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THE POLLINATION BIOLOGY OF *HELIOTROPIUM CURASSAVICUM* L. (BORAGINACEAE) FROM PAKISTAN

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Abstract

Heliotropium curassavicum L., of the family Boraginaceae is the only glabrous species from Pakistan. The species exhibits autogamous mode of reproduction while, the value of pollen–ovule ratio indicates the mixed pattern of pollination i.e., facultative xenogamy. A variety of insects frequently visit the flower as it simultaneously offers food and visual as well as olfactory attractions thus the plant enjoys both, direct as well as insect mediated selfing.

Key words: Heliotropium, Pakistan, Pollination, Pollinators.

Introduction

Heliotropium curassavicum L., belongs to the family Boraginaceae and the only glabrous heliotrope from Pakistan, mainly distributed from sea level to 300m. It is characterized by its fistular stems and halophytic nature (Nasir, 1989). One of the most important characteristics of plants is reproduction which depends on its breeding behavior. Similarly, the knowledge of breeding behavior is also important among all the approaches available to taxonomy as it does not only effect the pattern of group variation but also the evolutionary capabilities of the concerned group (Boreux *et al*., 2013; Rech *et al*., 2016; Kantsa *et al*., 2018; Linjun *et al*., 2019; Faheem *et al*., 2022). Moreover, study of reproductive biology may also help to plan conservation strategies in order to develop sustainable measures for cultivation (Yao *et al*., 2019). Regarding to the reproductive biology there are various reports available for the members of the family Boraginaceae (Opler *et al*., 1975; Dukas & Dafni, 1990; Ahmed *et al*., 1995; Boyd, 2004; McMullen, 2007; Taylor, 2008; Kantsa *et al*., 2018). However as far as *Heliotropium curassavicum* is concerned, no attention has been paid towards its reproductive biology, except for few scattered information about insect visitation on its flowers (Wiesenborn, 2010; Wiesenborn & Pratt, 2010) and on floral changes (Gori, 1983; Munz, 1974; Wiesenborn & Pratt, 2010). The present work is first of its kind from the area under consideration to determine the pollination biology of *Heliotropium curassavicum*.

Material and Methods

Study sites: All the field studies and observations were conducted within the vicinity of Karachi. (i) Adjacent to UBL, Karachi University Campus. (ii) Near Abu Bakar Mosque, Karachi University Campus. (iii) Near girls hostel, University of Karachi. (iv) Karachi University Employees Housing Society. (v) Near Paradise Point.

Floral phenology: Young buds (At least 10) from each of the population were tagged to study the floral phenology (all the changes from initial bud stage to fruit set).

Osmophores: Osmophores (odoriferous glands) were detected by Vogel's method (1962). All the floral parts including sepals, petals, anthers, stigma, style and ovary were dipped in Neutral dye for about 45 minutes. Then, these parts were washed under tap water for the removal of excessive stain and then observed under microscope. Presence of red spots, patches or streaks indicated the presence of osmophores in that floral parts.

Pollen fertility: Pollen grains from mature bud/freshly opened flowers were shed on a glass slide by tapping anthers. These pollen grains were then stained using cotton blue and acetocarmine for 30 minutes and then observed under microscope. Dark stained pollen grains were scored fertile whereas, light stained or unstained pollen were scored sterile.

Breeding studies

Pollen-ovule ratio: The flower buds were collected prior to anthesis and pollen ovule ratio was determined by dividing the total number of pollen grains/flower by the total number of ovules/flower and following counts were made:

(i) Number of anthers / flower, (ii) Number of pollen grains / anther, (iii) Number of pollen grains / flower (P), (iv) Number of ovary / flower, (v) Number of ovules / ovary, (vi) Number of ovules / flower (O).

Bagging experiments: Following treatments were given at flowering bud stage (At least 10 buds were used for each treatment).

Control (open pollination): Buds were tagged and left to study the normal seed set.

Self-pollination:

a) Direct autogamy: Buds were bagged and left to test direct autogamy.

b) Indirect autogamy: Pollinated by hand and bagged to test indirect autogamy.

Apomixis: Buds were emasculated and bagged to test the apomixis.

Cross-pollination:

a) Geitonogamy: Pollinated by hand with pollen grains from different flowers of the same plant and bagged to test the geitonogamy.

b) Xenogamy: Cross pollinated with pollen grains of different plants to test the xenogamy.

Insects behavior: Visitors (insects) of the flower were observed for their foraging behavior. Insects were collected by hand net, chloroformed and observed microscopically for pollen load. The insects carrying pollen were evaluated as pollinators.

Observations and Results

Phenology: The peak blooming period of *Heliotropium curassavicum* ranged from April-September. Normally 4-6 flowers on a cyme opened simultaneously. A young bud of *Heliotropium curassavicum* took 8-10 days to bloom and attained its maximum size (3mm). The opening time of the flower varied with the climatic fluctuation. However, it opened usually from 11:00 a.m.- 2:00 p.m. The centre of the corolla tube was yellow when the flower opened and during the course of pollination, turned bluish purple. After being fully bloomed for about 2-3 days, the flower closed generally between noon to 2:00 p.m. The petals crumpled, turned brown within 2-3 days and started wilting slowly and gradually. The stigma and anthers matured simultaneously. The stigma was conical, sessile and papillate with a prominent stigmatic ring at its base (Fig. 1A & B). The swelling of ovary indicated the fruit formation. The nectaries were spotted at the base of the ovary in the form of a ring.

Osmophores: Osmophores were frequently detected on sepals, petals, anthers, stigma and ovary in the form of red spots.

Pollen fertility: Pollen fertility was found to be 89.35% (Table 1).

Pollen-ovule ratio: The pollen-ovule ratio calculated was found to be 673.68. So the species seemed to have partially facultative autogamous to facultative xenogamous mode of reproduction (Table 1).

Bagging experiments: Bagging experiments showed that the fruits were set in control, direct autogamy and indirect autogamy but no fruit setting was observed in apomixis, geitonogamy and xenogamy (Fig. 3. Tables 2 and 3).

Insect (pollinators and visitors): A variety of insects including Moths, flies and bees visited the flower of *H. curassavicum* and usually got attracted towards the flowers for food (Nectar and pollen), colour and odour.

Lepidoptera: Two different kinds of Moths visited the flowers of *H. curassavicum*. As the size of the flower was too small, the moth was observed to alight on the neighboring flowers and insert its proboscis into the targeted flower. As it alighted on the neighbouring flowers, its legs came in contact with their sexual parts i.e. sternotribic (Fig. 2C). While getting its food from the flower, the moth kept on moving both of its lower wings slightly and also kept on rotating itself, seemed like it tried to suck nectar through the entire ring of the nectaries. It stayed on a single flower for about 4-10 seconds, and then targeted other flower of the same plant. It visited the flower just prior to opening and continued till about 2-3 hours (Fig. 1G-H, Fig. 2A-C).

Member of syrphidae: Flower fly belonging to Syrphidae was observed to start visiting the flowers in the morning hours of the day and continued until afternoon (9 a.m.-12noon). It alighted on the flowers next to the flower of interest and grasped them with the help of its legs (which came in contact with the sexual parts of those flowers) and inserted its proboscis into the targeted flower for nectar feeding. It stayed on a flower for about 5-8 seconds and then turned its attention towards other flowers of the cushion (Fig. 2D-F).

Table 1. Pollen fertility of *Heliotropium curassavicum* L.

Population No.	Total no. of pollen grains (mean)	No. of sterile pollen grains (mean)	No. of fertile pollen grains (mean)	Percentage of fertility (mean)
1.	103	5	98	95.14
2.	100	9	91	91.0
3.	110	8	102	92.72
4.	98	21	77	78.57
5.	68	8	60	88.25

Table 2. Pollen-ovule ratio of *Heliotropium curassavicum* L.

Population No.	Mean no. of anthers	Mean no. of pollen/anther	Mean no. of pollen/flower	Mean no. of ovary/flower	Mean no. of ovule/flower	P/O	Mean P/O
1.	5	607.2	3036.4	1	4	759.1	
2.	5	477.2	2386.8	1	4	596.74	679.5
3.	5	554.6	2773	1	4	693.25	
4.	5	481	2405	1	4	601.25	
5.	5	598	2990	1	4	747.5	

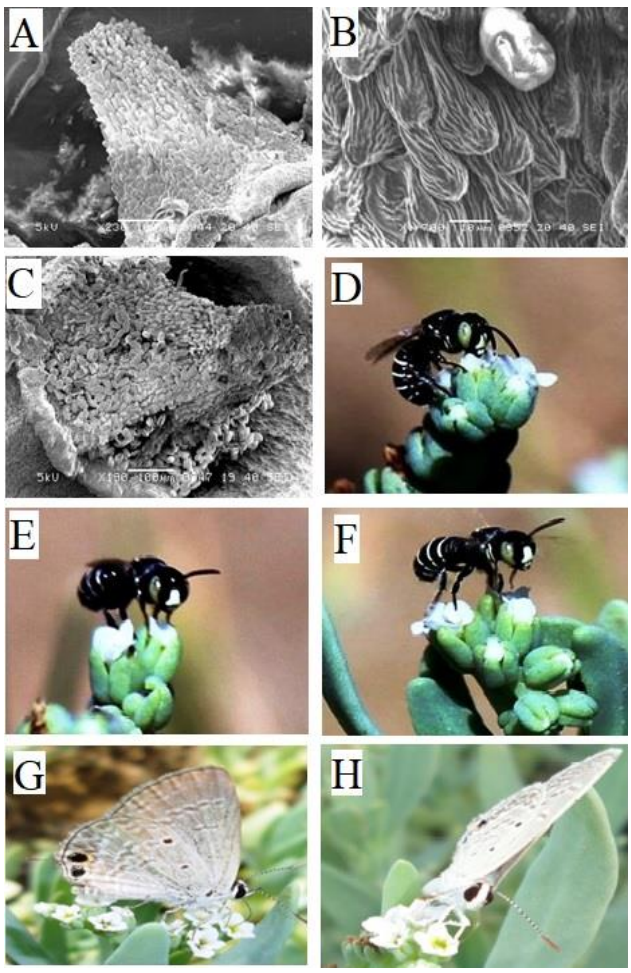

Fig. 1. "A" Stigmatic cone (Bar=100um), "B" Papillate stigmatic surface (Bar=10um), "C" Stigmatic surface covered by pollen grains (Bar=100um), "D" Fly (member of Apidae) feeding on nectar, "E" Fly (member of Apidae) inserted its proboscis, "F" Fly (member of Apidae) alighted on the flower and ready to insert its proboscis, "G-H" *Chilades parrhasius* 'A' inserted its proboscis deep into the flower and feeding nectar.

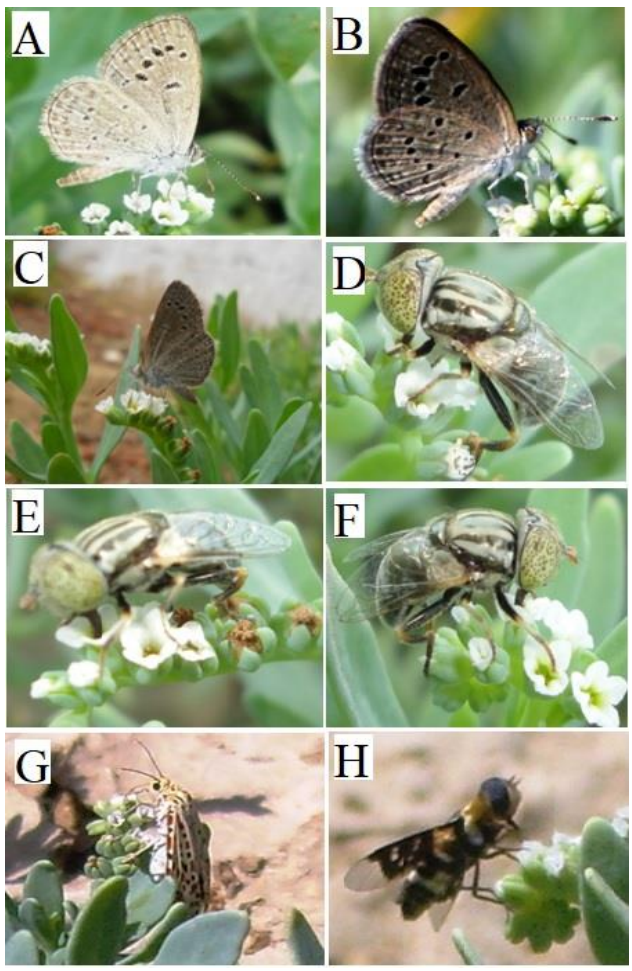

Fig. 2. "A-B" Moth 'B' Feeding on nectar, "C" Moth 'B' searching nectar and its legs touching the reproductive parts of the flower, "D" *Sarcophaga* sp., clasped the flower for feeding nectar, "E-F" *Sarcophaga* sp., feeding nectar, "G-H" Insect 'A' & 'B' visiting and obtaining food without playing any role in pollination.

Table 3. Bagging experiments of *Heliotropium curassavicum* L.

Population No.	Sample size. (No. of buds)	Control	Direct autogamy	Indirect autogamy	Apomixis	Geitonogamy	Xenogamy
1.	20	15	18	16	0	0	0
2.	30	30	28	23	0	0	0
3.	22	22	20	18	0	0	0
4.	18	16	18	16	0	0	0
5.	25	24	20	14	0	0	0

Member of apidae (fly): Although the pollinator itself was very small, but still larger than the flower, therefore, it alighted on the flower present next to the flower of interest and inserted its proboscis into it either in search of nectar or pollen in such a way that its head rested on the targeted flower while its body lying on the neighboring flower. In this way, the abdomen and legs of the fly came in contact with the reproductive parts of the flower. The fly continued to remain on a flower in the same posture for about 3-15 seconds. It generally then flied to neighboring inflorescence or moved on to other cushions. Peak visiting hours of this fly were observed to be from 11 a.m.-1 p.m (Fig. 1D-F).

Insect A: It was mostly observed in the afternoon. Due to its large size as compared to the size of the flower as well as to the size of the entire inflorescence, it usually settled itself on the leaves present adjacent to the flower of interest. It then used to insert its proboscis to obtain nectar and took 10-15 seconds. It departed from the population after visiting 2-3 flowers (Fig. 2G).

Insect B: This visitor was not observed on daily basis but whenever observed, it visited in the afternoon only (1 p.m.-3 p.m.). It was bigger in size compared to other pollinators but did not normally grasp the neighboring flowers like the former ones. Instead it settled itself

slightly on the neighboring flowers keeping most of its body in air. It then inserted its proboscis into the flower of interest for feeding nectar and continued to remain in the same position for about 8-15 seconds (Fig. 2H).

***Danaus plexippus*:** It was also one of the visitors of *H. curassavicum.* It rested its body on the leaves or the neighbouring inflorescence due to its very large size as compared to the flower. It visited the flowers mostly in the morning hours but sometimes in afternoon too. It inserted its proboscis into the targeted flower deeply to suck the nectar without touching the reproductive part and spent 10-30 seconds on a single flower. It then turned its attention towards other flowers of either the same or other inflorescence (Fig. 2D-F).

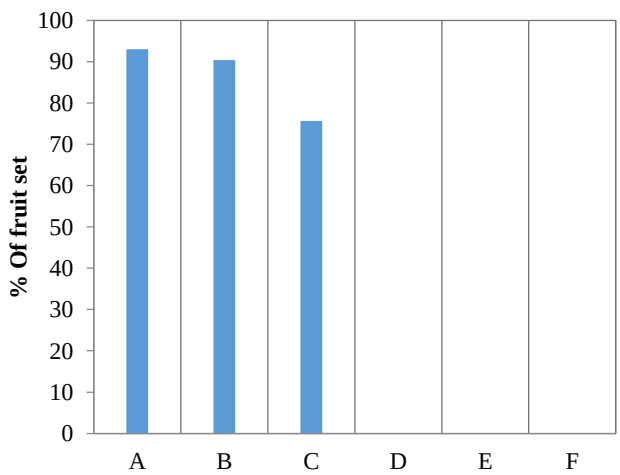

Fig. 3. Graphical presentation of percentage of fruit set among different pollination treatments: A= Control, B= Direct autogamy, C= Indirect autogamy, D= Apomixis, E= Geitonogamy, F= Xenogamy.

Discussion

The bagging experiments of *Heliotropium curassavicum* mainly indicated the autogamous mode of reproduction. While, the pollen-ovule ratio pointed out the mixed pattern of pollination i.e., facultative xenogamous. Present findings of bagging experiments did not support the pollen-ovule ratio. However, Cruden (1977) opined that pollen-ovule ratio was general indicator of breeding system, although our findings could be strengthened from the previous finding on various taxa where breeding system did not correlate with the pollen-ovule ratio (Lozada-gobilard *et al*., 2019; Faheem *et al*., 2022). Peak flowering period of *Heliotropium curassavicum* was observed from April-September. Opening and closing time of flower varied with climatic changes. The insect visitation was scarce in shady areas and also during low temperature. A flower once fully opened, remained in the same condition for the next 2-3 days and get closed by wilting of corolla after the completion of pollination. Similar to the previous observations of Gori (1983) and Munz (1974), the change in colour of corolla center from yellow to purple indicated completion of pollination process. The flower consisted of a conical stigma that was sessile and had a prominent stigmatic ring with papillate surface to grab as much pollen grains as possible (Fig. 1A-C). This conical stigma is a very unique characterstic of the order Boraginales (Schrad, 1819). The nectaries were present at the base of the ovary in a ring like structure. Kugler (1970), Weryszko-chmielewska (2003) and Bernardello (2007) also observed the same position of nectaries in various members of the family Boraginaceae. Regarding the mixed mode (direct and insect mediated selfing) of *H. curassavicum*, the flowers showed olfactory, visual and food devices that served as attractant for the insects. Insects' visitation on *H. curassavicum* was mainly observed in hot days whereas no or very few visitors came to visit the flower in colder climatic conditions. Visitation on *Heliotropium curassavicum* by *Hesperopsis gracielae* was also observed by Wiesenborn (2010) and Wiesenborn & Pratt (2010) where it was observed that the insects were attracted more towards yellow or purple centered flowers as compared to white ones and frequencies of insect landings were greater in sun plants than those present in shade. The flowers of *H. curassavicum* were mainly visited by flower fly (Syrphidae) and members of Apidae. *Danaus plexippus* and insect 'A' and 'B' visited the flowers but no pollen load was found on their bodies. So, they may be considered as opportunists as they suck the nectar without their bodies coming in contact with the reproductive organs (Abid *et al*., 2010) without performing the pollination. While, the moths, member of syrphidae (fly) and members of Apidae participated in the process of pollination as pollen load was found on their bodies.thus, a variety of insects visited the plant and flower simultaneously offer the opportunities as primary as well as secondary attractant and mutually benefitted through insect mediated selfing.

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