PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.2517

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Floral biology and phenological studies of *Datura metel* L. in Tripura, Northeast India, with special reference to floral morphotypes

Aparajita Das[1*], Somnath Kar[2], Panchatapa Bhattacharya[1], Sani Das[1], Dixit Bora[1] & B.K. Datta[1]

[1]Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University, Suryamaninagar -799022, Tripura, India

[2]Department of Botany, Holy Cross College, Jubatara, Lembucherra, Tripura, India

***Email:** dasaparajita0210@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 20 March 2023
Accepted: 23 July 2023

Available online
Version 1.0 : 07 October 2023
Version 1.1: 15 October 2023

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS,UGC Care etc. See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Das A, Kar S, Bhattacharya P, Das S, Bora D, Datta B K. Floral biology and phenological studies of *Datura metel* L. in Tripura, Northeast India, with special reference to floral morphotypes. Plant Science Today (Early Access). https://doi.org/10.14719/pst.2517

Abstract

Datura metel L. is an important medicinal plants of Tripura. There are four floral morpho-types found throughout India. The floral biology of four morpho-types of *D. metel* collected from different places of Tripura state have been investigated based on their morphological and palynological study. Initiation of the floral bud, anthesis, pollen viability, pollen germination, and pollen production are the topics covered in the present study. The present study includes photographic representations and UPGMA dendrogram for quick identification, as well as a detailed explanation of four morpho-types of the species.

Keywords

Floral morphs, Floral visitors, Pollen germination, Reproductive success

Introduction

Datura is an important genus of the *Solanaceae* family and comprises approximately 15 species. In India, five species of *Datura*, *Datura metel* L., *D. stramonium* L., *D. ferox* L., *D. innoxia* Mill. and *D. quercifolia* Kunth were recorded. As the name "Datur" is derived from the Indian word for the seed capsule "Dhatura", the term "Dhatura" commonly refers to *Datura metel* (1). However, any species belonging to the entire genus may also be referred to as "Dhatura" (2). *Datura metel* is a perennial herbaceous plant that can grow to a height of 1.5 metres. The leaves of the plant are dark green, glabrous, simple, alternate, broadly ovate, and shallowly lobed. Flowers are huge, solitary, trumpet-shaped, and have a sweet aroma that is typically savoured in the mornings and nights. Colour of the flowers vary from white to yellow and light to dark purple and pollination is through insects. Different colours of corolla and sweet fragrances of *D. metel* attract the insects. The fruit is a capsule and it is covered by tiny spines. The plant is typically found growing in waste areas and along roadsides. This plant tolerates mediocre soil but prefers rich, moist soil. Since 16th century, the use of the genus *Datura* has been established in traditional medicine, associated with its psychotropic, anticholinergic, and anti-inflammatory effects (3). Night-blooming *Datura* species, such as *D. innoxia*, *D. metel*, *D. stramonium* and *D. wrightii* are grown for their attractive, funnel-shaped and scented blooms (4).

Studies of reproductive biology or floral biology are essential for the successful cultivation, conservation and genetic improvement of plants (5-7). Knowledge of the floral structure and breeding systems is important for the controlled pollination and crossing of any species. Inflorescence structure influences the foraging efficiency of insects, the degree of outcrossing and other aspects of plant reproductive success (8). There has been no previous

record of published studies on reproductive biology, including the floral structure, floral morphs and development of *D. metel*.

Materials and Methods

Six populations of *D. metel* were selected for the present investigation in the natural conditions. The selected places were Damdamia (N 23°53 '48.9" and E 091°18'27.6"), Mohanpur (N 23°58'15.0" and E 091°22'20.5"); Khowai (N 24° 04 '03.5"and E 091°36'15.2"); Bishalgarh (N 23°33 '57.8" and E 091°25'22.9"); Dhalai (N 24°11 '48.7" and E 091°49'42.5") and Udaipur (N 23°33 '02.2"and E 091°27'48.9").

As per the field investigation, the species exhibited four different floral morphs (morphs 1, 2, 3, and 4) that could be distinguished by the colour of the corolla and shape of the capsules. The first morph type (morph 1) had an entirely purple corolla; the second morph (morph 2) displayed a completely purplish white corolla; the third morph (morph 3) had white corolla; and the fourth morph (morph 4) had yellow corolla. The shape of fruit capsules is an important character of *Datura* for proper identifications. Here, round to ovoid shape capsule were present in morph 1, oval shape capsule in morph 2, broad-ovoid capsule in morph 3 and ovoid-ellipsoid capsule in morph 4. The samples were collected when they were in full bloom and images of the flowers were taken with a Nikon (D5600) camera, and some fresh specimens were collected from the field for further taxonomic authentication. Morphological and morphometric analysis were performed using a stereo microscope (Stimi508, Carl ZEISS), binocular microscope (OLYMPUS CX23), and a scanning electron microscope (Sigma 300, Carl ZEISS) at the Central Instrument Centre, Tripura University, India. Various important characteristics of four floral morphs of *D. metel* L., UPGMA dendrograms (Fig. 4) were created based on the floral morphometric properties. The processed specimens (9) were placed in the Tripura University Herbarium (TUH).

Five healthy plants were chosen from each population and the phenological characters were observed periodically in the natural habitat. Habit of the plant, the time of anthesis, floral bud initiation and blooming were noted during systematic observational study. Morphological and morphometric studies of flowers were carried out using a hand lens and a dissecting microscope. The process and the pollinating agents of the flowers were noticed and documented (Plate 1 and 2). The morphology and morphometric analysis of acetolysed pollen, mounted in glycerine jelly was performed under oil-immersion (10, 11). *In-vitro* pollen germination and pollen tube germination was carried out following the standard methods (12, 13). Acetocarmine (2%) and 2, 3, 5-triphenyl tetrazolium chloride (TTC; 1%) solutions were used to examine pollen fertility and pollen viability respectively (14, 15). The pollen germination study was conducted using various concentrations of sucrose solution (2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, and 40%) and 10% sucrose solution combined with various concentrations of boric acid. Stigmas of various ages were fixed in Carnoy's fixative for 3 to 4 hours, stained with aniline blue-lactophenol, and then examined under a microscope to determine the stigma receptivity (16). The stigmas with the developing pollen grains were regarded to be receptive and the pollen-ovule ratio was determined using Dafni's method (17).

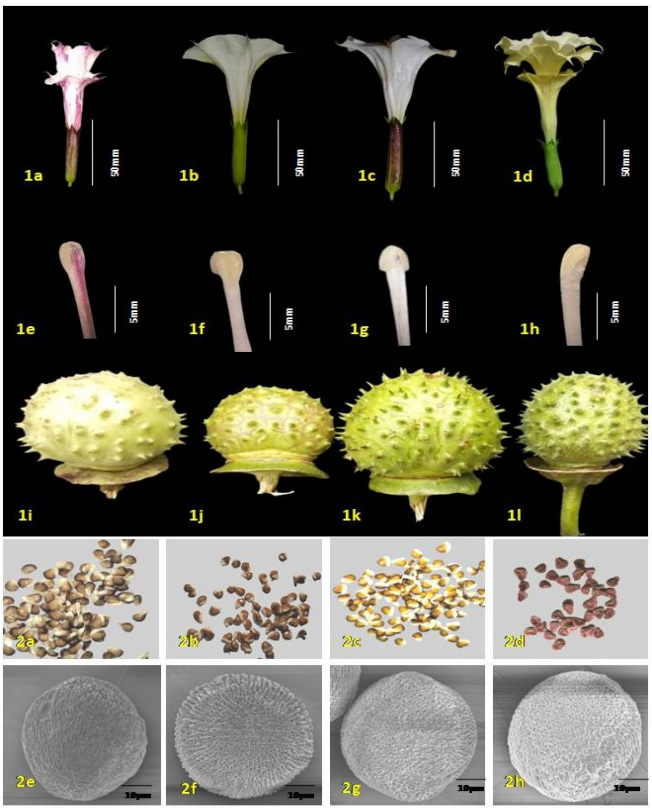

Plate 1: 1a)Flower,1e)Stigma, 1i)Fruit,2a) Seeds and 2e) Pollen of Morph 1; 1b)Flower, 1f)Stigma, 1j)Fruit, 2b) Seeds and 2f) Pollen of Morph 2; 1c) Flower,1g)Stigma, 1k) Fruit,2c) Seeds and 2g) Pollen of Morph 3 and 1d) Flower,1h)Stigma, 1l) Fruit,2d) Seeds and 2h) Pollen of Morph 4 of *Datura metel* .

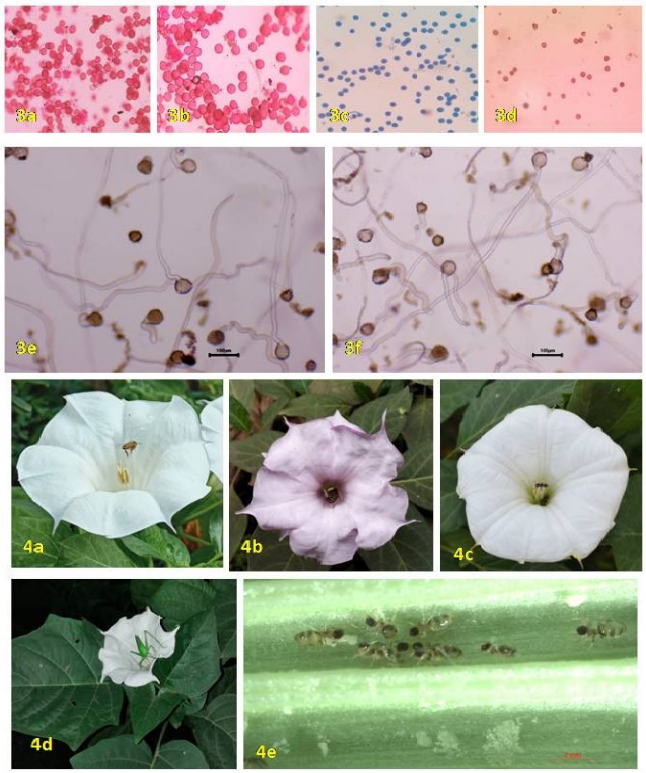

Plate 2: 3a) Muntzing's mixture, 3b) 2% Aceto-carmine, 3c) Lactophenol cotton blue, 3d) TTC pollen viability slides of *Datura metel* L.; 3e) Pollen germination in 10 % sucrose of *Datura metel* L., 3f) Pollen germination in 10 % sucrose with 500 ppm Boric acid solution of *Datura metel* L.;4a),4b),4c),4d) and 4e) Different pollinators of *Datura metel* L.

Results

Floral Phenology

All of the plant phenotypes were observed from April 2019 to October 2020, and then again from May 2021 to April 2022. The blooming period of all the plants ere lasted for 2-3 months; however in *D. metel* it was throughout the year, but its prime flowering time was from the end of March to the middle of December. It took 18-20 days for vegetative shoots to produce floral buds, however, it took only 12–15 days for the buds to bloom. The ovary produces ripe fruits after 15–25 days of pollination. It was discovered that on rainy days, the process of anthesis, when the flower emerges from its bud, is delayed by an hour. Between 4 and 5 O'clock, anthesis occurred, and between 5 and 6 O'clock, the bloom unfolds. The average lifespan of a flower was 37.1 ± 0.75 hours with a range of 35 - 40 hours.

Floral Morphology

Inflorescence is solitary, in fork of branches; pedicel 5-6 mm in length, pedicel indumentums densely hairy. Calyx 60-90 mm in length, 34-55 mm in width, calyx indumentums densely hairy, purple or green in colour, calyx teeth 5, 10-16 mm in length, unequal, apex acuminate. Corolla infundibuliform, lobes 5-10, purple/ yellow/white/purplish white in colour, 150-200 mm in length, 60-130 mm in width. Lobes 20-26 mm in length, apex acuminate. Stamens 5-7, filament 100-150 mm in length, hairy at adnate region, anther 13- 15 mm in length, 0.2-3.2 mm in width, yellowish white in colour, anther indumentums sparsely hairy. Ovary ovoid, carpel 2, 4-8 mm in length, 3-6 mm in width, style 118-150 mm in length, stigma 3-4 mm in length, 2-3 mm in width.

Pollinating agents

There were a total 10 genera of floral visitors out of which *Apis* sp., *Alphitobius diapernus*, and *Tapinoma melanoceophelum* were the significant floral visitors. Pollination activity was most conveniently operated during 6 am to 12 pm, and it slowly declined later. It was found that *Apis* sp. regularly visited the flowers during the day time for pollination. It has been recorded that *Alphitobius diapernus* visited during the pollination one to three flowers per spell. The duration of the visit was 10 to 30 seconds per flower. *Tetragonula sp*. were significant floral visitor in Morph 1, 2 & 3; *Alphitobius diapernus* were in Morph 2 & 4 and *Camponotus compressus* were in Morph 1, 2 & 3. Bees would visit one to six blooms at a time, staying for four to sixty seconds each time. The pollination method, which was determined to be most useful, involved *Apis sp.* visiting the flowers to gather pollen and nectar. They frequently move from one flower to another, which may facilitate pollen transport. After the flowers are opened, the visitors to the blooms begin their activities. During cloudy days, there is less insect activity. Flowers with morph 1 and morph 2 had the highest frequency of floral visitors, whereas flowers with morph 4 (entirely yellow) had the lowest frequency (Table 2).

Stigma receptivity

In *D. metel*, the stigma became receptive between 15 and 17 hours before flowering, and the flowers bloomed between 17 and 18 hours. During the second day of blossoming, the stigma was still open. The stigma was glossy and yellowish when in the receptive condition and turned blackish red when the receptivity is lost. In *D. metel*, the stigma receptivity lasted anywhere from a few hours to a few days. The time of day and the existence or absence of stigmatic exudates may have an impact on the susceptibility to stigma. Environmental elements like temperature and humidity have an impact on the receptivity period. Typically, the stigma's receptive period lasted until the third day of flowering on overcast and wet days. Also correlated with the shift in blossom colour is stigma receptivity. According to the findings, the stigma of morph 1 was more receptive than those of the other three morphs.

Pollen Viability

Pollen viability is the ability to successfully complete fertilisation on a suitable, receptive stigma and carry out the post-pollination activities. Pollen viability also known as pollen stainability as it depends on the staining method (18). In order to discriminate between viable and nonviable pollen grains as well as fertile and sterile pollen grains, all of the colours used in this experiment demonstrated good colour contrast.

A high likelihood of fertilisation may be ensured by the pollens of *D. metel*, which demonstrated great viability and fertility rates. The pollens of morph 2 showed higher percentages i.e. 95% than the other floral morphs in all tests for viability and fertility (Fig. 1).

Table 1. Comparison of different essential characters of four floral of morphs of *Datura metel* L.

Parameters		Morph 1	Morph 2	Morph 3	Morph 4
Calyx size		60 mm (<70mm)	69 mm (<70mm)	78mm (<100mm)	82mm (<100mm)
Calyx teeth no.		5 (2+1+2)	5 (2+3)	5 (2+1+1+1)	5 (2+1+1+1)
Corolla size		177mm(<200mm)	182mm(<200mm)	192mm(<200mm)	203mm (>200mm)
Stamen size		13 to 14 mm	15mm	15mm	14mm
Pollen size	**P**	50.71 ± 0.91	44.70 ± 0.63	47.66 ± 0.85	47.41 ± 1.08
	E	48.60 ± 1.14	47.92 ± 0.52	43.23 ± 0.95	43.43 ± 0.43
Style length		119 mm	126mm	124mm	148mm
Ovule number (Mean±SE)		205.4 ± 1.99	210.6 ± 2.85	208.8 ± 2.03	197.6 ± 1.46
Fruit size (Mean±SE)		43.8 ± 0.95	52.8 ± 1.37	50.3 ± 1.00	39.1 ± 0.86
Seed weight (Mean±SE)		0.0167 ± 0.00	0.0182 ± 0.00	0.0189 ± 0.00	0.0160± 0.00

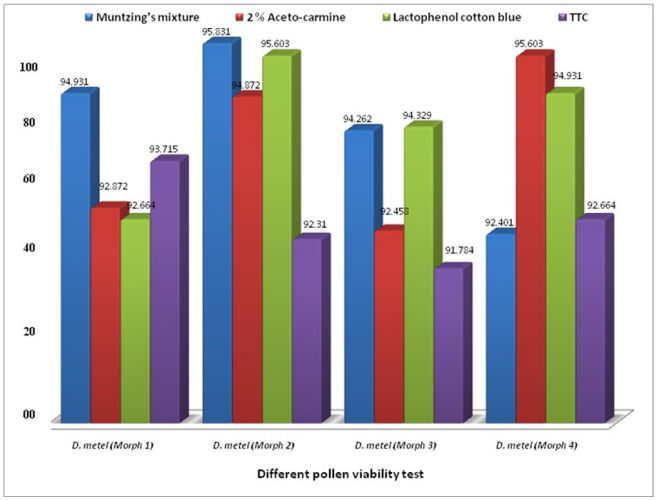

Figure 1: Pollen viability of four floral morphs of *Datura metel*.

Pollen germination

In this study, the pollen grains displayed the highest rates of germination (81.18% by *D. metel* morphs 1 respectively) and pollen tube formation (705.433±61.376 µm, 785.42± 18.95 µm by the morphs 1, 2 respectively) in 10% sucrose solution. The maximum pollen germination rate (80.01 ±1.16) by morph 1 in *D. metel* and the largest pollen tube development (814.37± 25.67 µm) by morph 3 were seen in 500 ppm Boric acid +10% sugar solution, which was one of several concentrations of boric acid and 10% sucrose solution (Fig. 2, 3).

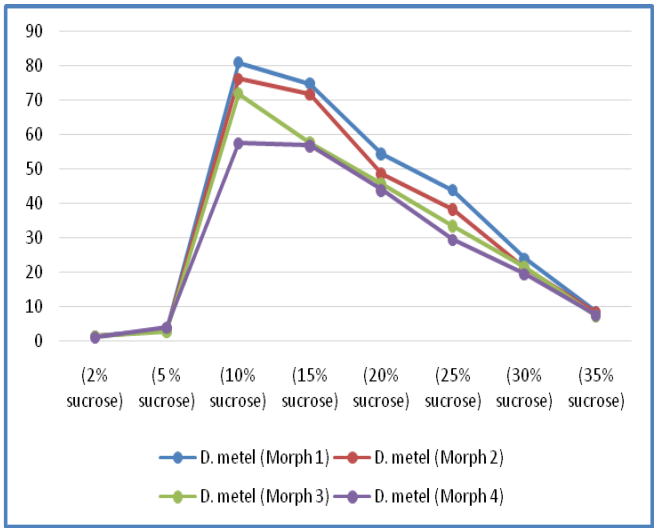

Figure 2: Extent of pollen germination in four floral morphs of *Datura metel* in different concentration of sucrose solution.

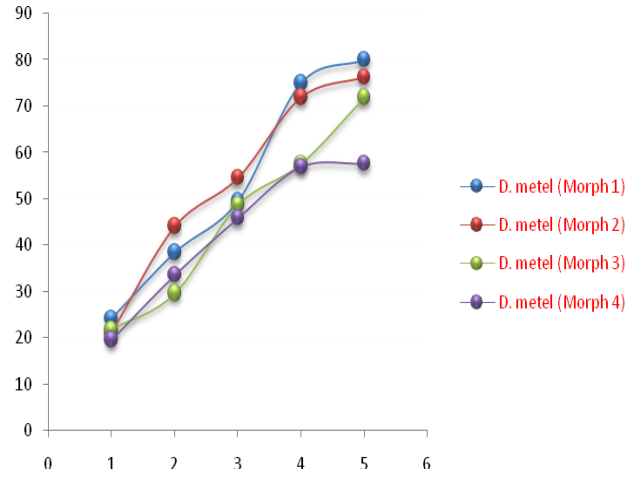

Figure 3: Extent of pollen germination in four floral morphs of *Datura metel* in different concentrations of boric acid along with 10 % sucrose solution.

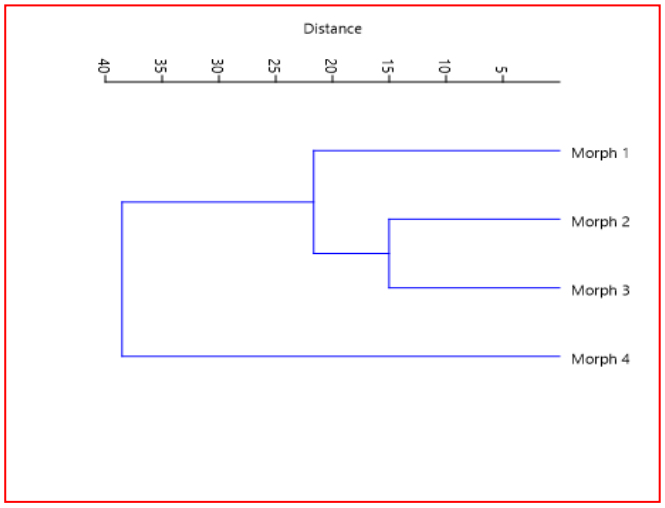

Figure 4: UPGMA dendrogram based on floral morphometric characters by Comparison of different essential characters of four floral of morphs of *Datura metel* .

Pollen: ovule ratio

According to Shivanna et al. (19), the pollen: ovule ratio is a more reliable indicator of reproductive success than the total amount of pollen in each flower or plant. During the present investigation estimated pollen: ovule ratio was found to be 8259.72 ± 153.50 in morph 1, 1994.25 ± 73.64 in morph 2, 2820.21 ± 175.922 in Morph-3 and 5251.4 ± 535.751 in morph 4 (Table 3). According to Cruden (20), xenogamous flowers had pollen: ovule ratios that ranged from 2108 to 19523. According to Cruden's findings and

Table 2. Floral visitors of four floral morphs of *Datura metel* L.

Sl. no.	Name of floral visitors	Family	Visiting time	Frequency of visitation			
				Morph 1	Morph 2	Morph 3	Morph 4
1.	*Tetragonula sp.*	Apidae	Day (8 am to 11am)	++	++	++	--
2.	*Alphitobius diapernus*	Tenebrionidae	Evening (5 pm) to Night (1 am), Day (7 am to 10 am)	--	++	--	++
3.	*Forficula auricularisa*	Forficulidae	Day (2pm to 4pm)	++	--	++	++
4.	*Maladera sp.*	Scarabaeidae	Day (9 am to 11am)	++	++	++	--
5.	*Eunconocephalus thunbergi*	Tettigoniidae	Evening (5pm to 7pm)	++	++	--	--
6.	*Apis sp.*	Apidae	Day (6am to 11 am and 3pm to 5pm)	++	++	++	++
7.	*Tapinoma melanoceophelum*	Formicidae	Night (9 pm) to Day (10 am)	--	++	--	++
8.	*Camponotus compressus*	Formicidae	Night (7 pm to 1am)	++	++	++	--
9.	*Aulacophora nigripennis*	Chrysomelidae	Day (6 am to 12pm)	++	--	++	++
10.	*Megacopta sp.*	Plastuspididae	Day (4 am to 11am)	--	--	++	++

the pollen-to-ovule ratio statistics for *D. metel*, the species is obligately xenogemous. Xenogamous flowers had pollen: ovule ratios that ranged from 2108 to 19523. According to Cruden's findings and the pollen-ovule ratio statistics for *D.metel*, the species is obligately xenogemous.

Reproductive Success

Morph 2 was able to produce the largest number of inflorescence per plant of 53 to 101 (mean ± SD, 53.4 ± 2.30, n = 50) and lowest in morph 4 varied between 34 and 84 (mean ± SD, 34.4 ± 4.602, n = 50), respectively. Whereas fruit set/inflorescence showed highest and lowest values in morph 4 (mean ± SD, 125.82 ± 4.91, n = 50) and morph 2 (mean ± SD = 108.212 ± 1.09, n = 50). The *D. metel* plant has the absolute/maximum reproductive potential (Rm) to produce 11320.6 ± 378.97, 10703.6 ± 579.82, 10857.8 ± 699.07 and 7242.6 ± 1013.99 seeds, respectively (Table 4). Morph 1 showed the highest value of maximum reproductive potential (Rm) and Ecological reproductive potential (Re) i.e. 11320.6 ± 378.97 and 11270.4 ± 400.92 (Table 4). This could be as a result of some significant ecological constraints.

Discussion

The four investigated floral morphs of *D. metel* of the family Solanaceae were characterized by some important floral morphological features which can serve as marker characters for the identified different morphs of this species (Plate. 1, 2). In previous studies morph 1 was considered as *D. metel* (21). But the present study clears the doubts abouts floral morphotypes of *D. metel.* It was observed that all studied floral morphs of *D. metel* showed that the initiation timing of floral buds takes 18 to 20 days to develop from vegetative shoots. Whereas, floral buds take 12-15 days to bloom. After pollination, the ovary takes 15-25 days to produce a mature fruit and the period of anthesis was 4.00 to 5.00 pm and the flower opens from 5.30 to 6.30 pm. In Table 1, there are comparisons of different essential characters (calyx size, calyx teeth, corolla size, pollen size, stamen size, style length, ovule no., fruit size and seeds weigth) of four floral morphs of *D. metel*. A total of 10 genera of floral pollinators were recorded. The important floral pollinators were *Apis* sp., *Alphitobius diapernus* and *Tapinoma melanoceophelum*. From 6 am to 12 pm, there was a spike in the pollinator's activity. In the late afternoon and at night, there were fewer floral pollinators. The *Apis sp.* frequently visited the flowers during the day, whereas Thrips did so both during the day and at night. Almost one to three flowers were visited in a single spell by *A. diapernus* (Table 2). Flowers begin to bloom between 17.00 and 18.00 hours before the stigma became receptive. One to three flowers were visited in a single spell by *Alphitobius diapernus* (Table 2). Flowers begin to bloom between 17.00 and 18.00 hours before the stigma becomes receptive. During the second day of blossoming, the stigma is still open. The stigma is glossy and yellowish when in the receptive condition and turns blackish red when the receptivity is lost. All the dyes used in the experiment for pollen viability of four morphs of *D. metel* (Fig. 1) showed good colour to differentiate between fertile and sterile pollens viz., larger percentages of Muntzing's mixture (95.83% in morph 2), Acetocarmine (95.60 % in morph 4) and Lactophenol cotton blue (95.60% in morph 2). In the TTC test the percentage of viable pollen was 93.71% in morph 1 (Fig. 1). Saha & Datta (22) also found similar results in their experiments, emphasizing that pollen grain viability assessment through the staining method seemed to express the germination potential, but not its occurrence. It may be explained by the fact that this technique overestimates the percentage of pollen tubes formed. Pollen viability is considered as an important parameter of pollen quality (23). Pollen size and viability are good markers of the course of microsporogenesis. The effect of sucrose on *in vitro* pollen germination of *D. metel* showed that the four morphs of this species required comparatively low sucrose concentration (10%) for their optimal germination (Fig. 2). It was also observed that to some extent boric acid also influences the percentages of pollen germination. However,

Table 3. Pollen production and pollen: ovule ration in four floral morphs of *Datura metel* L.

Floral Attribute	Values (Mean ± SE)			
	Morph 1	Morph 2	Morph 3	Morph 4
Number of Pollen per anther	339582.8 ± 9304.23	75390.2 ± 2211.45	115087±7857.53	77107 ±3144.16
Number of Pollen per flower	1697914 ± 46521.14	376951 ± 11057.24	587437 ± 32219.6	1034388 ± 99804.3
Number of ovule per flower	205.4 ± 1.99	210.6 ± 2.85	208.8 ± 2.03	197.6 ± 1.46
Pollen / Ovule Ratio	8259.72 ± 153.50	1794.25 ± 73.64	2820.21 ± 175.92	5251.40 ± 535.75
Assessment	The high P/O ratio along with high pollen production attributes to its high seed set.			

Table 4. Absolute and Ecological Reproductive Potential

Floral attributes	Morph 1(Mean±SE)	Morph 2(Mean±SE)	Morph 3(Mean±SE)	Morph 4(Mean±SE)
Inflorescences / plant	53.4 ± 2.30	48.7 ± 1.90	45 ± 2.93	34.4 ± 4.60
Fruit set	124.97 ± 9.21	108.21 ± 1.09	113.59 ± 5.99	125.82 ± 4.91
Seeds per fruit	202.5 ± 5.15	191.5 ± 2.50	179.5 ± 5.43	125 ± 5.51
Absolute / maximum reproductive potential (Rm)	11320.6 ± 378.97	10703.6 ± 579.82	10857.8 ± 699.07	7242.6 ± 1013.99
Ecological/realized reproductive potential (Re)	11270.4 ± 400.92	10650.6 ± 572.28	10776.2 ± 701.02	7104 ± 1020.75

the best result was obtained in 10% sucrose solution supplemented with 500 ppm boric acid (Fig. 2, 3). Lower concentrations than 300 ppm were shown to be toxic and to have the lowest germination rates in the four morphs of *D. metel*. Similarly, concentrations of boric acid, higher than 300 ppm showed larger pollen tubes and maximum germination percentages. Boron may increase the uptake of sucrose and induce germination because it is known to interact with sugar to form a sugar-borate complex, which is more easily transported than non-borate sugar molecules (19). Saha & Datta (24) and Kar & Datta (25) have also recorded similar results. The pollen: ovule ratio is 2108 to 19523. According to Cruden's findings and the pollen-ovule ratio statistics for *D. metel*, the species is represented obligately xenogemous. The present study represented the values of ecological reproductive potential (Re) i.e. 11320.6 ± 378.97 and 11270.4 ± 400.92 (Table 4). This could be as a result of some significant ecological constraints.

Conclusion

This article is the first to include full information on the floral biology and phenological investigations on floral morphotypes of *D. metel* L. This species has night blooming flowers and the floral opening occurs between 05:00 h to 06:00 h. Funnel shaped with different coloured corolla and are the main attractive structure to pollinating insects. The shape of fruit capsules is an important character to properly identify the four morphs of *D. metel*. The field experiment indicated classification *D. metel* as xenogamous flower according to the P/O ratio. The different concentrations of sucrose (2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%) and boric acid (100ppm, 200ppm, 300ppm, 400ppm, 500ppm) used in the study of *in vitro* pollen germination. This study has to be expanded to include more *Datura* species in order to find cross-promoters for future crosses that will result in hybrids with the potential to be useful in agriculture, pharmacological, and ornamentation. This present work discusses that the floral biology and phenological studies of *D. metel* is valuable for understanding the reproductive biology and pollination ecology of other *Datura* species in addition to its critical implications for the preservation and management of this important plant.

Acknowledgements

Authors are grateful to Department of Botany and Central Instruments Centre (CIC) of Tripura University for providing necessary and FE SEM (Field Emission Scanning Electron Microscope) facilities during work.

Authors' contributions

AD and PB carried out the field survey and collected the specimens. SK and BD identified the specimens and conceived the study. AD carried out the field data analysis. SD and DB participated in design and coordination the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any competing interests to declare.

Ethical issues : None

References

1. Bouziri A, Hamdi A, Borgi A, Hadj SB, Fitouri Z, Menif K, Jaballah NB. *Datura stramonium* L. poisoning in a geophagous child: A case report. Int J Emerg Medic. 2011; https://doi: 10.1186/1865-1380-4-31.
2. Freye E. Pharmacology and abuse of cocaine, amphetamines, ecstasy and related designer drugs. Springer Dordrecht Heidelberg London New York. 2010; Pp: 215-18. http://dx.doi.org/10.1007/978-90-481-2448-0.
3. Benítez G, March-Salasb M, Villa-Kamelc A, Cháves-Jiménez U, Hernándezc J, Montes-Osunad N, Moreno-Chocanoa J, Cariñanos P. The genus *Datura* L. (Solanaceae) in Mexico and Spain – Ethnobotanical perspective at the interface of medical and illicit uses. J Ethnopharmacol. 2018;219:133-51. http://dx.doi.org/10.1016/j.jep.2018.03.007.
4. DeWolf GP. Notes on cultivated Solanaceae 2 *Datura*. Baileya. 1956;4:12-13.
5. Baskorowati L. Controlled pollination methods for *Melaleuca alternifolia* (Maiden & Betche) Cheel. ACIAR Technical Reports Series, Issue 63. (Australian Centre for International Agricultural Research: Canberra, ACT). 2006.
6. Moza MK, Bhatnagar AK. Plant reproductive biology studies crucial for conversation. Curr Sci. 2007; 92:1207.
7. Baskorowati L, Moncur MW, Doran JC, Kanowski PJ. Reproductive biology of *Melaleuca alternifolia* (Myrtaceae) 1. Floral biology. Austral J Bot. 2010;58:373-83. http://dx.doi.org/10.1071/BT10035.
8. Wyatt R. Inflorescence architecture – howflower number, arrangement and phenology affect pollination and fruit-set. Amer J Bot. 1982;69:585-94. https://doi.org/10.2307/2443068.
9. Jain SK, Rao RR. A handbook of field and herbarium methods. Today and Tomorrow Printers and Publishers, New Delhi. 1977; p.157.
10. Erdtman G. Pollen morphology and plant taxonomy-Angiosperms. Almqvist and Wiksell, Stockholm, Sweden. 1952;p.539. https://doi.org/10.1080/11035895209453507
11. Wodehouse RP. Pollen grains. McGraw-Hill Book Co., New York and London. 1935.
12. Taylor LP, Hepler PK. Pollen germination and tube growth. Plant Physiol Plant Mol Biol. 1997;48:461-91. https://doi.org/10.1146/annurev.arplant.48.1.461.
13. Nair PK, Rastogi K. Pollen production in some allergenic plants. Curr Sci. 1963;32:566-67.
14. Radford AE, Dickinson WC, Massey JR, Bell CR. Vascular plant systematics. New York, Harper and Row. 1974.
15. Norton JD. Testing of plum pollen viability with tetrazolium salts. Am Soc Hort Sci. 1966;89:132-34.
16. Hauser EJP, Morrison JH. The cytochemical reduction of nitroblue tetrazolium as an index of pollen viability. Amer J Bot. 1964;51:748-52. https://doi.org/10.1002/j.1537-2197.1964.tb06696.x.
17. Dafni A. Pollination ecology. Oxford University Press, New York. 1992; Pp.1- 57.
18. Bhowmik S, Datta BK. *In vitro* pollen germination in *Eichhornia Crassipes* (Mart.) Solms: An insight into its preferred mode of clonal reproduction. Notul Sci Biol. 2012;4 (2):65-71. http://dx.doi.org/10.15835/nsb.4.2.7419.

19. Shivanna KR, Johri BM. The angiosperm pollen structure and function. Wiley Eastern Ltd. Publisher, New Delhi. 1985;Pp.5-83.

20. Cruden RW. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. Evolution. 1977;31:32-46. https://doi.org/10.2307/2407542.

21. Das A, Kar S, Datta BK. Phenological and micro-morphological study on two *Datura* species of Tripura, North East India. Biosystematics and Bioresources: The proceedings of the International conference on "Algae, Fungi and Plants: Systematics to Applications''. Chapter-8. 2022; pp.129-13.

22. Saha M, Datta BK. Reproductive biology of *Solanum viarum* Dunal (Solanaceae) in Northeast India. Pleione. 2014;8(2):258-66.

23. Dafni A, Firmage D. Pollen viability and longevity practical, ecological and evolutionary implications. Plant Syst Evol. 2000;222:113-32. https://www.jstor.org/stable/23644330. https://doi.org/10.1007/978-3-7091-6306-1_6

24. Saha M, Datta BK. Reproductive biology of *Solanum sisymbriifolium* Lamk. (Solanaceae) in Tripura, North-East India. Int J Plant Reprod Biol. 2017;9(1):59-62.

25. Kar S, Datta BK. Floral biology of *Cajanus cajan* (L.) Millsp. (Leguminosae) in Tripura (India). Pleione. 2017;11(1):104-15.