The Study of Pollen Biology, Palynology and Pollen Production to Estimate Pollen-Ovule Ratio in Datura innoxia Mill.

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Abstract

Both flowering plants and plants that produce male strobilus require pollen grains for sexual reproduction. Every pollen grain has the male gametes essential for fertilization. Palynology is the scientific study of both living and fossilized pollen grains. Pollen is categorized according on its structural characteristics. Size, shape, aperture count, and surface ornamentation of pollen grains vary remarkably. These distinguishing features enable the identification and classification of plants at the family, genus, and frequently even species levels. The majority of pollen grains are spherical, ovoid, triangular, or disc-shaped. These fundamental geometric concepts have a wide range of variations, including elongated and flattened. Pollen kinds have a remarkably wide range in their surface ornamentation. The exine, outermost wall of pollen, which varies greatly between species, is frequently ornately sculpted. It is believed that the relationship between the pollen grain and stigma, which aids in germination, plays a significant role in species-specific identification mechanisms. Calculating pollen production is challenging since it varies from species to species and is influenced by a variety of biotic and abiotic factors. The study of pollination requires an understanding of anthesis and pollen formation in order to build a useful model for forecasting pollen concentration and to get deeper insight into the ecological context of pollen dissemination

Keywords- Reproduction, Fertilization, Palynology, Fossilized, Ornamentation, Classification.

Introduction

Pollens are a collection of microspores found in seed plants and typically occur in the form of a fine dust. Each pollen grain is a tiny entity with a unique shape and structure that develops in the male reproductive organ, or anthers, of plants that bear seeds. Pollen is transported by a variety of means, including wind, water, and insects. Pollen production is the most important factor in determining the fitness and reproductive success of a natural population (La Deau and Clark 2006).It is fact that the actual pollen production per plant might vary greatly from year to year due to ecological considerations (Stanley and Linskens, 1974).Various terminology, including pollen sterility, stainability, germinability, fertilization ability, and pollen quality, have been used to describe the viability of pollen grains and their capacity to germinate and fertilize ovules (Dafni and Firmage, 2000; Klein, 2000).Pollen viability is regarded as a crucial indicator of pollen quality (Dafni and Firmage, 2000). For plants where cross fertilization predominates over self fertilization, pollen viability is an essential characteristic for plant genetic alterations (Lyra et al., 2011). Pollen production, viability, and germination rates are known to be constrained by both internal and external influences (Yaegaki et al., 2002).The in-vitro pollen germination and pollen tube growth can provide an explanation for the lack of fertility (Pfahler et al., 1997). This is explained by the requirement of sucrose for appropriate pollen nutrition, osmotic regulation, and in combination with boric acid increased pollen germination (Sidhu and Malik, 1986). Boron may increase the absorption of sucrose and also promote the ability to germinate (Pal et al., 1989; Gupta et al., 1989; Mandal et al., 1982; Bhattacharya et al., 1997).Pollen grain adherence, hydration, activation, germination, and subsequent expansion of the pollen tube to reach the ovule are all aspects of the interaction between pollen grains and pistils (Edlund et al., 2004). Fertilization occurs when suitable pollen interacts positively with the pistil; otherwise, some processes may not be initiated or the pistil may reject the pollen tube (Cheung, 1995).

Study site

Study was performed in Kota district of Rajasthan in year 2021 and 2022 during the peak flowering period (January-April) and (July- October). 10 different sites were selected at Kota to carry out this work. These areShrinathpuram sector- E (Site-1),R.K Puram (Site-2), University of Kota (site-3), Hanging bridge road (site-4), Dadabadi (site-5), Chambal garden road (site-6), Nauapura (Site-7), Kunhadi (Site-8), Behind Aerodrome (Site-9), Borkheda(Site-10).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 26.7°C (max.) to 12.0°C (min.). The Kota district receives 660.6 mm of rain on average per year.

Material and Methods

***Datura innoxia* Mill**.- *Datura innoxia* Mill. is aresearch plant for the study of pollen biology, palynology and pollen production to estimate pollen-ovule ratio, which is a wild hazardous invasive species of flowering plant belong to family Solanaceae. It is native to the South Western United States, Central and South America and introduced in Africa, Asia, Australia and Europe. It is

annual herb and widely grows in temperate and moderate climate across the world. *Datura innoxia* is an annual herbaceous plant that grows upright and bushy. The leaves are long stalked with unequal basal lobe, simple, alternate, often entire and with a little wave at the margin. The stems, branches, and leaves are totally pubescent with simple and glandular hairs. Typically, the branches are purple. The pendulous, pubescent, and soft-spined fruit with curved stalk has these characteristics. Flowers are large solitary axillary, large, white coloured, trumpet/ bell shaped, pentamerous, hypogynous, gamosepalous, gamopetalous, valvet arrangement, stamen 5 polyandrous epipetalous, ovary superior bicarpellary with axile plecentation. Fruit ispendulous, pubescent; ovoid to subspheroidal and long soft-spined capsule with curved stalk are the main characteristics of *Datura innoxia* Mill.

Total pollen production and pollen ovule ratio - The anthers of *Datura innoxia* Mill. (10 samples from each studied site) selected randomly, were collected in the month of March 2022. The pollen grain production in *Datura innoxia* was evaluated by means of "Burker" haemocytometer following the procedure described by Cruden (1977). The ovary of randomly selected same flowers was dissected under a dissecting microscope and the ovules per flower were counted.In order to find out the pollen ovule ratio the number of pollen grains per flower divided by the number of ovule per flower, method proposed by Cruden in (1977). Buds just before anthesis were used to find out pollen ovule ratio (N=10) per site.

$$\frac{\textbf{\textit{Pollen}}}{\textbf{\textit{Ovule}}} = \frac{\textbf{\textit{Average no.of pollen per flower}}}{\textbf{\textit{Average no.of ovule per flower}}}$$

Pollen fertility and viability- Different tests were carried out for determining pollen fertility and viability of the studied species such as 0.2% TTC (2, 3, 5- Triphenyl tetrazolium chloride) test, 2% Aceto-carmine test and Lectophenol /cotton blue test were used in different interval of time before and after the anthesis. The tetrazolium test is based on the reduction of a colourless soluble tetrazolium salt to a reddish insoluble substance called formazan, in the presence of dehydrogenase.

Pollen size, morphology and palynology of *Datura innoxia* Mill.-The size of pollen grain were measured by the using a standerd ocular micrometer. Pollen grains of freshly dehisced anthers of 20 flowers (5 anthers each) were used to measure the size of pollen grain. The terminology used in accordance with Erdtman(1952), Faegrie and Iversen (1964), Walker and Doyle(1975) and Nair (1962). For Scanning electron microscopy (SEM), pollen grains collected from freshly opened flower were fixed in carnoys fluid. These were dehydrated through aqueous acetone series, dried with CO in jumbo Critical Point Dryer (Polaron). The samples were coated with gold in a Balzer Union SCPO sputter coater and observed in MIRA3 TESCAN with 2.0kv Acc. Voltage and EDS image analyzer system at Banasthali Vidyapith, Niwai Rajasthan.

***In vitro* pollen germination**- The study of *In vitro* pollen germination were carried out in various concentration of sucrose 10%, 20%, 30%, 40% and 50% respectively. Germination percentage of pollen grains and the length of pollen tube measured in different concentration of sucrose solution with the combination of boric acid, following the method of Shivanna and Rangaswamy (1993).

***In vivo* pollen germination on stigmatic surface**-Development and *in vivo* pollen germination on stigmatic surface was checked by aniline blue fluorescence method given by Martin in 1959. The pistils were fixed at different intervals, 3 h, 6 h, 8 h etc. up to 48 h after pollination in Carnoy's fixative (absolute ethanol: chloroform: glacial acetic acid 6:4:1) for 24 h and then they were transferred to 70% ethanol. The pistils were processed, stained using multiple stain and pollen tubes located in the pistil. There by the rate and growth of pollen tubes and approximate time taken by the pollen tube to reach the embryo sac were determined.

Observation and findings

Total pollen production, ovule production and pollen ovule ratio

TABLE-1

Representing the average number of pollen grain/anther/flower, average number of ovule/flower and pollen-ovule ratio.

S.N.	Experimental sites	Number of pollen/anther Mean±S.D.	Number of pollen / flower Mean±S.D.	Number of ovule/flower Mean±S.D.	Pollen: Ovule Mean±S.D.
1	Shrinathpuram sector- E (Site1)	12577±1688.13	62860±8440.67	314.2±38.63	200.19±218.47
2	R.K Puram (Site2)	12620±3386.94	63100±16934.71	303.1±32.05	208.18±528.38
3	University of Kota, road(site3)	13948±4149.95	69740±20749.74	298.6±37.29	233.55±556.32
4	Hanging bridge road (site4)	14356±3941.46	71780±19707.29	305.9±38.91	234.65±506.47
5	Dadabadi (site5)	13288±4598.04	66440±22990.20	304.5±28.11	218.19±817.92
6	Chambal garden	12572±2469.87	62860±12349.377	297.5±27.71	211.29±445.67

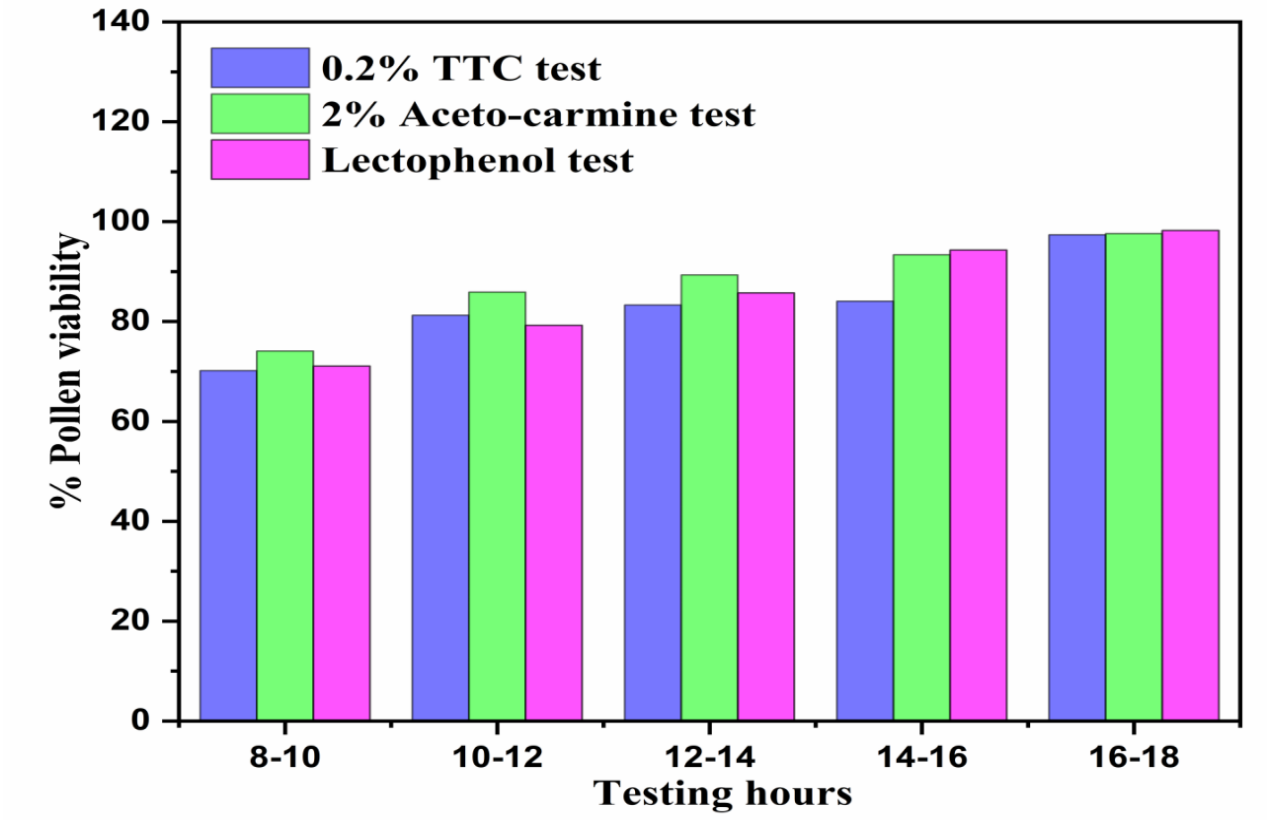
	road (site6)				
7	Nauapura (Site7)	13240±3133.42	66200±15667.09	296.9±23.03	222.97±680.33
8	Kunhadi (Site8)	13240±3275.06	66200±16375.32	322±32.41	205.59±505.25
9	Behind Aerodrome(Site9)	14264±4370.09	71320±21850.44	303±19.08	235.38±1144.93
10	Borkheda (site10)	12928±3513.92	64640±17569.62	299.3±27.72	215.97±633.89

Pollen fertility and viability-

TABLE-2

Representing the pollen viability/fertility of *Datura innoxia* in different tests.

S.N.	**Name of test**	**% pollen viability at different hours**				
		0800h-1000h (before anthesis)	**1000h-1200h (before anthesis)**	**1200h-1400h (before anthesis)**	**1400h-1600h (before anthesis)**	**1600h-1800h (at the time of anthesis)**
1	0.2% TTC test	70.14±8.29	81.23±8.08	83.29±5.82	84.05±5.61	97.37±2.33
2	2% Aceto-carmine test	74.09±7.10	85.86±4.53	89.31±4.11	93.37±2.59	97.59±2.99
3	Lectophenol/cotton blue test	71.08±3.16	79.23±5.81	85.73±3.02	94.32±5.02	98.23±2.89



1. Graphical representation of pollen viability in different test.

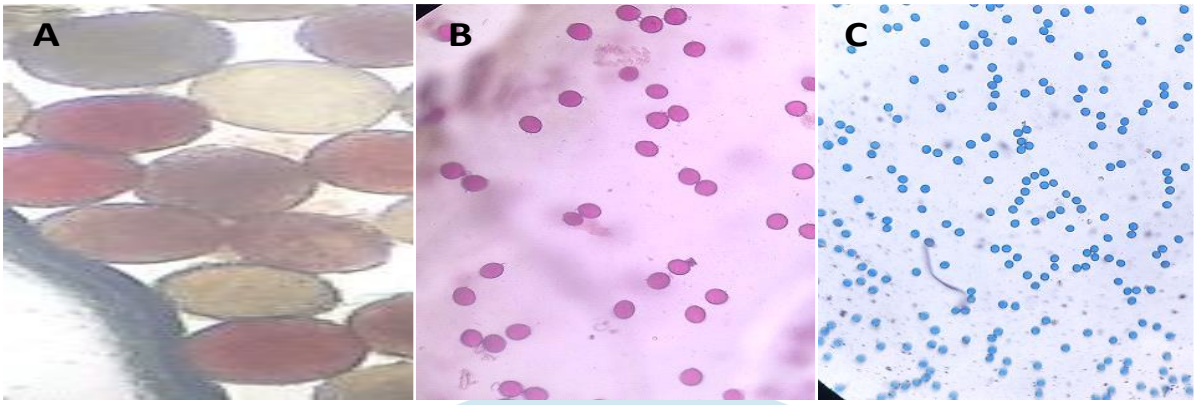


Figure-1: Pollen viability in different tests A- In TTC, B- In Acetocarmine, C- In Lectophenol.

Pollen size, morphology and palynology of *Datura innoxia* Mill.

TABLE-3

Morphological characteristics of pollen grain in *Datura innoxia* Mill.

Name of species /Family	**Characteristics of pollen grain**				
	Shape	**Colour**	**Amb**	**Exine ornamentation**	**Type of apertures**
Datura innoxia Mill. (Solanaceae)	Subspheroidal to Ovoidal	Light gray	Circular to ovoidal	Psilate (thick)	Colpate (Trizonocolpate)

TABLE-4

Size of pollen grain from polar axis and equatorial axis.

Name of species	**Diameter of Polar axis (P)** Mean±SD	**Diameter of Equatorial axis (E)**Mean±SD	**P :E Ratio** Mean±SD
Datura innoxia Mill.	56.4±7.88µm.	50.4±7.38µm.	1.12±1.07

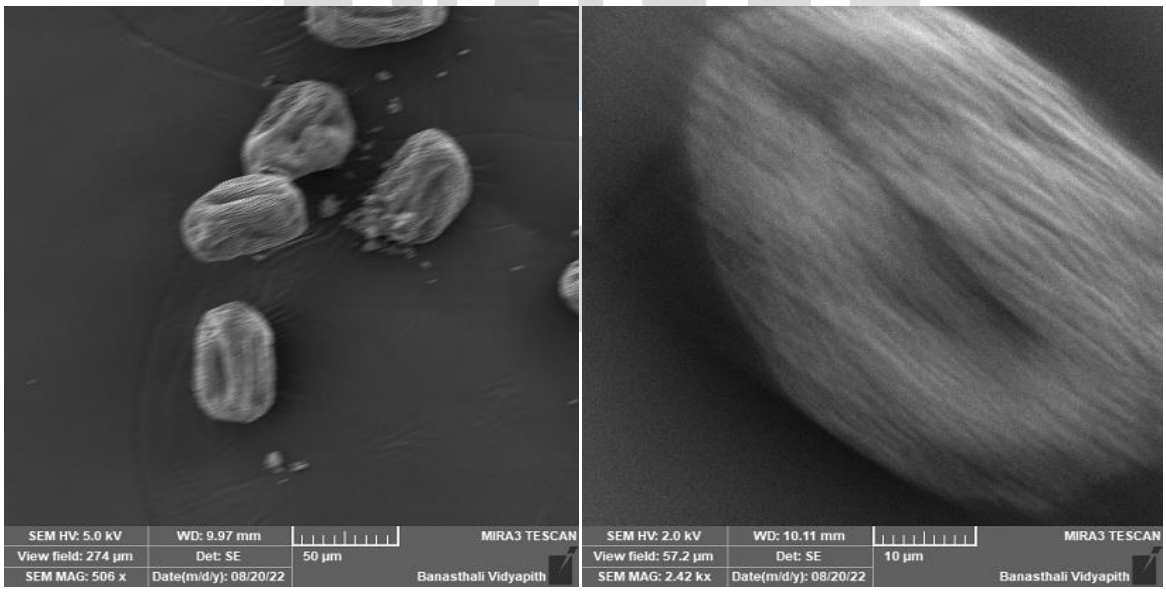


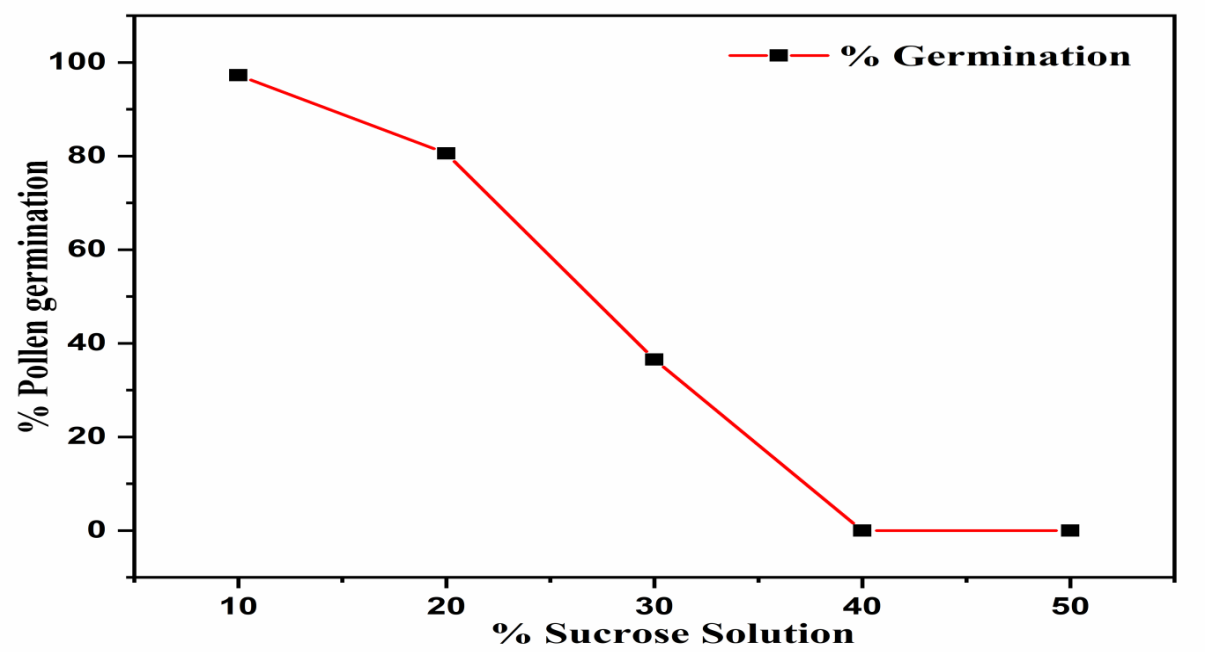
Figure-2 SEM photographs of *Datura innoxia* Mill. pollen grains.

***In vitro* pollen germination**

TABLE- 5

In vitro pollen germination showing germination rate and length of pollen tube.

S.N.	Sucrose solution	Pollen germination % Mean±SD	Length of pollen tube Mean±SD
1	10%	97.26±1.87%	77.3±14.53µm.
2	20%	80.58±7.55%	34.2±10.50µm.
3	30%	36.51±9.37%	12±3.55µm.
4	40%	Nil	-
5	50%	Nil	-



2. Graphical representation of pollen germination in different concentration of sucrose.

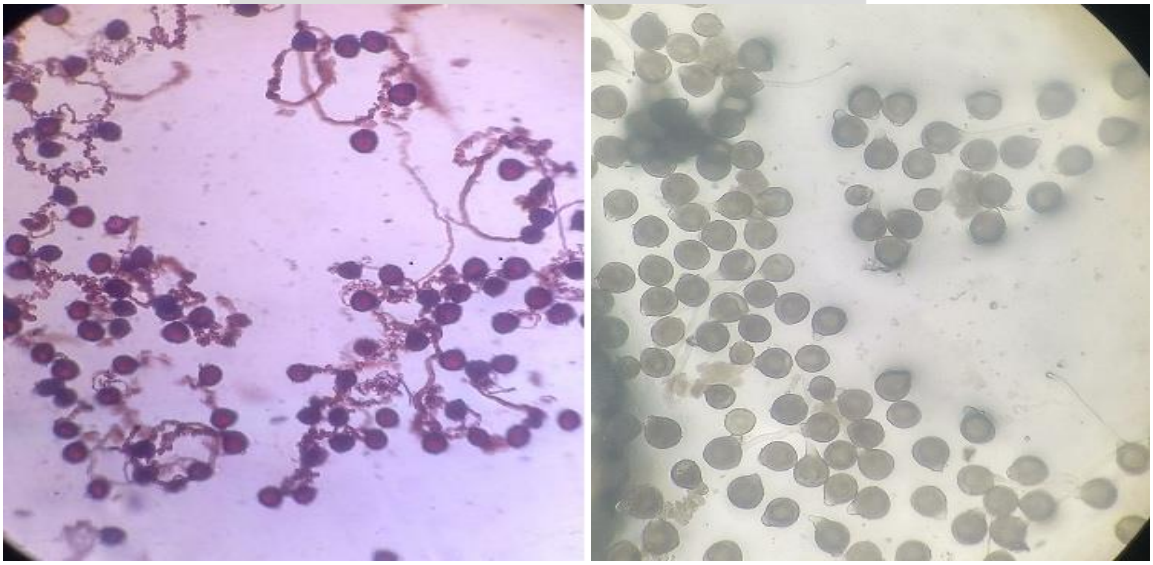


Figure- 3 *In vitro* pollen germination in Sucrose solution

***In-vivo* pollen germination on stigmatic surface**

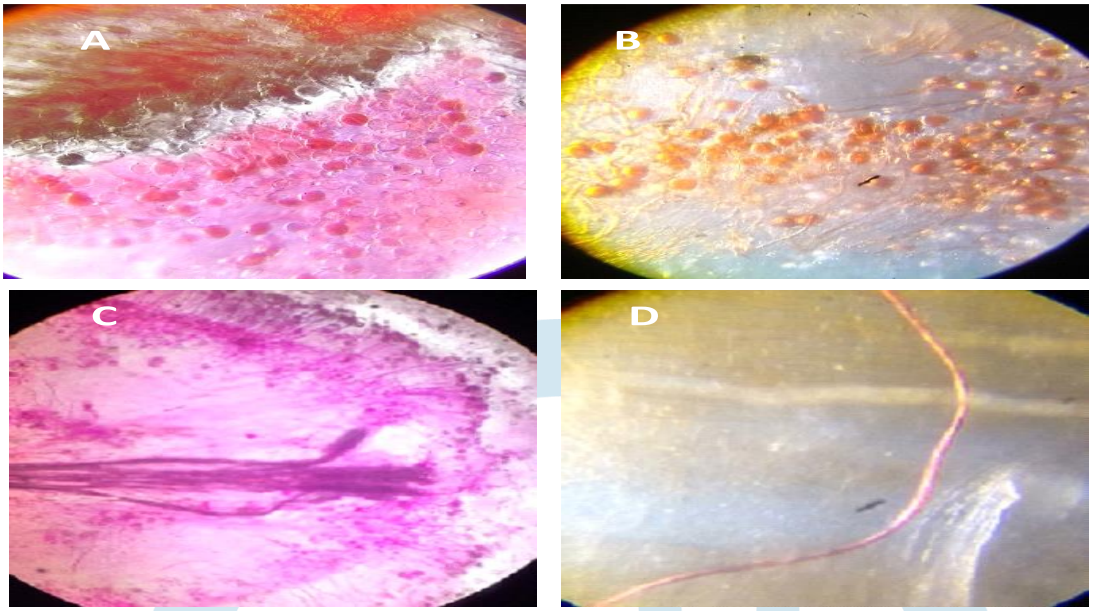


Figure-4
A, B & C- *in vivo* pollen germination on stigmatic surface, D- Pollen tube growth in style.

Result and Discussion

Total pollen production and pollen ovule ratio

The study of pollination requires an understanding of anthesis and pollen formation in order to build a useful model for forecasting pollen concentration and to get deeper insight into the ecological context of pollen dissemination (Davidson, 1941; Khanduri, 2011). In present study all 10 study sites shows high rate of pollen production of per anther and per flower which is ensures high reproductive success given table-1. Average number of pollen grain in all 10 sites was calculated 13302.8±677.61 per anther and 66514±3388.03 per flower. Faegri and Inversen (1975) discovered that anemophilous species typically produced huge numbers of little pollen with a smooth and non-sticky surface, whereas zoophilous plants typically produce modest amounts of large pollen with a rough sticky surface. Number of ovules per flower determines the seed sets in a fruit, the mean number of ovules evaluated in different studied sites given in table-1. The average number of ovules in all studied sites counted as 304.5±7.99 and average of pollen:ovule ratio calculated as 218.59±12.73. Which is shows the high rate of reproductive success. As reported by Cruden (1977), the pollen-ovule ratio has a positive relationship with the level of outcrossing and reveals if a plant species is autogamous or xenogamous, obligatory or facultative. Cruden's work on the pollen-ovule ratio has drawn criticism for stating that the main purpose of producing pollen is to assure ovule fertilization (Charnov, 1979) but this pollen: ovule ratio studies can be used to evaluate new evolutionary models (Kearns and Inouye, 1993).

Pollen fertility and viability

Pollen viability and size serve as reliable indicators of how microsporogenesis is progressing. While highly viable pollens have a consistent size and reproduce normally through meiosis, less viable pollens have an uneven size, which can be caused by autoploidy, segmental allopolyploidy, and a number of other factors, such as environmental impacts (Stace, 1991). The simplest and fastest method for assessing viability is pollen diameter measurement (Kelly et al., 2002) confide on different tests. The present study reveals that in the species of *Datura innoxia* Mill. the pollen collected at the time of 0800h-1000h on the day of anthesis represents lowest viability and pollen collected between 1600h-1800h at the time of anthesis shows the highest percentage of pollen viability than other times. Among three tests of pollen viability given in table-2, lectophenol / cotton blue test shows the highest pollen viability 98.23±2.89% at the time of anthesis and lowest in TTC test 97.37±2.33% at 1600h-1800h respectively. Pollen viability in 0.2% TTC test at 0800h-1000h before anthesis was determined 70.14±8.29%, at the time of 1000h-1200h before anthesis 81.23±8.08%, 1200h-1400h before anthesis 83.29±5.82%, 1400h-1600h pollen viability of *Datura innoxia* estimates 84.05±5.61% and highest pollen viability presented at the time of anthesis 1600h-1800h. In aceto- carmine test pollen viability at different hours as shown 0800h-1200h, 74.09±7.10%, 1000h-1200h, 85.86±4.53, 1200h-1400h, 89.31±4.11%, 1400-1600h, 93.37±2.59 and highest at the time of just before of anthesis 97.59±2.99% viability. In lectophenol cotton blue test the pollen viability at different hours as follows- 71.08±3.16% at 0800h-1000h, 79.23±5.81% at 1000h-1200h, 85.73±3.02% at the time of 1200h-1400h, 94.32±5.02% at 1400h-1600h and pollen viability at the time of anthesis calculated 98.23±2.89%. There are a little difference shown in all three tests, and result of test reveals that the pollens of *Datura innoxia* are highly viable at the time of anthesis as well as at the bud stage on same day of anthesis and remains viable after 12-14 hours of anthesis till corolla withered. The observation of study reveals that the anther dehiscence occurs in bud stage before the anthesis and stigma takes also receptive before the anthesis, this type of mating system promotes autogamous self pollination in studied species.

Pollen size, morphology and palynology of *Datura innoxia* Mill.

Palynology is the study of pollen and spores and it can provide incredible amount of information from a small material in alittle time (Walker and Doyle, 1975). Numerous genera in the family Solanaceae have examined using palynological methods (Punt and Monna- Brands, 1977; Barth and Duarate, 2008; Martins et al., 2013). Although the pollen morphology of the Solanaceae family is highly diverse, tricolpate pollen grains are always present. The morphology of pollen from Solanum species was examined by (Perveen and Qaiser, 2007; Alwadi and Lashin, 2007; Franklim and Esteves, 2008; Lashin, 2012; Saha. Moumita, 2017). In present study scanning electron microscopy were used to analyze pollen morphology, shape, structure and aperture type of pollen grains. Pollen grains of *Datura innoxia* Mill. determined spheroidal to ovoidal in shape with prolate amb, exine ornamentation- psilate , aperture- trizonocolpate or tricolpate, diameter of Polar axis 56.4±7.88µm, diameter of equatorial axis 50.4±7.38µm. and P/E ratio 1.12±1.07µm. were found in study. In comparison to other studies pollen grains of *Datura stramonium* L. and *Datura tatula* L. form were isopolar, radially- symmetrical, trizonocolporate, with regulate- reticulate exine ornamentation, in term of size pollen grains of both studied form were found medium sized with mean polar axis of 29.03-29.07µm, equatorial diameter of *Datura stramonium* L. form was larger than the *Datura tatula* L. form 28.45 and 26.46 µm, respectively. Pollen grain shape was prolate- spheroidal in both form according to P/E ratio 1.03-1.14. In *Datura tatula* form colpus were sunken and deeply sunken colpi were found (Rania, A. Hassan and Wafaa, M. Amer, 2019). Barnali, Bera; Jayanta, kumar. Kundu; Sanjukta, Mondal and Amal, Kumar.Mondal, (2018) studied on the allergenic protein profile in Datura pollen, *Datura metal* L. and *Datura innoxia* Mill. had lower protein concentrations in immature than mature pollen, while *Datura stramonium* L. had greater immature than mature protein concentrations. According to Kshirsagar, S.R. (2020) the pollen grains of *Datura innoxia* Mill. were observed more/less rounded in shape, trizonocolpate, light gray in colour and and exine ornamentation were psilate thick, they have studied the pollen morphology of 42 plant species.

***In vitro* pollen germination**

Boron combines with sugar to generate a sugar-borate complex, which is more effectively translocated than non-borate sugars, according to Gauch and Duggar (1953). It has been noted that the addition of CaCo3 to a sucrose solution causes a rise in the percentage of pollen germination as well as an expansion of the pollen tube. Calcium dependent kinases control the ion channels in pollen (Steinhorst and Kudla, 2013). In the present study, a high concentration of sucrose solution suppresses the formation of pollen tubes and the germination of pollen in *Datura innoxia* Mill. This is because pollen grains accumulate in the 40% and 50% sucrose solutions. It exhibit rate of high pollen germination and pollen tube growth in 10% as well as 20% sucrose solution with combination of boric acid. In 10% sucrose solution germination rate of pollen grains was 97.26±1.87% and the growth of pollen tube was 77.3±14.53µm, in 20% sucrose solution rate of germination 80.58±7.55% and the length of pollen tube 34.2±10.50µm were observed. Often as the concentration of sugar increased the rate of pollen germination decreased. In 30% sucrose solution germination percentage was 36.51±9.37% and pollen tube length was 12±3.55µm. Due to the accumulation of pollen at high sugar concentrations, which prevents germination, the pollen grains in solutions containing 40% and 50% sucrose show no germination and no pollen tube formation. In conclusion, the results show that the 10% sucrose solution is the best medium for the germination of pollen and the growth of pollen tubes in *Datura innoxia* Mill. Additionally, boric acid promotes the growth of pollen tubes.

***In vivo* pollen germination on stigmatic surface**

The pollen's arrival triggers a sequence of reactions that cause the pistil to discriminate between its own pollen and, in many circumstances, pollen from the same plant. Surface interaction controls this remarkable series of recognition occurrences. These are probably chemical and lock-and-key devices. Signals are exchanged between pollen and stigma cells as a result of the interaction. Rejection inhibits the contact from happening, acceptance causes pollen to germinate, and acceptance causes the growth of pollen tube to ovule (Knox, R.B., 1986). Non-specific esterases, which are concentrated on the stigma's surface, are crucial for pollen adherence and pollen tube development. After two to four hours following pollination, pollen grains begin to germinate on the stigmatic surface. Because of the rapid expansion of the pollen tube, it takes roughly 15 to 18 hours to reach the ovule. Stigma receptivity is maximum on the day of anthesis and lasts only through the following day. Pollen grain availability is not the limiting factor for fruit set, as determined by the success rate of *in vivo* pollen germination and pollen tube expansion (Deo, S.S.; Pant, P. and Bhatnagar,A.K., 2018).

Conclusion

The study of pollen biology reveals that pollen grain or *Datura innoxia* are medium size, prolate, subspheroidal to ovoidal shape, psilate and tricolpate, trizonocolpate. High average number of pollen production per anther and per flower ensures the high reproductive success in *Datura innoxia* Mill according to pollen ovule ratio.Result of test reveals that the pollens of *Datura innoxia* are highly viable at the time of anthesis as well as at the bud stage on same day of anthesis and remains viable after 12-14 hours of anthesis till corolla withered. The observation of study reveals that the anther dehiscence occurs in bud stage before the anthesis and stigma takes also receptive before the anthesis, this type of mating system promotes autogamous pollination in studied species. Non-specific esterases, which are concentrated on the stigma's surface, are crucial for pollen adherence and pollen tube development in *in vivo* pollen germination.

References

1. Al-Wadi, H. M. and Lashin, G. M. A. (2007). Palynological and cytological characters of three species of Genus *Solanum* (Solanaceae) from Saudi Arabia. Journal of Biological Sciences.7(4), 626-631.
2. Barth, O. M. and Duarte, S. G. (2008). *Morfologia polinica* de species arboreas de Solanaceae do Estado de Santa Catarina, Brasil. Hoehnea. 35(3), 379-386.
3. Bera, B.; Kundu, J.K.; Mondal, S. and Mondal, S.K. (2018). Allergenic Protein Profile of The Pollen Of Datura species – A Comparative study. IJCRT. 6(2).
4. Bhattacharya, A. and Mandal, S. (1997). *In vitro* pollen germination of *Delonix regia* (Boj.) Raf. Science and Culture. 63(5/6), 143-144.
5. Charnov, E. L. (1979). Simultaneous hermaphroditism and sexual selection. Proceedings of the National Academy of Science. 76(5), 2480-2484.
6. Cheung, A.Y.(1995). Pollen- pistil interaction in compatible pollination. Proceedings of the national academy of sciences USA. 92, 3077-3080.
7. Cruden, R.W.(1977). Pollen- ovule ratio: A conservative indicator of breeding systems in flowering plants. Evolution.31, 32-46.
8. Dafni, A. and Firmage, D. (2000). Pollen viability and longevity practical, ecological and evolutionary implications. Plant Systematics and Evolution. 222, 113-132.
9. Davidson, R.L. (1941). A note on anthesis in some common grasses near Johannesburg and the relation of anthesis to collection of pollen for medical.
10. Deo, S.S.; Pant, P. and Bhatnagar, A.K. (2018). Reproductive biology of *Excoecaria agallocha* L. (Euphorbiaceae), A mangrove associate species. The international journal of plant reproductive biology. 11(1), 38-50.
11. Edlund, A.F.; Sanson, R. and Preuss, D. (2004). Pollen and stigma structures and functions, The role of diversity in pollination. The Plant Cell. 16, 884-897.
12. Faegri, K. and Iversen, J. (1975). Textbook of Pollen Analysis: 3rd edition Hafner, New York.
13. Franklim, C. P. R. B. and Esteves, V. G. (2008). Palynology of species of *Solanum* L.(Solanaceae A. Juss.) from the salt marshes of Rio de Janeiro State, Brazil. Acta Botanica Brasilica. 22, 782-793.
14. Gauch, Hg. and Dugger, W. M. Jr. (1953). The role of boron in the translocation of sucrose. Plant Physiology. 28, 457-466.
15. Gupta, A.; Sharma, B. M. and Kerni, S. (1989). Comparative efficiency of *Azospirillum*, *Azotobacter* and blue-green algae in submerged rice culture. Res Develop Reporter. 6, 169- 171.
16. Kearns, C. A. and Inouye, D. W. (1993). Techniques for pollination biologist. University press of Colorado, Niwot, Colorado, USA.
17. Kelly, J. K.; Rascha, A. and Kalisz, S. (2002). A method to estimate pollen viability from pollen size variation. American Journal of Botany. 89(6), 1021-1023.
18. Khanduri, V. P. (2011). Variation in Anthesis and Pollen Production in Plants. American-Eurasian J. Agric. & Environ. Sci.11 (6):834-839.
19. Klein, M. (2000). Pollen studies in plant breeding and selection. *Botanical Guidebooks*.24, 171-193.
20. Knox, R.B.(1986). Pollen- pistil interaction. Cellular interaction. 17, 508-608.
21. Kshirsagar, S.R. (2020). Roll of pollen morphology in taxonomy and detection of adulterations in curd drugs. Journal of plant science and phytopathology. (4), 24-27.
22. La Deau, S. L. and Clark, J. S. (2006). Elevated CO2 and tree fecundity: the role of tree size, inter-annual variability and population heterogeneity. Global Change Biology. 12(5), 822-833.
23. Lashin, G. M. A. (2012). Ultrastructures and Pollen Morphology Significance of some species of *Solanum* (Solanaceae). Egyptian Journal of Botany. 141-156.
24. Mandal, S.; Barui, N. C. and Ganguly, P. N. (1982). *In vitro*pollen germination in *Arachis hypogoea*. Scientific Culture.48: 115-116.
25. Martins, K. C.; Souza, S. A. M.; Pereira, T. N. S.; Rodrigues, R.; Pereira, M. G. and Da Cunha, M.(2013). Palynological characterization and genetic divergence between accessions of chilli and sweet pepper. HorticulturaBrasileira. 31, 568- 573.
26. Nair, P.K.K. (1970). Pollen morphology of Angiosperm, A historical& phylogenetic study. Scholar publication house.
27. Pal, J. K.; Datta, B. K.; Mandal, S, and Bhattacharya, G. N. (1989). Studies on the *in vitro* pollen germination of *Cassia fistula* L. Mendel. 6(3/4), 311-315.
28. Parfitt, D.E.; Ganeshan, S. (1989). Comparison of procedures for estimating viability of Prunus pollen. Horticultural Science 24, 354–356.
29. Perveen, A. and Qaiser, M. (2007). Pollen morphology of family Solanaceae from
30. Pakistan. Pakistan Journal of Botany. 39(7), 2243-2256.
31. Pfahler, P. L.; Pereira, M. J. and Barnett, R. D. (1997). Genetic variation for *in vitro* sesame pollen germination and tube growth. Theoretical and Applied Genetics. 95, 1218- 1222.
32. Punt, W. and Monna-Brands, M. (1977). Solanaceae. Review of Paleobotany and Palynology. 23, 1-30.
33. Rania A, Hassan. & Wafaa M, Amer. (2019). Biosystematic study of Egyptian *Datura stramonium* (Solanaceae).phytotaxa 408(3), 178-194. Available at: https://www.researchgate.net/publication/334149414.
34. Saha, M.; Dutta, B.K. (2017). Reproductive biology of some Solanum species. Sodhganga. Available at- https://hdl.handle.net

35. Shivanna, K. R. and Rangaswamy, N. S. (1993). Pollen Biology–A laboratory manual. Narosa Publishing House, New Delhi.
36. Sindhu, R.J. and Malik, C.P. (1986). Metabolic role of boron in germinating pollen and growing pollen tube. Springer New York. 373-378.
37. Spice, (2011). The University of Western Australia./ spice.wa.edu.au/usage/ast0075/ version 1.0 Food and energy. Available at: https://www.uwa.edu.au/study//media/Faculties/Science/Docs/What-is-pollen.pdf
38. Stace, C. A. (1991). Plant Taxonomy and Biosystematics 2nd edition, Cambridge University Press, Cambridge.
39. Stanley, R. G. and Linskens, H. F. (1974). Pollen biology, biochemistry and management. Springer, Verlag. Berlin, Heidelberg, New York.
40. Steinhorst, L. and Kudla, J. (2013). Calcium: a central regulator of pollen germination and tube growth. Biochimica et Biophysica Acta. 1833(7), 1573-1581.
41. Stelly, D.M.; Peloquin, S.J.; Palmer, R.G. and Crane, C.F. (1984). Mayer's hemalum methyl salicyate; a stain clearing technique for observation within whole ovules. Stain Technology. 59, 155-161.
42. Walker, J. W. and Doyle, J. A. (1975). The bases of Angiosperm Phylogeny: palynology. Annals of the Missouri Botanical Garden. 62(3), 664-723.
43. Walker, I.W. (1976). Evolution of exine structure in the pollen of primitive angiosperm. Amer. Journal of Botany. 61, 891-902.
44. Wodehouse, R. P. (1935). Pollen Grains. Mc Graw-Hill Book Co. New York and London. 574.
45. Wodehouse, R. P. (1936). Pollen grains in the identification and classification of plants. VIII.The Alismataceae. American journal of Botany. 23, 535-539.
46. Yaegaki, H.; Haji, T. and Yamaguchi, M. (2002). Cultivar differences of pollen quantity, rate of stained pollen and rate of germinated pollen in Japanese apricot (*Prunus mume* Sieb.et Zucc.) cultivars. Bulletin of the National Institute of Fruit Tree Science. 1, 47-53.