



Pollination biology and breeding behaviour of *Datura innoxia* Mill

Dr. Vijay Laxmi Hada

Assistant Professor, Under Affiliated College, University of Rajasthan, Rajasthan, India

Abstract

The pollination process is essential to a plant's long-term viability because it produces seeds and transmits genetic material to the offspring. Additionally, the wide range of pollination mechanism provides excellent opportunities to study the degree of co-evolution that occurs between pollinators and plants. Many plant features, such as floral appearance, phenology, self-incompatibility and floral structure have an impact on plant mating systems and pollinator behaviour. *Datura innoxia* Mill. (Family- Solanaceae) is an evergreen, invasive, branched herb producing a large amount of white colored flowers that have an appealing appearance and secrets copious amount of nectar. In the present study floral visitors and the breeding behaviour of *Datura innoxia* Mill. have been studied, aiming to contribute the knowledge of pollination biology of the studied species. In order to find out the pollination biology, floral visitors and breeding behaviour of *Datura innoxia* Mill, field experiments were conducted in the selected study sites in Kota district of Rajasthan throughout the flowering season in year 2021 and 2022. Floral visitors of *Datura innoxia* observed are *Manduca sexta* (Hawk moth), *Apis indica* (Indian honey bee), *Apis dorsata* (Giant honey bee), *Pantala flavescens* (Dragonfly), *Dysdercus cingulatus* Fab. (Red cotton bug), *Eristalinus megacephalus* Rossi. (Spot eye hover fly) and *Aulacophora intermedia* Jacoby. (Pumpkin beetle). Due to their ability to transfer numerous pollen grains from one flower to another, Lepidoptera and Hymenoptera are the primary groups of pollinators in *Datura innoxia* Mill. As they enter the corolla tube of a flower in the searching of nectar and pollen, pollen grains stick to their body parts and move with them from one location to another. The primary pollinator of *Datura* species is *Manduca sexta* L. It visits flowers at dusk and at night and plays an important role in pollination. Some other most frequent pollinators of *Datura innoxia* are *Apis indica* Fab. (Indian honey bee), *Apis dorsata* Fab. (Giant honey bee) and *Pantala flavescens* Fab. (Dragonfly). They visit flowers in the morning and afternoon for pollen as well as nectar reward and play a significant role in pollination. The results of breeding experiments revealed that *Datura innoxia* Mill. is a highly self-compatible as well as cross pollinated invasive plant species, it exhibits xenogamy, geitonogamy and thus shows various ways of breeding system. Instead Apomixis, all five types breeding experiments produced good percentage of fruit sets.

Keywords: Pollination, diversity, invasive, geitonogamy, viability, dispersal mechanism

Introduction

Studies on the reproductive biology are an important interdisciplinary field that are essential for developing effective strategies for both ex situ and in situ conservation of the species. Plant reproduction and pollination have long piqued the interest of experts and laypeople alike in the natural sciences. From gametogenesis to seed germination, reproductive biology encompasses a number of processes. Structure of the flower or inflorescence, viability of the pollen and ovules, various pollination mechanisms, pollen pistil interaction as determined by compatibility, breeding and mating systems, fertilization, embryogenesis, seed development, dispersal, and germination are some of the factors studied under reproductive biology of angiosperm (Marbaniang *et al.* 2018) [24]. There are many different ways in which a plant and a pollinator interact. The efficacy and accuracy of pollen transfer are often significantly increased by the anatomical and behavioral traits of effective pollinators (Faegri and van der Pijl, 1979) [14]. Plants entice pollinators in a number of ways, including flowers color and size, scent, heat, nectar glands and by the edible pollen grains. The timing of flowering and the number and size of flowers is the other two factors that play a significant role in the sexual reproduction of flowering plants, in addition to the change involving the aforementioned structures. Because of its significance in understanding the plant breeding system, floral initiation, foraging theory, and animal behaviour pollination biology is experiencing a

renaissance. It offers a thoughtful synthesis that advances our comprehension of variety based on numerous energetic elements in terms of resource production (Becker and Becker, 1973) [6], Foraging strategy, adaptive radiation (Grant and Grant, 1965) [16], and community structure (Parrish & Bazzaz, 1978) [28]. Floral fossil evidence suggests that there were interactions between plant and pollinator during the Eocene epoch. Insect pollination followed by fertilization is a crucial component of the plant breeding system required for the development of new and improved species. Insect pollinators have been essential in the development of angiosperm as the most widespread and technologically advanced class of plants. Pollinators serve an essential part in maintaining healthy ecosystems. In many ecosystems, a higher level of total biodiversity or variety among living organism is correlated with a bigger range of pollinators. An established method for evaluating plant breeding systems is controlled pollination studies (Dafni, 1992) [9]. According to Cruden, (1977) [8] the ratio of pollen grains to ovules in a flower could serve as a good indicator of a plant's breeding strategy, including whether it is autogamous, allogamous-xenogamous, or geitonogamous as well as if it engages in mandatory or facultative outcrossing. The study of Pollination biology is fundamental for Systematic, evolution and conservation analysis (Anerson, 1995). Every conservation and restoration program must have a thorough understanding of the pollination and breeding systems (Weller 1994) [37]. Observation of floral

traits as well as pollination studies can provide inferences in to plant breeding systems. In order to advance our understanding in pollination biology of the species, breeding habits and pollination biology of *Datura innoxia* Mill. have been studied.

Material and Methods

Study sites: Study on pollination biology and breeding experiments of *Datura innoxia* was performed in Kota district of Rajasthan in year 2021 and 2022 during the flowering and fruiting period (January-April) and (July-October). The city of Kota is located in the southeast of Rajasthan, along the banks of Chambal River. District Kota is situated between latitudes 24.25° and 25.51° in the north and 75.37°- 77.26° in the east. The range of temperature is 36.7°C (max.) to 12.0°C (min.). The Kota district receives 660.6 mm of rain on average per year.

***Datura innoxia* Mill:** The study of pollination biology and breeding behaviour was carried out on *Datura innoxia* Mill. The genus *Datura* belong to family Solanaceae, there are about 15 species which distributed naturally in world wide. It is widely grow at the location where the species has escaped cultivation and established small population surrounding ruderal habitats including waste lands. All species of *Datura* are toxic to animals and humans. *Datura innoxia* is tuberous rooted, erect, branched, evergreen herb. Its stem and leaves are covered with short and soft grayish hairs. The flowers are white coloured, trumpet shaped 18 - 21cm long giving a pleasant appearance when blooming at night.

Flower visitors and their behaviour: Species dense population sites were observed day as well as night during the peak flowering season in order to examine the behaviour of floral visitors. Visiting time of floral visitors on each flower, frequency of visits, pollinator reward, and foraging activities were all closely monitored. The floral visitors were photographed, captured and preserved and identified by the experts' consultation in the department of entomology, Govt. Agriculture College, Kaithun, Kota (Rajasthan).

Nectar analysis: The volume and sugar concentration of the nectar samples from 20 randomly selected protected and unprotected flowers were measured by microcapillary tube and light refractometer after the method of Dafni (1992) [9]. The values on refractometer were considered as sucrose equivalents method proposed by (Inouye *et al.* 1980).

Breeding experiments: The breeding system of studied species was established by following pollination treatments.

- 1. Apomixis:** Randomly selected 50 buds were emasculated and bagged before anthesis to prevent pollination.
- 2. Bagged/Spontaneous autogamy:** Mature buds were bagged without emasculation prior to anthesis to examine the self pollination.
- 3. Emasculated open flowers or uncontrolled pollination** was carried out in presence of pollinators.

4. Geitonogamy: Mature buds were emasculated before anthesis, pollinated using pollen grains from other flowers of the same plant and bagged to study the geitonogamy.

5. Xenogamy: Mature buds were emasculated, pollinated using pollen from different plants and bagged before anthesis.

Observation and findings

Flower Morphology of *Datura innoxia* Mill

Flowers of *Datura innoxia* are large 20.06±1.21cm long, trumpet/ tubular shape, hypogynous, actinomorphic, hermaphrodite, complete, pentamerous, gamosepalous and gamopetalous with valvet arrangement. Stamen 5 polyandrous, epipetalous, equal in size, 14.86±1.20cm long, ovary superior bicarpellary with axile placentation, style 13.92±1.42cm long yellow or pale green, stigma bilabiate, with wet and sticky surface (Plate-1).



Plate- 1: A-Elongated stigma shows protogyny stage. B- Different stages of flower (bud to mature flower). C- Flowers of *Datura innoxia* having different size. D- L.S. of mature flower

Flower visitors of *Datura innoxia* Mill.

Nocturnal plant *Datura innoxia* produce white coloured, large, trumpet shape flowers and have five nectaries surrounding the ovary situated at the base of long corolla tube. Nectar of flower accumulates at the base of flower funnel so only few insect are able to reach and suck the nectar, also all part of plant emit a foul odor so a few insect visit on the flowers and play a role of pollinator. These are *Manduca sexta* L. (Hawk moth), *Apis indica* Fab. (Indian honey bee), *Apis dorsata* Fab. (Giant honey bee) and *Pantala flavescens* Fab. (Dragonfly) given in table- 1. These

all are most frequent visitors and pollinators of *Datura innoxia* Mill. and aid a significant role in fruit and seed set. *Blattella germanica* L. (German cockroach) has also visited during rainy season and helped in pollination as its body parts touched anthers and stigma while entering inside the corolla tube (Plate-3 C & D). Some other floral visitors were

also observed on *Datura innoxia* flower, these were merely visitors and their function as a pollinator was unknown. These are *Dysdercus cingulatus* Fab. (Red cotton bug), *Erastalis megacephalus* Rossi. (Spot eye hover fly) and *Aulacophora intermedia* Jacoby. (Pumpkin beetle) shown in plate-3.

Table 1: Flower visitors, foraging behaviour and pollination efficacy

S.N.	Name and family of pollinator	Visiting time	Foraging nature	Foraging hours (hrs.)	Time spent /flower	Frequency	Pollination Efficacy Mean±SD
1	<i>Manduca sexta</i> L. Order-Lepidoptera Family-Sphingidae	Night	Nectar	2000h-2400h	15-20 sec.	+++	0.52±0.001
2	<i>Apis indica</i> Order- Hymenoptera Family-Apidae	Day	Pollen +nectar	0600h-1200h	5-10 sec.	+++	0.40±0.02
3	<i>Apis dorsata</i> Order- Hymenoptera Family- Apidae	Day	Pollen +nectar	0800h-1800h	40-60sec.	+++	0.50±0.02
4	<i>Pantala flavescens</i> Order-Odonata Family-Libellulidae	Day	Pollen	0700h-1000h	4-6sec.	++	0.39±0.01

Note: + : occasional, ++ : moderate, +++ : high

Table 2: Visitation Frequency

S.N.	Floral visitors	Visiting Frequency in Different hours							
		0700-1000h	1000-1200h	1200-1400h	1400-1600h	1600-1800h	1800-2000h	2000-2200h	2200-2400h
1	<i>M.sexta</i> Lin.	8.33	0	0	0	0	0	23.3	46.66
2	<i>A.indica</i> Fab.	31.66	23.33	23.33	0	0	0	0	0
3	<i>A.dorsata</i> Fab.	36.66	33.33	46.66	36.66	30	0	0	0
4	<i>P.flavescens</i> Fab.	13.33	21.66	8.33	0	0	0	0	0

$$\text{Visitation Frequency}\% = \frac{\text{Number of visits per flower}}{\text{per minute}} \times 100$$

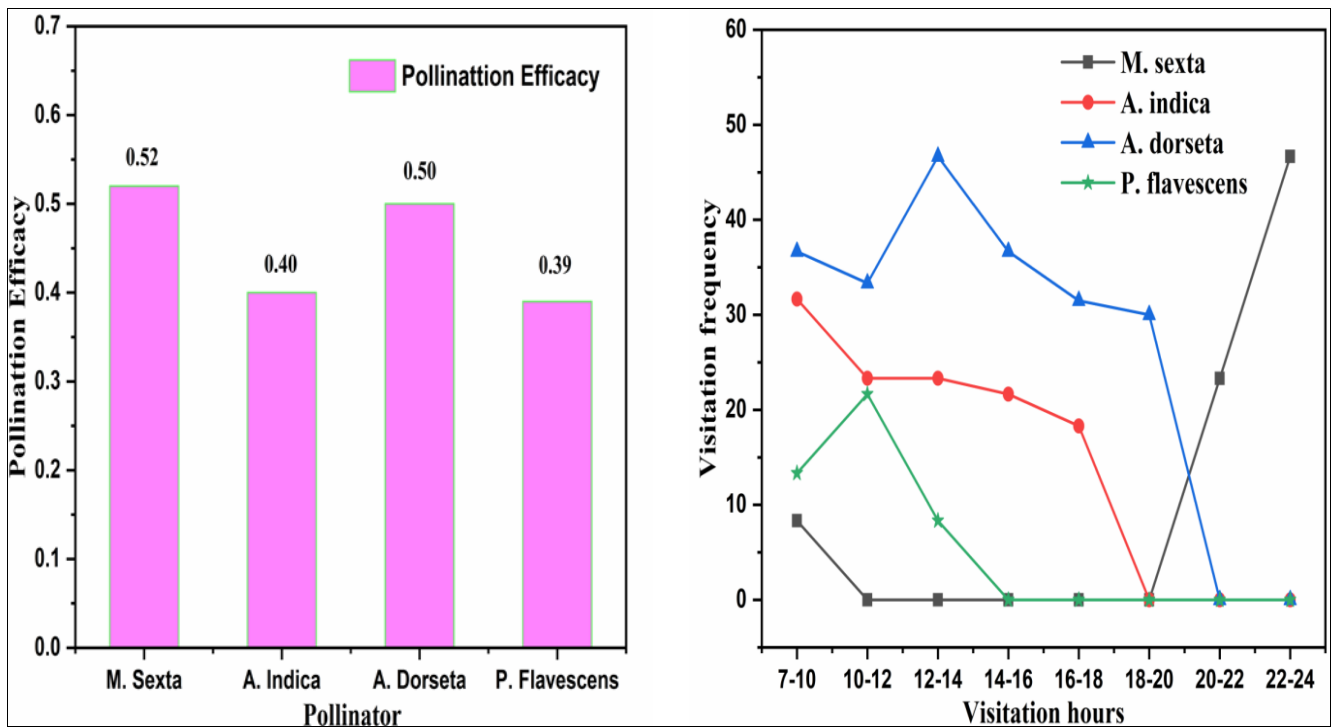


Fig-1: Graphical presentation of pollination efficacy and visitation frequency

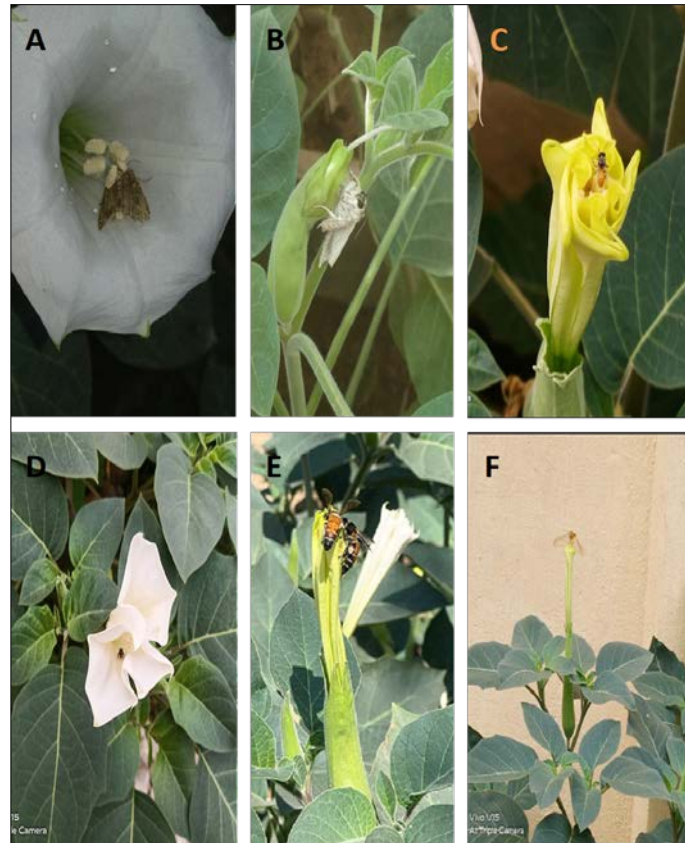


Plate- 2: Floral visitors of *Datura innoxia* Mill. A & B- *Manduca sexta* Lin., C& D- *Apis indica* Fab., E- *Apis dorsata* Fab., F- *Pantala flavescens* Fab.

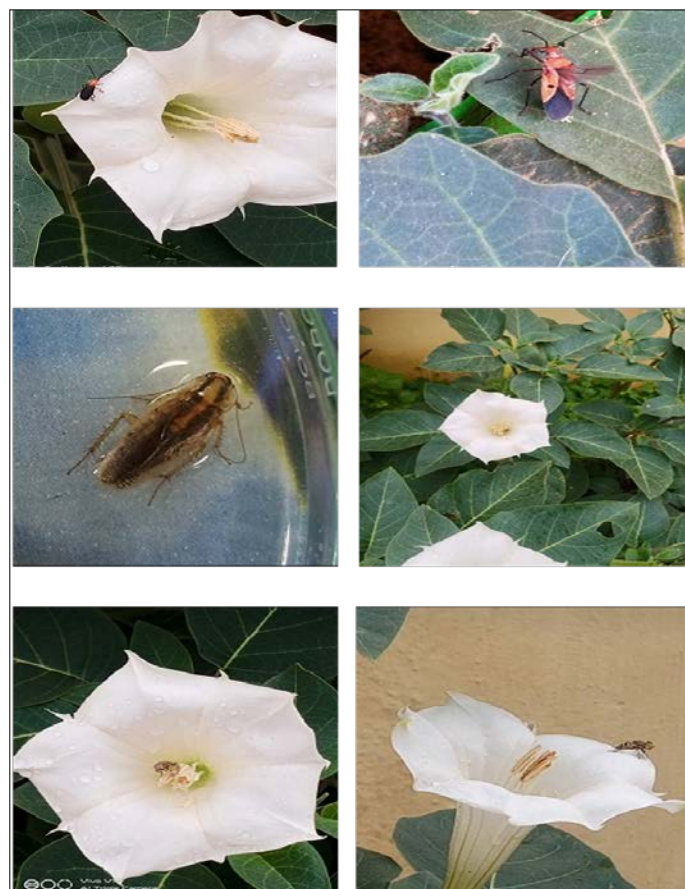


Plate 3: A-*Aulacophora intermedia* Jacoby. (Pumpkin beetle), B- *Dysdercus cingulatus* Fab (Red cotton bug), C & D- *Blattella germanica* L. (German cockroach) and *Eristalinus megacephalus* Rossi. (Spot eye hover fly)

Nectar analysis

For the analysis of nectar amount and sugar concentration the samples were collected at the time of anthesis (protected flower) and next day after anthesis (unprotected flower) from 20 randomly selected flowers each. The Benedict test of nectar reveals the presence of fructose and sucrose rich nectar by showing a cherry red color.

Table 3: Volume and sugar concentration present in nectar of *Datura innoxia* flower.

Protected flower		Unprotected flower	
Volume of nectar Mean±SD	Sugar/ brix % Mean±SD	Volume of nectar Mean±SD	Sugar/ brix % Mean±SD
92±34.58µl.	4.05±0.76%	54±16.026µl	4±0.29%

Breeding Experiments

For the study of breeding behaviour in *Datura innoxia* some breeding experiments such as Apomixis, Natural autogamy (bagged flower), Emasculated open flower, Geitonogamy and Xenogamy were carried out continue two years 2021-2022 and observation were made as follow. Among all breeding experiments conducted in 2021 and 2022, the cross-pollination results demonstrated that geitonogamy and xenogamy provided the highest percentage of fruit set 100% in 2021 and 84%, 96% fruit set in 2022. The result of

autogamy exhibited 56% and 64% of the fruit set respectively in 2021 and 2022. While 64% in 2021 and 82% in 2022, fruits were produced from emasculated open flowers (table-4). Thus, based on the experiment's findings we can say that *Datura innoxia* Mill is an invasive plant that is highly self-compatible as well as cross-pollinated. It demonstrates both xenogamy and geitonogamy, demonstrating a variety of reproductive strategies. In contrast to Apomixes, all four types of breeding trials produced a substantial percentage of fruit sets.

Table 4: Breeding experiments in *Datura innoxia* Mill.

Types of pollination treatment	Breeding Experiments in 2021			Breeding Experiments in 2022			Mean of fruit set % in 2021-22 Mean±SD
	No. of flower pollinated	Fruit set	Fruit set%	No. of flower pollinated	Fruit set	Fruit set%	
Apomixis	50	0	0	50	0	0	0
Natural autogamy (Bagged flower)	50	28	56%	50	32	64%	60±5.65
Emasculated open flower	50	32	64%	50	41	82%	73±12.72
Geitonogamy	50	50	100%	50	42	84%	92±11.31
Xenogamy	50	50	100%	50	48	96%	98±2.82

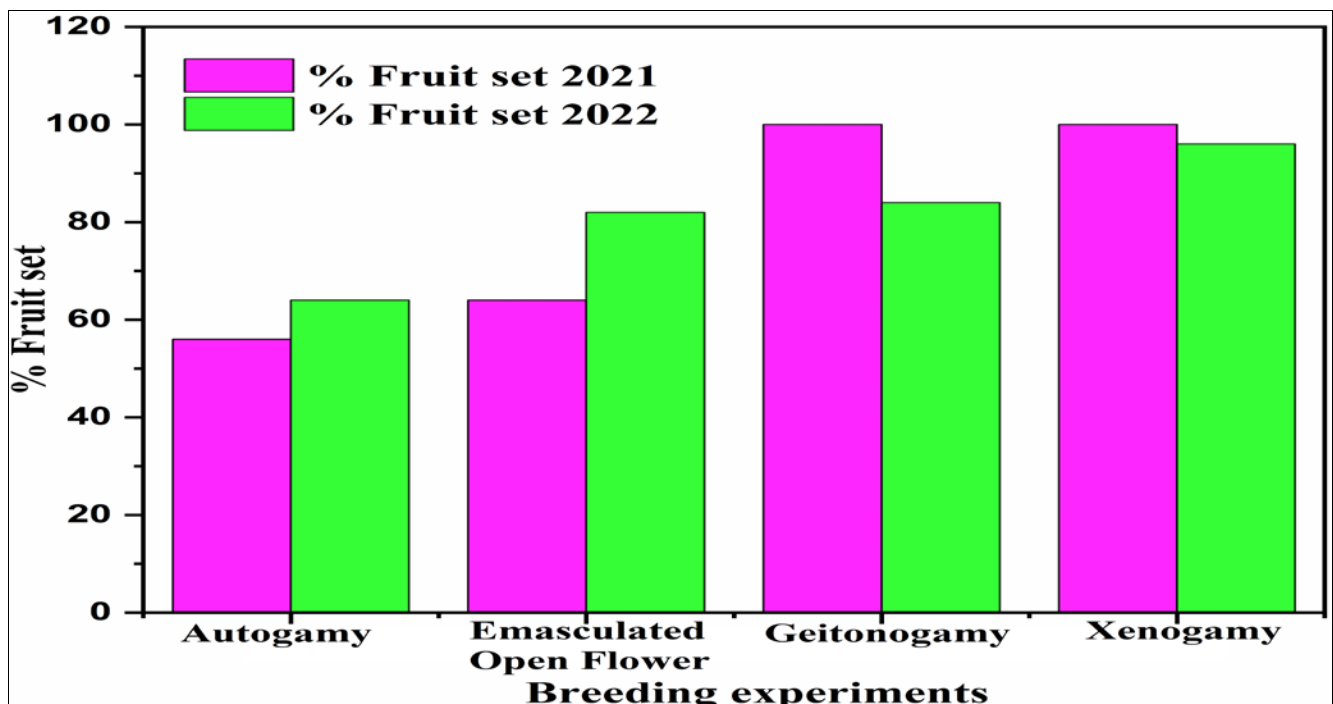


Fig 2: Graphical presentation of breeding experiments in *Datura innoxia* Mill.



Plate 4: A- Experimental site, B- Bagged flower for autogamy, C- Apomixis experiment, D-Geitonogamy breeding experiment, E & F- outcross breeding experiment xenogamy.

Result and Discussion

Flower visitors and their behaviour

In order to ensure successful pollination, flowers rely on insects that feed on pollen grains and produce enormous amounts of nutrients (USDA, United States Department of Agriculture). Flower visitors of *Datura innoxia* observed are *Manduca sexta* L. (Hawk moth), *Apis indica* Fab. (Indian honey bee), *Apis dorsata* Fab. (Giant honey bee) and *Pantala flavescens* Fab. (Dragonfly). The hawk moth visits on flower observed at dusk and at night between 2000h-2400h. Time spent on each flower is 15-20 seconds for sucking the nectar. Visitation frequency is very high and efficacy of pollination observed is 0.52 ± 0.001 . *Apis indica* Fab. belong to order Hymenoptera. These are the secondary group of pollinator in *Datura innoxia*, they visit flowers at early morning 0600h to 1200h and start foraging flowers for nectar as well as pollen transfer. Visitation frequency of *Apis indica* was observed high 36.65%, time spent on each flower is 5-10sec. and pollination efficacy was calculated as 0.40 ± 0.02 . *Apis dorsata* is the member of family- Apidae and belongs to order- Hymenoptera commonly known as giant honey bee. They start visits of flower from early morning 0600h to whole day at the dusk 1800h. They visit flower to flower for nectar as well as eating pollen grain on buds and open flowers and spent approx 40-80 second on each flower. It is found that they enter in flower at the bud stage and spent 5-10 sec in corolla tube before opening the flower. The pollination frequency is highest 83.30 % observed among other pollinators of *Datura innoxia* Mill. Pollination efficacy of *Apis dorsata* calculated 0.50 ± 0.02 . *Pantala flavescens* Fab. is the member of family- Libellulidae and Order- Odonata. It is commonly known as wandering glider and dragonfly. *Pantala flavescens* observed on *Datura innoxia* flower only in rainy season. It starts foraging at 0700h to 1000h and time spent on each flower 4-6 sec. It visits flowers for eating pollen grains. Pollination frequency of *Pantala flavescens* observed moderate with percentage of 13.33% and pollination efficacy was calculated 0.39 ± 0.01 which is lowest among all pollinators of *Datura innoxia* Mill. Some other floral visitors were also observed on *Datura innoxia* flower, these were merely visitors the function as a pollinator was unknown. These are *Dysdercus cingulatus* Fab. (Red cotton

bug), *Eristalinus megacephalus* Rossi. (Spot eye hover fly) and *Aulacophora intermedia* Jacoby. (Pumpkin beetle) showing in plate-3.

Similar benefits, but more widespread, are provided by the hawkmoth's relationship with *Datura wrightii* Regel. (Solanaceae). Adequate pollen grains transfer by *Manduca sexta* L. to produce fruit and seed set (Judith, L. Bronstein.; Huxmans, T. *et al.* 2009) [21]. Hawk moth pollinators and honeybee foragers are frequently seen on the enormous, white flowers of the night-blooming *Datura wrightii*, especially during the evening and dusk. Their foraging behaviour focuses on the anthers, indicating that they may be pollen eaters (Andrew, C.M. *et al.* 2018) [3].

Nectar analysis

In present study, nectar volume was measured using a micro capillary tube from protected and unprotected flowers of *Datura innoxia* Mill. Sugar concentration was assessed using a light refractometer. The volume of nectar in protected flower evaluated $92 \pm 34.58 \mu\text{l}$ and brix percentage was found $4.05 \pm 0.76\%$. The nectar amount in unprotected flower that visits by many pollinators measured $54 \pm 16.026 \mu\text{l}$ and sugar concentration estimated $4 \pm 0.29\%$. In *Datura innoxia*, only a small amount of nectar was produced prior to anthesis; nearly all of nectar was released after the flower opened. Sugar concentration was constant throughout the flowering season and across populations. Baker and Baker, 1983 [5] found that flowers that attract hawk moths in particular tend to have nectars that are high in sucrose. Usually, flowers with nocturnal anthesis and hawk moth pollination exhibit a certain set of characteristics, such as night blooming flower, whitish corolla, potent, pleasant odors, and an abundance of diluted nectar (Faegri and Van der Pijl, 1979) [14].

Breeding experiments

Pollination is essential for gene recombination in angiosperms and it is crucial for plant breeding initiatives (Aswathi *et al.*, 2015). Autogamy and inbreeding are generally causes a decline in genetic variability and also considered as fatal for the evolution of angiosperms (Silva and Goring, 2001) [32]. Mc Cubbin and Kao (2000) [26] assert that the most effective widely distributed plant strategy is

the ability of a plant to identify and reject its own pollen grains from the pistil in order to prevent inbreeding and promote outcrossing. In present study the results of breeding experiments reveals that all emasculated flowers without pollination did not produce fruit, showing no evidence of apomixis in *Datura innoxia* Mill. The result of cross pollination showed that geitonogamy and xenogamy produced highest percentage of fruit set (100%) in 2021. Natural autogamy (bagged flower) produced 56% fruit set in 2021 and 64% in 2022 respectively. Emasculated open flowers produced 64% in 2021 and 82% fruit set in 2022. So we can conclude after observing the results of experiments that *Datura innoxia* Mill is a highly self-compatible as well as cross pollinated invasive plant species, it exhibits xenogamy, geitonogamy and thus shows various ways for reproduction. Instead Apomixis all five types breeding experiments produced good percentage of fruit sets. In *Datura* abortion of fruit is rare occurrence. According to Stephenson (1981), the most prevalent causes of pollination failure are sexual selection, predation, and lack of nutrition, which results in a high abortion rate and poor fruit set rate. Compared to other *Datura* species, *Datura ferox* L. which is self-compatible and autogamous produced a higher percentage of fruits through natural pollination, and it possesses a variety of traits that give it an ecological advantage during the establishment and survival processes in agroecosystems, which makes it difficult to control the species (Toress *et al.*, 2013). *Datura wrightii* Regel. is known to be self-compatible, self-pollination happens when anthers and stigmas come into touch during flower opening and corolla shed the following day, as well as when pollinators spread pollen across flowers on the same plant (Elle and Hare, 2002)^[13]. The amount of open flowers on a plant had a significant impact on the outcrossing rate of *Datura wrightii* Regel. This is most likely a result of enhanced geitonogamy, internal plant pollinator movement, and a plant with many open flowers (De Jong, Waser and Klinkhamer, 1993). Flowers of *Datura stramonium* L. produce a continuous variation in herkogamy that affects the relationship between autogamous and outcrossed flowers (Motten and stone, 2000).

Conclusion

Datura innoxia is a wild, hazardous nocturnal plant species. The nectar of *Datura innoxia* gathers at the base of long corolla funnel, making difficult for most insects to access and ingest it. Additionally, all parts of the plant exude a foul smell, which encourages a small number of insects to visit the flower and act as pollinators. The primary pollinator of *Datura* species is *Manduca sexta* L. It visits on flower at dusk and at night and plays an important role in pollination. Some other most frequent pollinators of *Datura innoxia* Mill. are *Apis indica* Fab. (Indian honey bee), *Apis dorsata* Fab. (Giant honey bee) and *Pantala flavescens* Fab. (Dragonfly). They visit flower at the morning and afternoon for pollen as well as nectar reward and play a significant role in pollination. After observing the results of breeding experiment we can conclude that *Datura innoxia* Mill. is a highly self-compatible as well as cross pollinated invasive species. It exhibits autogamy, xenogamy and geitonogamy and thus shows various ways for reproduction. Instead apomixis, all five types breeding experiments produced good percentage of fruit set.

References

1. Ali S, Ripley SD, Dick JH. A pictorial guide to the Birds of the Indian subcontinent. Bombay Natural History Society, Mumbai, 1995.
2. Anderson GJ. Systemics and reproductive biology. In: Hoch, PC and Stephenson, A.G. Experimental and molecular approaches to plant biosystematics. Mongr. Systematic Botany, 1995:53:263-272.
3. Andrew CM, Richman S, Thomson E. Do honeybees acts as pollen thieves or pollinator of *Datura wrightii*. Journal of pollination ecology, 2018:24(8):164-171.
4. Aswathi P, Aswani K, Sabu M. Pollination Biology of *Costus woodsonii* Maas. (Costaceae). The International Journal of Plant Reproductive Biology, 2015:7(2):120-127.
5. Baker HG. Baker I. Floral nectar sugar constituents in relation to pollinator type. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, 1983, 117-141.
6. Becker KM. A comparison of angiosperm classification systems. Wiley Online Library, 1973:22(1):19-50.
7. Chauhan S. Floral and Pollination biology of *Saraca asoca* (Roxb.) De Wilde (Caesalpinioideae). International Journal of Plant Reproductive biology, 2018:11(1):31-37.
8. Cruden RW. Pollen- ovule ratio: A conservative indicator of breeding systems in flowering plants. Evolution, 1977:31:32-46.
9. Dafni A. Pollination ecology: A practical approach. Oxford University Press, Oxford, UK, 1992.
10. Dafni A, Firmage D. Pollen viability and longevity practical, ecological and evolutionary implications. Plant Systematics and Evolution, 2000:222:113-132.
11. Davidson RL. A note on anthesis in some common grasses near Johannesburg and the relation of anthesis to collection of pollen for medical, 1941.
12. De Jong TJ, Klinkhamer PGL. Evolutionary Ecology of Plant Reproductive Strategies. Cambridge University Press, 2005.
13. Elle E, Hare JD. Environmentally induced variation in floral traits affects the matting system in *Datura wrightii*. Functional Ecology, 2002:16(1):79-88.
14. Faegri K, Van der Pijl L. The principals of Pollination ecology. Oxford: Pergamon Press, 1979.
15. Godini A. Counting Pollen grains of some almond cultivars by means of a haemocytometer. CIHEAM, 1981, 83-86.
16. Grant V, Grant K. Flower pollination in the phlox family. Columbia University press, New York, 1965.
17. Harder LD, Barrett SC. Pollen dispersal and matting patterns in animal pollinated plants. In: Lloyd, DG and Barrett, SC (eds.) Floral biology: Studies on floral evolution in animal- pollinated plants. Chapman and Hall, New York, USA, 1996, 140-190.
18. Holsinger KE. The scope and the limits of conservation genetics. JSTOR, 1996:50(6):2558-2561.
19. Jafar Palot M, Balkrishanan VC, Babu K. Keralathile Chithra Shala bhanganl (Butterflies of Kerela- A photo field guide). Malabar Natural History Society, Kalicut, 2003.
20. Jothish PS, Kumar AC. Pollination Biology of *Cassine kedarnathii* Sasidh. & Swarup. (Celastraceae), A rare endemic tree species of the western ghats, India.

- International Journal of Plant Reproductive Biology,2018:11(1):24-30.
21. Judith LB, Huxman T, *et al.* Reproductive biology of *Datura wrightii*: The benefits of a herbivorous pollinator. *Annals of Botany*,2009:103:1435-1443.
 22. Kaul Moza M, Bhatnagar AK. Plant Reproductive biology studies crucial for conservation. *Current Science*, 2007: 92(9):1207.
 23. Lloyd DG, Webb CJ. The evolution of heterostyly. In Barrett, S.C.H., Ed, *evolution and function of heterostyly*. Springer Verlag, New York, 1992, 151-178.
 24. Marbaniang EJ, Venugopal N, Verma S, Raina R, Khajuria A, Gautam A. Floral biology and embryological studies are important for conservation of threatened plants having reproductive bottlenecks: A case study of *Illicium griffithii* Hook. F. & Thomson. *Current Science*,2018:114(3): 576-587.
 25. Maslo S, Saric S. *Datura innoxia* Mill. (Solanaceae), a new alien species in the flora of Bosnia and Herzegovina. *THAISZIA Journal of Botany*, 2019: 29(2): 225-230.
 26. Mc Cubbin AG, Kao T. Molecular recognition and response in pollen and pistil- interactions. *Annual Review of Cell and Developmental Biology*, 2000:16:333-364.
 27. Mottem AF, Stone JL. Heritability of stigma position & the effect of stigma anther separation on out crossing in a predominantly self-fertility weed, *Datura stramonium* (Solanaceae). *American Journal of Botany*,2000:8:339-347.
 28. Parrish JAD, Bazzaz FA. Pollination niche separation in a winter annual community. *Oecologia*, 1978: 35(2): 133-140.
 29. Richards AJ. Breeding systems in flowering plants and the control of variability. *Flora Geobotanica and phytotaxonomica*, 1996: 31(3): 283-293.
 30. Richards AJ. *Plant breeding systems*. Garland Science, 1997.
 31. Sengupta TM, Tapan A. *Insects of India*. Estern Book Corporation, New Delhi, 2005.
 32. Silva NF, Goring DR. Mechanisms of self-incompatibility in flowering plants. *Cellular and Molecular Life Sciences*, 2001:58:1988-2007.
 33. Stephenson AG. The regulation of maternal investments in an indeterminate flowering plant (*Lotus corniculatus*). *Ecology*, 1984:65(1):113-121.
 34. Torres C, Mimosa M, Galetto L. Nectar ecology of *Datura ferox* (Solanaceae): An invasive weed with nocturnal flowers in agro ecosystems from central Argentina. *Plant systematics and evolution*, 2013: 299(8):1433-1441. Available at: <https://www.researchgate.net/publication/257447641>
 35. USDA. United States Department of Agriculture. Available at- <https://www.fs.usda.gov/wildflowers/pollinators/plant>.
 36. Walker IW. Evolution of exine structure in the pollen of primitive angiosperm. *Amer. Journal of Botany*, 1976:61:891-902.
 37. Weller SG. The relationship of rarity to plant reproductive biology. In: Bowles, ML and Whelan, C.J. (eds.) *Restoration of endangered species*. Cambridge University Press, UK, 1994, 90-117.
 38. Wyatt R. Inflorescence architecture: how flower number, arrangement and phenology affect pollination and fruit set. *American Journal of Botany*,1982:69:585-594.