly rubbed on the double-sided tape mounted on the stubs and bulb-dried for 5 h.

To detect the pollen grains (if any were present) on the cuticle of the workers of the plant-visiting ant species, the worker ants of each of the three most abundant ant species were collected immediately following their visit to *E. hirta* inflorescence and bulb dried for 12 h. Sputter Coater (SC7620, Quorum Technologies Ltd., UK) was used for gold coating the plant parts as well as the ant samples and then these were examined with the help of scanning electron microscope (EVO 18, Zeiss).

Statistical analyses

The abundance of *E. hirta* plant-visiting ant species during the different months of the study period was analyzed by using Friedman's test, followed by Bonferroni corrections. The spatial patrolling patterns of the various ant species on the three plant parts (inflorescence, stem, and leaves) was analyzed by Kruskal Wallis test followed by Duncan's post hoc test. Spatial distribution of ant nests at different distances from *E. hirta* plant clusters was analyzed by Two-way ANOVA test followed by Duncan's post hoc test. The plant-visiting patterns of the different ant species in three m^2 quadrats was analyzed by One-way ANOVA followed by Tukey's post hoc test. The variation in the numbers of fruits produced in different treatments during exclusion experiments was analyzed by Kruskal Wallis test followed by Duncan's post hoc test. All the statistical analyses were performed using SPSS v. 25.

Results

Abundance and diversity

Worker ants belonging to seven species represented by five genera belonging to three subfamilies were recorded on *E. hirta* plants, during the entire study period. These included the minor caste workers of three species of carpenter ants, *Camponotus compressus* Fabricius, *Camponotus parius* Emery, *Camponotus sericeus* Fabricius, (Subfamily: Formicinae), the seed harvester ant, *Trichomyrmex scabriceps* Mayr, the acrobat ant, *Crematogaster subnuda* Mayr, *Lepisiota frauenfeldi* Mayr, (Subfamily: Myrmecinae) and the tiny ghost ant, *Tapinoma melanocephalum* Fabricius (Subfamily: Dolichoderinae) (Fig. 1). Significant differences were found in the mean abundance of each of the seven ant species on the plants (Friedman's test: Feb: $\chi^2 = 225.186$; March: $\chi^2 = 153.828$; April: $\chi^2 = 178.518$; May: $\chi^2 = 64.600$; June: $\chi^2 = 27.523$; July: $\chi^2 = 17.402$; $df = 6$ and $P < 0.001$ for all cases) (Fig. 2). The results (after Bonferroni corrections) revealed that the mean abundance of *Tr. scabriceps* was significantly higher than the other co-visiting ant species recorded during the months of February, March and May, whereas in the month of April the mean abundance of three species, *Tr. scabriceps*, *Ca. compressus*, and *Ca. parius* was significantly higher than that of the other co-visiting ant species. In the month of

May, there was a significant increase in mean abundance of *L. frauenfeldi* ($P < 0.05$). No significant differences were found in the mean abundance of the seven co-visiting ant species during the months of June and July. *Tapinoma melanocephalum* and *Ca. sericeus* ants were recorded on *E. hirta* plants only during the month of July and their number was consistently lower relative to that of *Tr. scabriceps* and *Ca. compressus* ants. The highest abundance was recorded for *Tr. scabriceps* followed by *Ca. compressus* as compared to the other ant species. No other arthropods were recorded on *E. hirta* plants (the only exception being the spider myrmecomorph, *Myrmarachne melanocephala*, which reportedly uses *Ca. compressus* ants as its model; (Kumari and Rastogi 2018), which was recorded on the inflorescence of *E. hirta* plants on five separate instances), although winged insects such as honeybees were observed on the flowers of other wild herbaceous plant species, such as *Ageratum houstonianum* (Mill.) and butterflies such as *Danaus chrysippus* (L.) were observed on *Lantana camara* (L.) plants.

Spatial patrolling pattern of ants

Significant variations were found in the mean time spent on the three plant parts by each of the seven ant species, *Ca. compressus* ($H = 37.682$, $df = 2$, $P < 0.001$), *Ca. parius* ($H = 38.996$, $df = 2$, $P < 0.001$), *Ca. sericeus* ($H = 35.472$, $df = 2$, $P < 0.001$), *Cr. subnuda* ($H = 37.675$, $df = 2$,

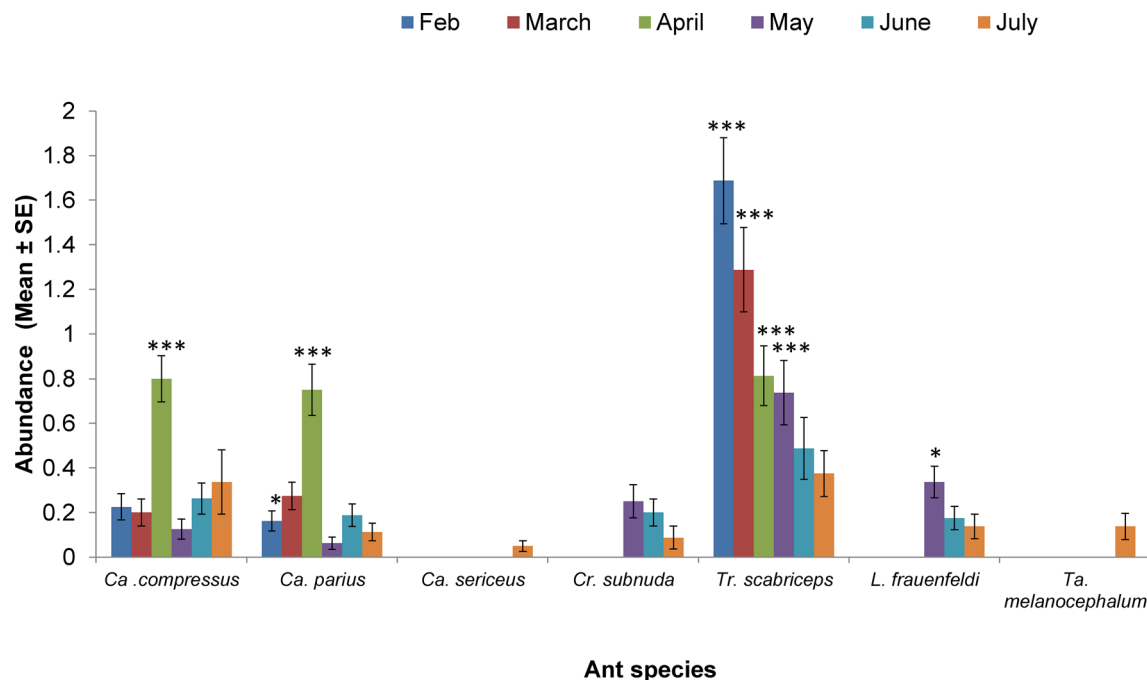


Fig. 2 Abundance (Mean \pm SE) of seven ant species recorded on the *Euphorbia hirta* plants during the 6-month study period, February–July (2018 and 2019). Friedman's two-way analysis of variance followed by Bonferroni post hoc test: * $P < 0.05$, *** $P < 0.001$

$P < 0.001$), *Tr. scabriceps* ($H = 38.006$, $df = 2$, $P < 0.001$), *L. frauenfeldi* ($H = 36.364$, $df = 2$, $P < 0.001$) and *Ta. melanocephalum* ($H = 30.863$, $df = 2$, $P < 0.001$). Duncan's post hoc test revealed that all the ant species spent significantly higher time on the inflorescence than on the leaves and stem of *E. hirta* plants ($P < 0.001$) (Fig. 3).

Spatial distribution of ant nests with reference to *E. hirta* plant clusters

Significant differences were found in the number of nests of the six plant-visiting ant species located at different distances from the clusters of *E. hirta* plants (*Ca. compressus*: $F = 9.995$, $P < 0.001$; *Ca. parius*: $F = 2.731$; $P < 0.05$; *Cr. subnuda*: $F = 28.445$, $P < 0.001$; *Tr. scabriceps*: $F = 27.379$, $P < 0.001$; *L. frauenfeldi*: $F = 4.737$, $P < 0.001$, *Ta. melanocephalum*: $F = 3.845$, $P < 0.01$; $df = 4$ in each case) (Fig. 4). Duncan's post hoc test revealed that significantly higher number of nests of *Ca. compressus* was found within 3 m distance of *E. hirta* plant cluster. In case of *Ca. parius* the number of nests at different distances did not differ significantly up to the distance of 7 m from *E. hirta* plants but the number declined significantly ($P < 0.001$) at 9 m distance. Very few nests of *Ca. sericeus* were located within 1–9 m distance from *E. hirta* plants as compared to those of other *Camponotus* species. Significantly higher number of nests of *Cr. subnuda* was found between 3 and 4 m distance from *E. hirta* plants. The number of nests decreased as the distance from the plants increased. In case of *Tr. scabriceps*, the number of nests was significantly higher within 3 m distance of *E. hirta* plants. Although, the nests of *L. frauenfeldi* and

Ta. melanocephalum were very few in number, the number of nests within 1 m distance of *E. hirta* plants was significantly higher ($P < 0.001$, in both cases) as compared to those located further away from the plants.

Plant visitation patterns of ants

One-way ANOVA revealed significant differences in the number of plants visited per foraging trip by worker ants of different species ($F = 6.288$, $P < 0.001$, $df = 6$) (Fig. 5). Tukey's post hoc test revealed that two species of *Camponotus*, (*Ca. compressus* and *Ca. parius*) visited significantly higher number of plants ($P < 0.001$) per foraging trip than other ant species. *Crematogaster subnuda* and *Ta. melanocephalum* ants visited significantly lower number of plants per foraging trip ($P < 0.001$).

Exclusion experiments to investigate the role of ants as pollinators of *E. hirta* plants

Significant variations were found in the number fruits ($H = 211.006$, $P < 0.001$, $df = 3$) produced under different pollination treatments (Fig. 6). Duncan's post hoc test revealed that the number of fruits produced was significantly higher ($P < 0.001$) in open pollination treatment than in case of other treatments. Although the number of fruits produced per plant was higher when only ants were allowed access to the plants as compared to that when the pollination was by winged insects and/or wind and also when the plants were under the self-pollination treatment conditions; there was no significant difference between the number of fruits produced

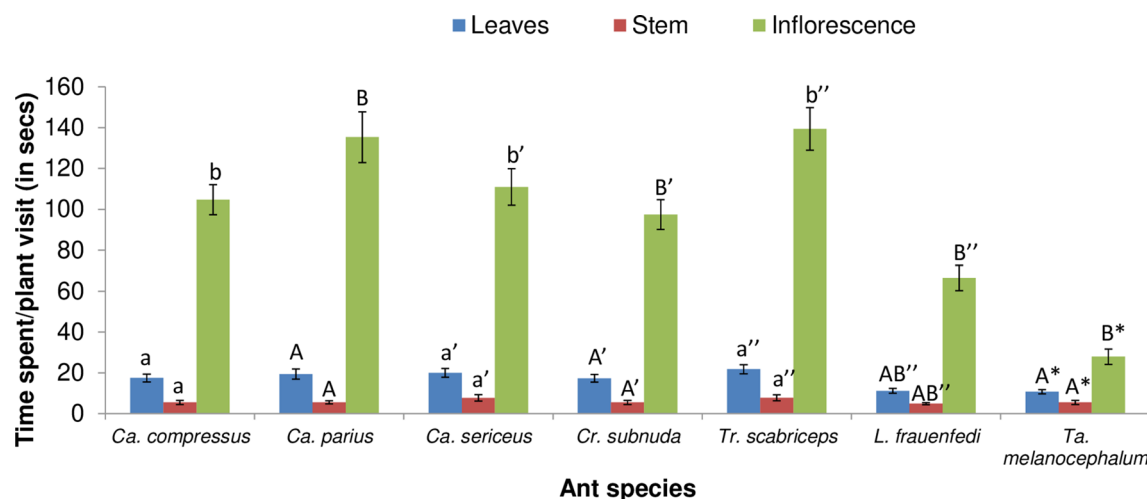


Fig. 3 Spatial patrolling patterns of the seven ant species on *E. hirta* plants and the time (Mean \pm SE) spent by each ant species on each plant part per visit. Kruskal Wallis followed by Duncan's post hoc test, $P < 0.001$. Means with different letters (lowercase: *Camponotus compressus*, uppercase: *Ca. parius*, lowercase with dash: *Ca. seri-*

ceus, uppercase with dash: *Crematogaster subnuda*, lowercase with double dash: *Trichomyrmex scabriceps*, uppercase with double dash: *Lepisiota frauenfeldi* and uppercase with star: *Tapinoma melanocephalum*) in the panel show significant difference

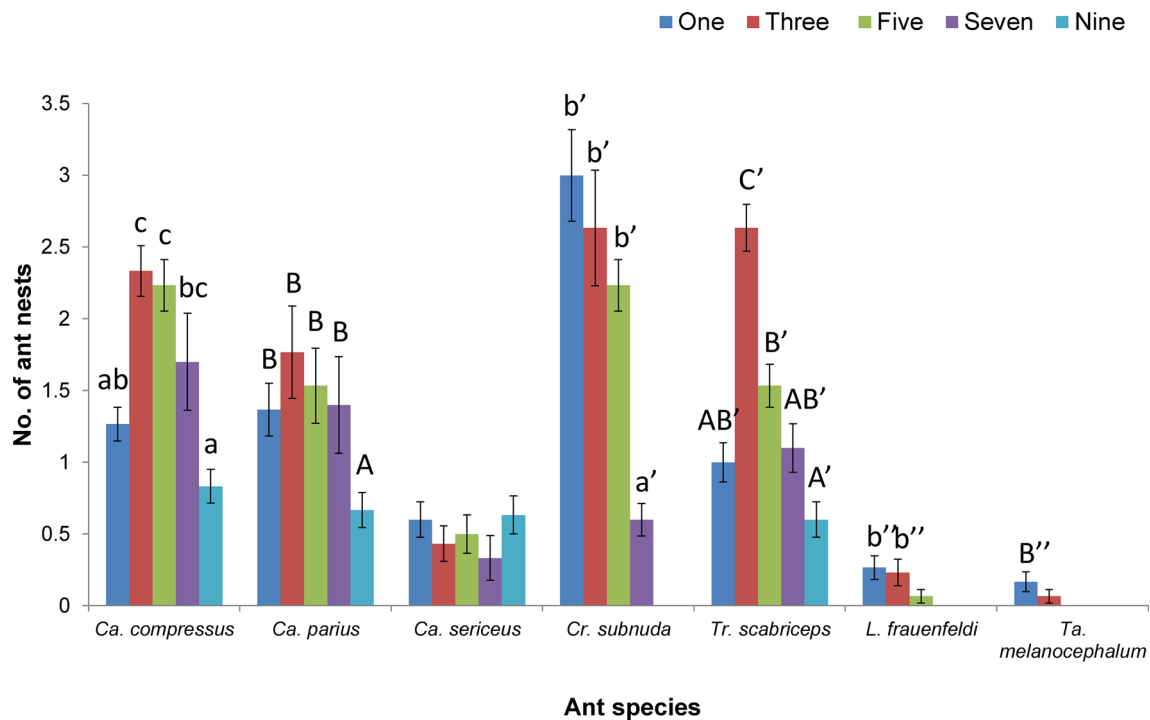


Fig. 4 Number (Mean \pm SE) of nests, of each of the seven plant-visiting ant species, present at different distance (1–9 m) from *E. hirta* plants. Two-way ANOVA test followed by Duncan's post hoc test. Different letters in a panel indicate significant differences at $P < 0.05$

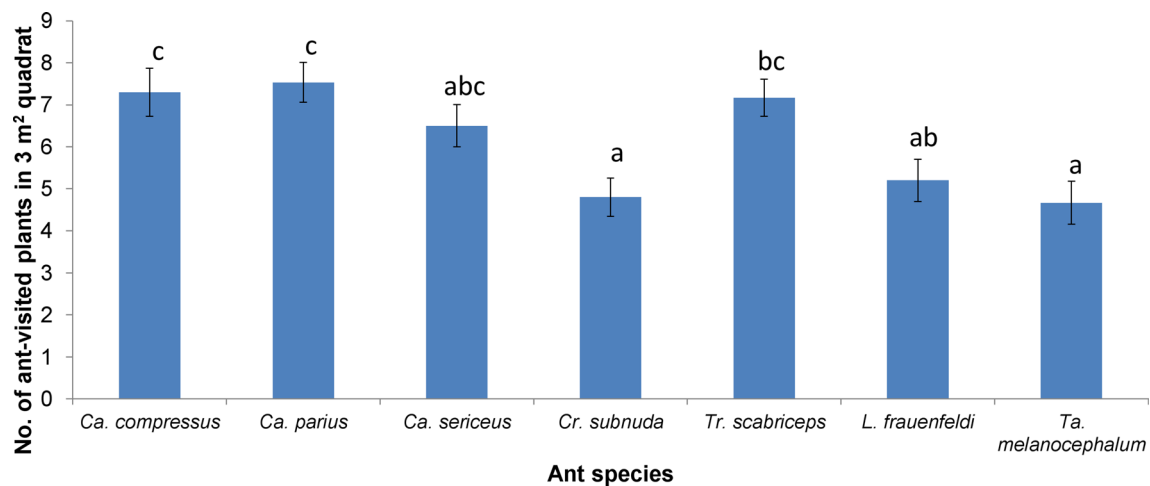


Fig. 5 Number (Mean \pm SE) of *Euphorbia hirta* plants visited by different ant species in 3 m² quadrat. One-way ANOVA followed by Tukey's post hoc test. Different letters in a panel indicate significant differences at $P < 0.05$

per plant in case of plants placed under winged insect and/or wind pollination and ant pollination treatment conditions.

Scanning electron microscopy

The SEM images of the cyathia of *E. hirta* revealed that the presence of bilobed anthers (Fig. 7A–C) which were located around the pistil which were three in number. Four

glandular nectaries were located at the periphery of the involucre of each cyathium (Fig. 7D, E). The pollen grains of *E. hirta* were tricolpate and elliptical in shape (Fig. 7F–I), ~26–28 μ m in length and ~13–16 μ m in width.

The pollen grains of *E. hirta* were recorded on the mouth-parts of three abundant plant-visiting ant species including, *Ca. compressus* (Fig. 8A, D), *Ca. parius* (Fig. 8B, E) and *Tr. scabriceps* (Fig. 8C, F). They were usually found adhering

Fig. 6 Number (Mean \pm SE) of fruits per *Euphorbia hirta* plant produced under different treatment conditions. Kruskal Wallis test followed by Duncan's post hoc test. Different letters indicate significant differences at $P < 0.05$

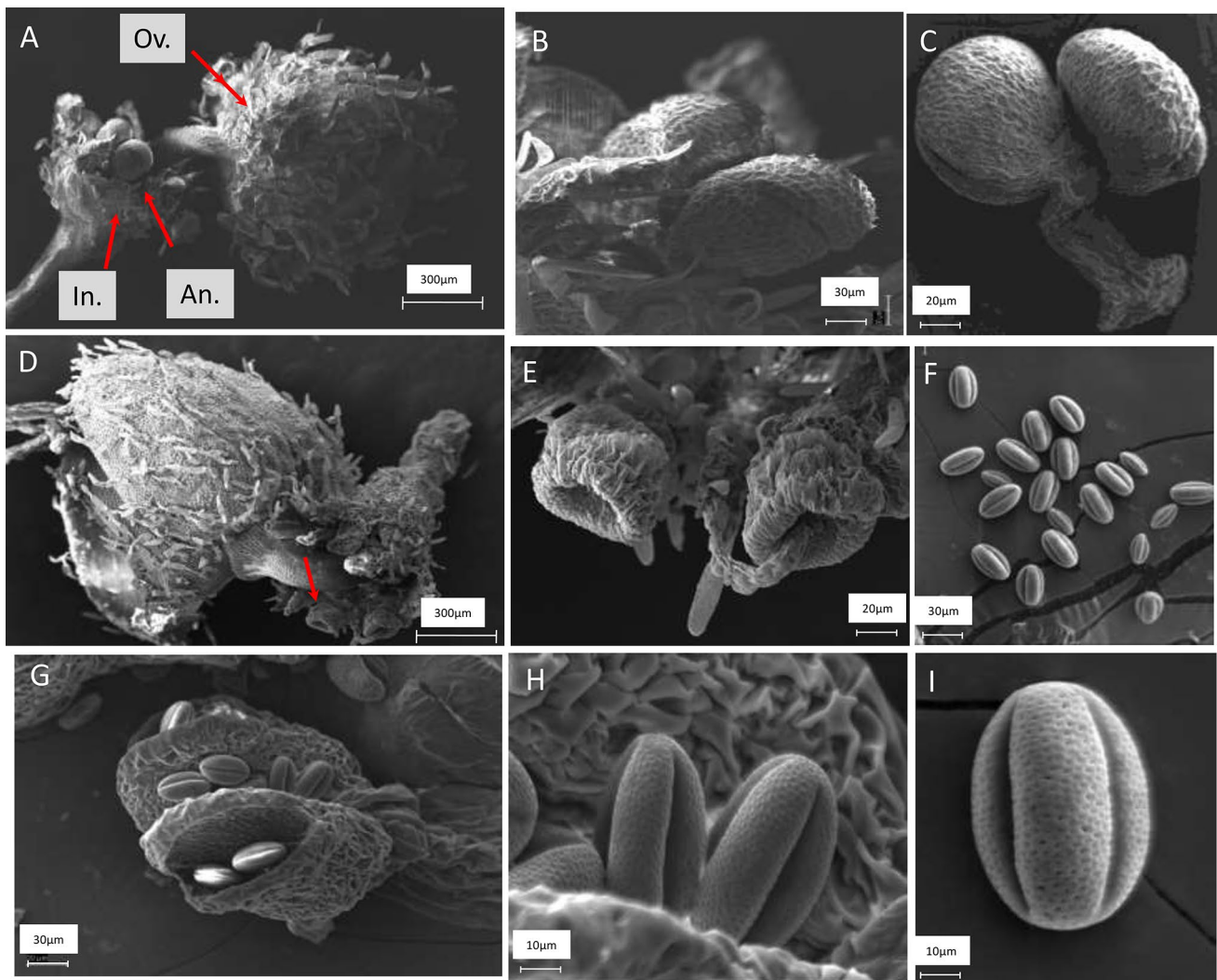
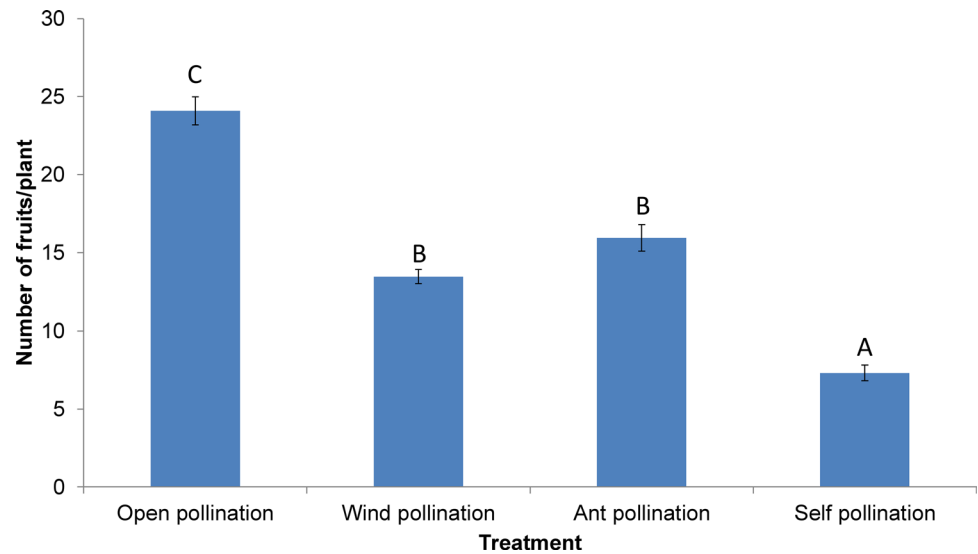


Fig. 7 Inflorescence of *Euphorbia hirta* showing: **A** whole cyathium with anthers (An.), ovary (Ov.) and involucre (In.), **B** enlarged view of anthers **C** bilobed anthers separated from the cyathium **D** cyathium

showing inflorescence-associated nectaries (red arrow), **E** enlarged view of nectaries, **F** pollen grains of *E. hirta*, **G** pollen grains with exposed anthers, **H–I** enlarged view of pollen grains

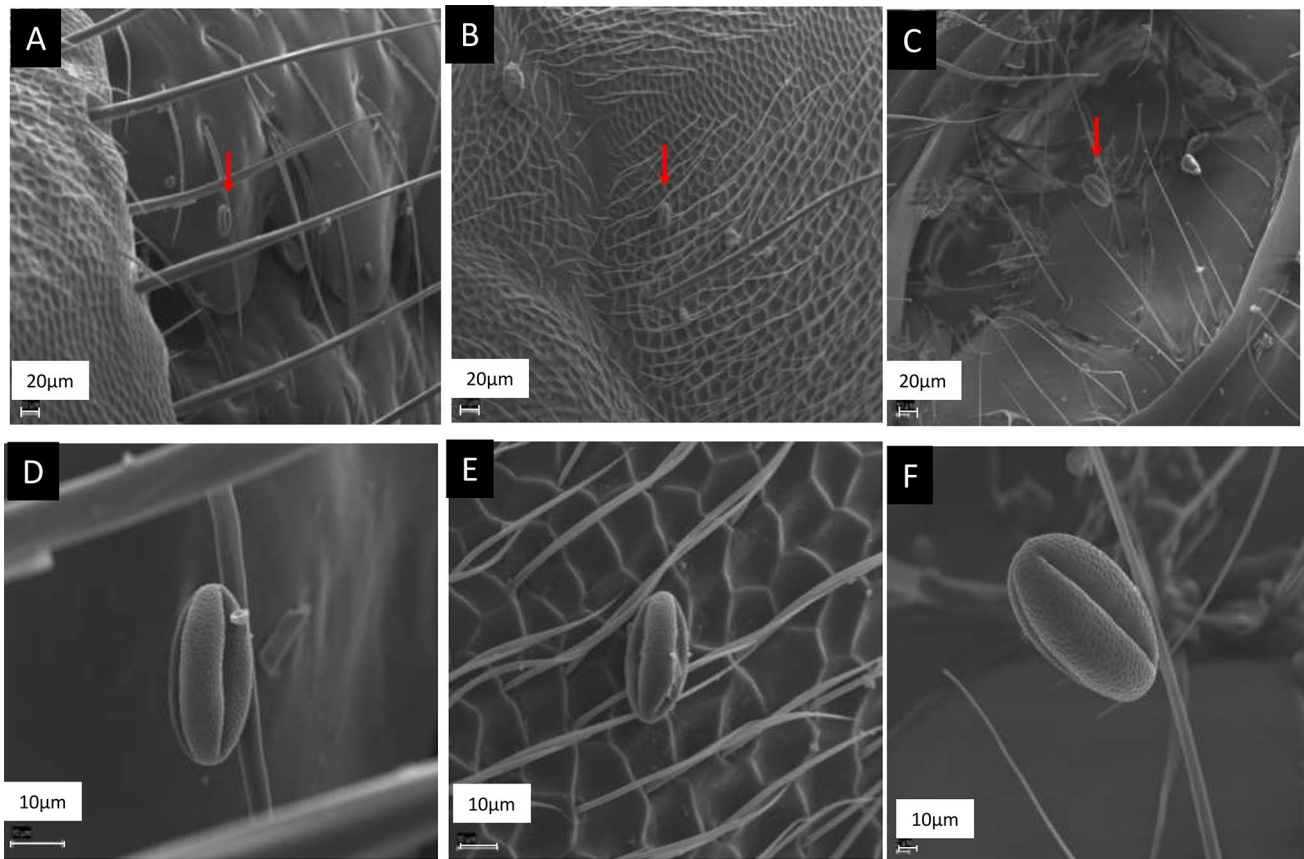


Fig. 8 Scanning electron microscopy (SEM) images of oval-shaped, tricolpate pollen grains trapped in the bristles located on the mouth-parts of different ant species: **A** *Camponotus compressus* **B** *Cam-*

ponotus parvus and **C** *Trichomyrmex scabriceps*, **D–F** enlarged view of same, i.e., *E. hirta* pollen grains present on the respective mouth-parts of the same three ant species

to the bristles of the mouth-parts of the minor caste worker ants. The pollen grains were never found to be gripped between the mandibles.

Discussion

The results of the field-based study revealed that seven species of ants belonging to five genera and three subfamilies (Formicinae, Myrmicinae and Dolichoderinae) are potential pollinators of *E. hirta* plants. While *E. hirta* plants showed adaptive traits related to pollination by ants, such as small inconspicuous unisexual flowers, small-sized prostrate or semi-erect stem, tiny nectaries (located on the involucre of the inflorescence but not on the unisexual flowers) and a large number of inflorescence clustered close to the stem of each plant, the ants showed several attributes of being potential insect pollinators, well-adapted for providing pollination services to this herbaceous plant species. The spatial patrolling patterns of all the ant visitors on the plants showed that the worker ants spent significantly higher time at the inflorescence than on the other plant parts. The movement

of the wingless ants on several inflorescence of the same plant is likely to promote self-pollination. *Trichomyrmex* and *Camponotus* ant species were the most abundant as well as regular visitors of *E. hirta* plants. Further, members of *Camponotus*, *Trichomyrmex* and *Crematogaster* genera constitute the most frequently recorded species at the inflorescence-associated nectaries of tropical plant species from a large number of families (Haber et al. 1981; Vanitha et al. 2017). They are also known to exhibit palatability to nectar containing phenolics and alkaloids (Haber et al. 1981). We found that the abundance of *Tr. scabriceps* and to a slightly lesser extent of *Ca. compressus* ants was consistently high on the *E. hirta* plants. *Trichomyrmex scabriceps*, *Ca. compressus*, and *Ca. parvus* were recorded on the plants throughout the study period, *Cr. subnuda* and *L. fraunfeldti* ants visited the plants all through the summer season and also during the month of July, while *Ta. melanocephalum* and *Ca. sericeus* were recorded only during July, i.e., after the onset of the rainy season. This may be related to their colony requirements and/or a relatively lower abundance of nests and of plant-visiting foragers (Kumari and Rastogi 2018; Shukla et al. 2016) of *Ta. melanocephalum* and *Ca. sericeus*, in

managed ecosystems. The spatial patrolling patterns of the two most abundant species of ant visitors on plants revealed a significant preference for the inflorescence of *E. hirta* plants as compared to other plant parts.

It has been reported that the nectar of various species of genus *Euphorbia* is rich in fructose, glucose and sucrose content (Papp 2004). Although according to some earlier studies, butterflies like *Danaus chrysippus*, *Acraea violae*, *Zizeeria* sp. are suggested to serve as pollinators of *E. hirta* (Chandra et al. 2013) we did not find any butterfly species on the plants during our field observations. The inflorescence of *E. hirta* attracted a number of sugar-loving ant species whose workers foraged upon the nectar produced by the small inflorescence-associated nectaries and a significant number of these foragers preferred to extend their foraging activity elsewhere and did not return to the nest. In this pursuit they visited higher number of *E. hirta* plants in one foraging trip. The results demonstrate that the three species of *Camponotus* visited up to 9–10 plants in one bout before returning to their nest. Since these ant species visited a number of *E. hirta* plants during one foraging trip, there is a strong possibility that they are involved in enhancing *E. hirta* reproductive fitness by providing cross-pollinating services (Del Claro et al. 2019). Examination of the mouth-parts of ants by SEM, following the visits of the foragers to *E. hirta* inflorescence, further revealed the presence of adhering pollen grains. Although, we did not conduct experiments to ascertain the viability of pollens present on ant cuticle, these results provide conclusive evidence of the role of ants as pollinator vectors of *E. hirta* plants. Moreover, the absence of other winged pollinators indicates that the tiny nectaries do not usually attract larger insect pollinators.

Our field experiments revealed that the plants produced the highest number of fruits under natural field conditions. However, the number of fruits produced in ant-pollinated plants was higher than those produced in winged insect and/or wind pollinated plants and self-pollinated plants. The least number of fruits were produced when both the pollinating agencies (i.e., ants and winged insects) were barred. Thus, it appears that *E. hirta* exhibits ambophily, where pollination services are accomplished by insects as well as the wind. *Euphorbia hirta* represents a pollination system which is adapted for pollination by ants as well as the wind since the monoecious flowers with raised stigma constitute an easily accessible receptacle for pollen grains which are numerous, minute and easily transported by air. The uniformly low plant height allowing easy access to the ants, the high density of plants and clustering of inflorescence, along with minute nectaries characterize the occurrence of the ‘ant-pollination syndrome’ (Wyatt 1981).

Our field observations further showed that clusters of *E. hirta* plants are invariably surrounded by nests of the seven plant-visiting ant species indicating that ant colonies may be

preferentially constructing their nests in close vicinity of *E. hirta* plants. This would be mutually beneficial to both, the ant colonies (which obtain the nectar from the inflorescence-associated nectaries) and the *E. hirta* plants which would benefit from enhanced reproduction due to cross-pollination activities of the ants. It is well known that returning ant foragers bring large amounts of food (such as honeydew, insect prey, floral nectar, leaves etc.) into their nests (Hölldobler and Wilson 1990). Hence, it appears that the returning ant foragers bring back the nectar collected from *E. hirta* inflorescence-associated nectaries.

The presence of various traits common to ant-pollinated plants ensures the attraction of ants to the inflorescence and is presumably adapted to accomplish cross-pollination. Although the self-grooming behavior of ants makes it difficult to be the vector of cross-pollination our results show that the presence of bristles on the mouth-parts of the minor caste worker ants help in trapping the pollen grains to the mouth-parts of ants. As the ants probe the nectaries of other *E. hirta* plants, the adhering pollen grains are likely to get transferred to stigma, thereby accomplishing cross-pollination.

Our field experiments reveal the role of ants in enhancement of seed setting by promoting cross-pollination although from the results of the bagging experiment it is evident that *E. hirta* plants are also self-compatible. As far as we are aware, this is the first report of ants as pollination vectors of the ant-adapted, *E. hirta* plants. These findings are also notable because *E. hirta* plants were found to be visited by seven ant species, and the occurrence of several pollinating ant species is not commonly recorded on ant-pollinated plant species studied till date. Our findings support earlier reports of the significance of ants in cross-pollination and seed set enhancement of the self-compatible, *Euphorbia cyparissias* (Schürch et al. 2000). While *Euphorbia brevitorta* (Martins 2010) and *Euphorbia nicaeensis* are also reported to be self-compatible, partial self-incompatibility is reported in *Euphorbia boetica* (Narbona et al. 2011). However, most species including *E. boetica* and *E. nicaeensis* rely on insect pollinators (Ehrenfeld 1979; Narbona et al. 2011), while ambophily involving ants as pollinators is reported in *Malotus* spp. (Euphorbiaceae) (Yamasaki and Sakai 2013) and more recently in the ant and wind pollinated gymnosperm, *Ephedra triandra* (Aranda-Rickert et al. 2021). Honeybees and flies have been implicated in increasing the fruit and seed sets (via geitonogamy and xenogamy) in the biodiesel plant, *Jatropha curcas* L. (Euphorbiaceae), but ants and thrips influenced pollination only via geitonogamy (Raju and Ezradanam 2002). However, further long-term investigations which took into consideration the bloom phenology of the biodiesel plant indicated a major role of ants as effective pollinators of *J. curcas*, particularly when honeybees were absent (Luo et al. 2012; Samra et al. 2014).

The diversity and abundance of ants on *E. hirta* plants, their spatial patrolling patterns on plants, multiplicity of plant visits during one foraging trip, presence of entangled pollens on ants' mouthparts and bristles and low fruit yield in ant-excluded plants provide ample evidence that there exists a mutualistic association between ants and *E. hirta* plants in which ants provide pollination service to plants in exchange for nutritional resources.

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References

- Albuquerque AA, de Lima HA, Gonçalves-Esteves V, Benevides CR, Rodarte AT (2013) *Myrsine parvifolia* (Primulaceae) in sandy coastal plains marginal to Atlantic rainforest: a case of pollination by wind or by both wind and insects? *Rev Bras Bot* 36:65–73. <https://doi.org/10.1007/s40415-013-0007-4>
- Aluri RJ (1990) Studies on pollination ecology in India: a review. *Proc Ind Natl Sci Acad B* 56:375–388
- Aranda-Rickert A, Torr  ns J, Yela NI, Brizuela MM, Di Stilio VS (2021) Distance dependent contribution of ants to pollination but not defense in a dioecious, ambophilous gymnosperm. *Front Plant Sci* 2021:1979. <https://doi.org/10.3389/fpls.2021.722405>
- Beattie AJ, Turnbull C, Knox RB, Williams EG (1984) Ant inhibition of pollen function: a possible reason why ant pollination is rare. *Am J Bot* 71:421–426. <https://doi.org/10.1002/j.1537-2197.1984.tb12527.x>
- Beattie AJ, Turnbull C, Hough T, Jobson S, Knox RB (1985) The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *Am J Bot* 72:606–614. <https://doi.org/10.1002/j.1537-2197.1985.tb08315.x>
- Berry PE, Calvo RN (1989) Wind pollination, self-incompatibility, and altitudinal shifts in pollination systems in the high Andean genus *Espeletia* (Asteraceae). *Am J Bot* 76:1602–1614. <https://doi.org/10.1002/j.1537-2197.1989.tb15145.x>
- Byk J, Del-Claro K (2010) Nectar-and pollen-gathering *Cephalotes* ants provide no protection against herbivory: a new manipulative experiment to test ant protective capabilities. *Acta Ethol* 13:33–38
- Cembrowski AR, Tan MG, Thomson JD, Frederickson ME (2014) Ants and ant scent reduce bumblebee pollination of artificial flowers. *Am Nat* 183:133–139
- Chandra PH, Ramana KV, Krishna JR, Rani DS, Rao SP, Raju AS (2013) A study on interactions of insects with herbaceous plants in Visakhapatnam. In *Advances in Pollen Spore Research*. Today & Tomorrow's Printers and Publishers: New Delhi, pp 69–79.
- Claessens J, Seifert B (2017) Significant ant pollination in two orchid species in the Alps as adaptation to the climate of the alpine zone. *Tuexenia* 37:363–374. <https://doi.org/10.14471/2017.37.005>
- Czechowski W, Mark   B, Er  s K, Csata E (2011) Pollenivory in ants (Hymenoptera: Formicidae) seems to be much more common than it was thought. *Annales Zoologici* 61:519–525. <https://doi.org/10.3161/000345411X603364>
- de Vega C, Arista M, Ortiz PL, Herrera CM, Talavera S (2009) The ant-pollination system of *Cytinus hypocistis* (Cytinaceae), a Mediterranean root holoparasite. *Ann Bot* 103:1065–1075. <https://doi.org/10.1093/aob/mcp049>
- de Vega C, Herrera CM, D  tterl S (2014) Floral volatiles play a key role in specialized ant pollination. *Perspect Plant Ecol Evol Syst* 16:32–42. <https://doi.org/10.1016/j.ppees.2013.11.002>
- Del-Claro K, Rodr  guez-Morales D, Calixto ES, Martins AS, Torezan-Silingardi HM (2019) Ant pollination of *Paepalanthus lundii* (Eriocaulaceae) in Brazilian savanna. *Ann Bot* 123:1159–1165. <https://doi.org/10.1093/aob/mcz021>
- Delnevo N, van Etten EJ, Clemente N, Fogu L, Pavarani E, Byrne M, Stock WD (2020) Pollen adaptation to ant pollination: a case study from the Proteaceae. *Ann Bot* 126:377–386. <https://doi.org/10.1093/aob/mcaa058>
- Dutton EM, Frederickson ME (2012) Why ant pollination is rare: new evidence and implications of the antibiotic hypothesis. *Arthropod Plant Interact* 6:561–569. <https://doi.org/10.1007/s11829-012-9201-8>
- Ehrenfeld JG (1979) Pollination of three species of *Euphorbia* subgenus *Chamaesyce*, with special reference to bees. *Am Midl Nat* 1:87–98. <https://doi.org/10.2307/2424904>
- Ekka PA, Rastogi N (2019) A single lycaenid caterpillar gets an ant-constructed shelter and uninterrupted ant attendance. *Entomol Exp Appl* 167:1012–1019. <https://doi.org/10.1111/eea.12859>
- Ekpo OE, Pretorius E (2007) Asthma, *Euphorbia hirta* and its anti-inflammatory properties: news & views. *S Afr J Sci* 103:201–203
- Faegri K, Van Der Pijl L (2013) Principles of pollination ecology. Pergamon Press
- G  mez JM, Zamora R (1992) Pollination by ants: consequences of the quantitative effects on a mutualistic system. *Oecologia* 91:410–418
- G  mez JM, Zamora R, H  dar JA, Garc  a D (1996) Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. *Oecologia* 105:236–242. <https://doi.org/10.1007/BF00328552>
- Haber WA, Frankie GW, Baker HG, Baker I, Koptur S (1981) Ants like flower nectar. *Biotropica* 13:211–214
- Hickman JC (1974) Pollination by ants: a low-energy system. *Science* 184:1290–1292. <https://doi.org/10.1126/science.184.4143.1290>
- H  lldobler B, Wilson EO (1990) The ants. Springer, Berlin
- Huang L, Chen S, Yang M (2012) *Euphorbia hirta* (Feiyangcao): a review on its ethnopharmacology, phytochemistry and pharmacology. *J Med Plant Res* 6:5176–5185. <https://doi.org/10.5897/JMPR12.206>
- Ibarra-Isassi J, Sendoya SF (2016) Ants as floral visitors of *Blutaparon portulacoides* (A. St-Hil.) Mears (Amaranthaceae): an ant pollination system in the Atlantic Rainforest. *Arthropod-Plant Interact* 10:221–227. <https://doi.org/10.1007/s11829-016-9429-9>

- Kumari S, Rastogi N (2018) Can a common and abundant plant-visiting ant species serve as a model for nine sympatric ant-mimicking arthropod species? *Curr Sci* 114:2189–2192
- Kuriakose G, Sinu PA, Shivanna KR (2018) Ant pollination of *Syzygium occidentale*, an endemic tree species of tropical rain forests of the Western Ghats, India. *Arthropod-Plant Interact* 12:647–655. <https://doi.org/10.1007/s11829-018-9613-1>
- Lach L (2005) Interference and exploitation competition of three nectar-thieving invasive ant species. *Insectes Soc* 52:257–262. <https://doi.org/10.1007/s00040-005-0807-z>
- Luo CW, Li K, Chen XM, Huang ZY (2012) Ants contribute significantly to the pollination of a biodiesel plant, *Jatropha curcas*. *Environ Entomol* 41:1163–1168. <https://doi.org/10.1603/EN12042>
- Martins DJ (2010) Pollination and seed dispersal in the endangered succulent *Euphorbia brevitorta*. *J East Afr Nat Hist* 99:9–17. <https://doi.org/10.2982/028.099.0104>
- Martins C, Oliveira R, Aguiar L, Antonini Y (2020) Pollination biology of the endangered columnar cactus *Cipocereus crassisepalus*: a case of close relationship between plant and pollinator. *Acta Bot Brasilica* 34:177–184. <https://doi.org/10.1590/0102-33062019ab0219>
- Merrill ED (1981) Plant life of the pacific world. C.E. Tuttle Co., Washington, Rutland
- Narbona E, Ortiz PL, Arista M (2011) Linking self-incompatibility, dichogamy, and flowering synchrony in two *Euphorbia* species: alternative mechanisms for avoiding self-fertilization? *PLoS ONE* 6:e20668. <https://doi.org/10.1371/journal.pone.0020668>
- Newstrom L, Robertson A (2005) Progress in understanding pollination systems in New Zealand. *N Z J Bot* 43:1–59. <https://doi.org/10.1080/0028825X.2005.9512943>
- Ollerton J (1996) Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant–pollinator systems. *J Ecol* 84:767–769
- Papp N (2004) Nectar and nectary studies on seven *Euphorbia* species. *Acta Bot Hung* 46:225–234. <https://doi.org/10.1556/abot.46.2004.1-2.16>
- Rahman AH, Akter M (2013) Taxonomy and medicinal uses of Euphorbiaceae (Spurge) family of Rajshahi, Bangladesh. *Res Plant Sci* 1:74–80. <https://doi.org/10.12691/plant-1-3-5>
- Raju AS, Ezradanam V (2002) Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). *Curr Sci* 10:1395–1398
- Raju AS, Ramana KV (2018) Pollination ecology of *Rhynchosia heynei* Wight and Arn. (Leguminosae), an endemic medicinal herbaceous shrub species of peninsular India. *J Inst Sci Tech* 22:32–44. <https://doi.org/10.3126/jist.v22i2.19592>
- Rastogi N (2007) Seasonal pattern in the territorial dynamics of the arboreal ant *Oecophylla smaragdina* (Hymenoptera: Formicidae). *J Bombay Nat Hist Soc* 104:13–17
- Regupathy A, Ayyasamy R (2011) Ants in biofuel, *Jatropha* ecosystem: pollination and phoresy. *Hexapoda* 18:168–175
- Samra S, Samocha Y, Eisikowitch D, Vaknin Y (2014) Can ants equal honeybees as effective pollinators of the energy crop *Jatropha curcas* L. under Mediterranean conditions? *Gcb Bioenergy* 6:756–767. <https://doi.org/10.1111/gcbb.12105>
- Schürch S, Pfunder M, Roy BA (2000) Effects of ants on the reproductive success of *Euphorbia cyparissias* and associated pathogenic rust fungi. *Oikos* 88:6–12. <https://doi.org/10.1034/j.1600-0706.2000.880102.x>
- Shukla RK, Singh H, Rastogi N (2016) How effective are disturbance-tolerant, agroecosystem–nesting ant species in improving soil fertility and crop yield? *Appl Soil Ecol* 108:156–164. <https://doi.org/10.1016/j.apsoil.2016.08.013>
- Sinu PA, Sibisha VC, Reshmi MN, Reshmi KS, Jasna TV, Aswathi K, Megha PP (2017) Invasive ant (*Anoplolepis gracilipes*) disrupts pollination in pumpkin. *Biol Invasions* 19:2599–25607. <https://doi.org/10.1007/s10530-017-1470-9>
- Srivastava AK (2001) Some studies on the climate of Varanasi. M.Sc. project work. Department of Geophysics, Banaras Hindu University
- Unni AP, Mir SH, Rajesh TP, Ballullaya UP, Jose T, Sinu PA (2021) Native and invasive ants affect floral visits of pollinating honey bees in pumpkin flowers (*Cucurbita maxima*). *Sci Rep* 11:1–7. <https://doi.org/10.1038/s41598-021-83902-w>
- Urbani CB, De Andrade ML (1997) Pollen eating, storing, and spitting by ants. *Naturwissenschaften* 84:256–258
- Vanitha K, Bhat PS, Raviprasad TN, Srikumar KK (2017) Species composition of ants in cashew plantations and their interrelationships with cashew. *Proc Natl Acad Sci India Sect B-Biol Sci* 87:399–409. <https://doi.org/10.1007/s40011-015-0600-3>
- Villamil N, Boege K, Stone GN (2018) Ant-pollinator conflict results in pollinator deterrence but no nectar trade-offs. *Front Plant Sci* 9:1093. <https://doi.org/10.3389/fpls.2018.01093>
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. *Ecology* 77:1043–1060. <https://doi.org/10.2307/2265575>
- Wyatt R (1981) Ant-pollination of the granite outcrop endemic *Diamorpha smallii* (Crassulaceae). *Am J Bot* 68:1212–1217. <https://doi.org/10.1002/j.1537-2197.1981.tb07827.x>
- Yamasaki E, Sakai S (2013) Wind and insect pollination (ambophily) of *Mallotus* spp. (Euphorbiaceae) in tropical and temperate forests. *Aust J Bot* 61:60–66. <https://doi.org/10.1071/BT12202>

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