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Anther dehiscence, pollen dispersal, stigma receptivity and anthesis of *Solanum indicum L.*

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ABSTRACT

Diurnal and seasonal time schedule of anther dehiscence, pollen dispersal, duration of stigma receptivity and anthesis of the medicinally important plant of *Solanum indicum* L. have been studied. The species produces flowers of effectively one day longevity. Anther dehiscence is gradual and is initiated by the appearance of a terminal thecal pore; with the progress of floral longevity the two terminal pores of an anther coalesce to relatively wide single pore, with a markedly necrotic margin. Anther contains 150923 (approx) pollen grains which are released steadily from the dehisced anthers throughout the duration of flower. The stigma assumes receptivity little before (20-30 mins) the flower opening. However, the pollen deposition over stigma dose not takes place until a flower opens and pollinators activity begins.

Key-words: *Solanum indicum,* anther dehiscence, pollen dispersal, stigma receptivity, anthesis.

INTRODUCTION

The male sex organ of a flower is referred to as stamens, each of which is typically constituted by a slender proximal filament, terminating into an anther and produce a profuse amount of pollen grains. For sexual reproduction of a flowering plant, pollen should be transferred from anther to the stigma in right time and it is an important phenomenon during the time of sexual reproduction. The process involves the mediation of several abiotic or biotic agents.

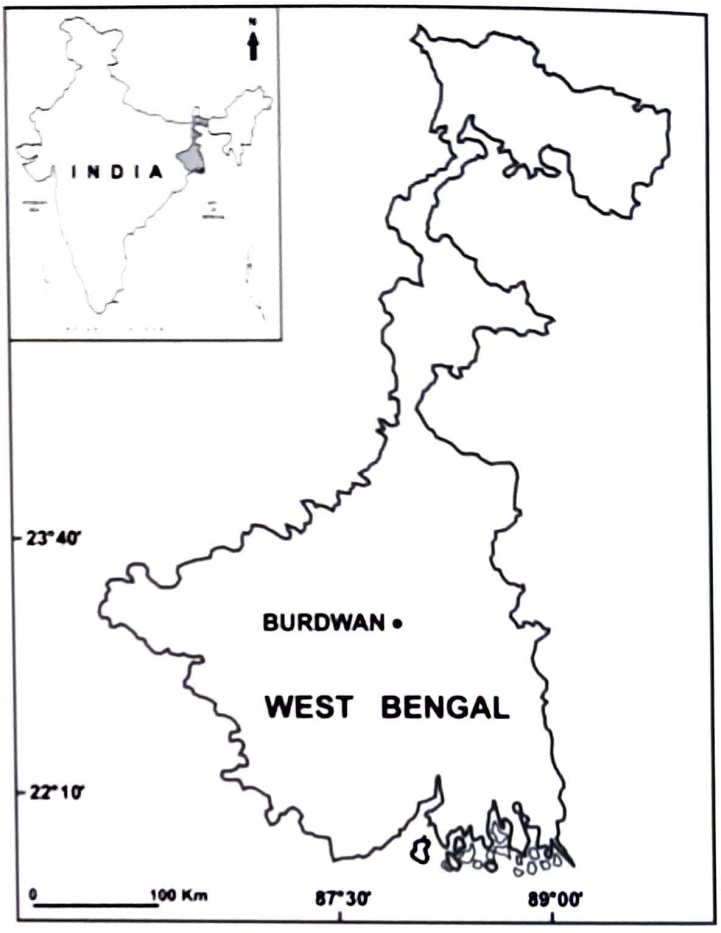
Every flower has a specific stage of anthesis, during which pollen grains are released from the anthers and are transferred to the receptive stigma (Faegri & van der Pijl 1979, Vogel 1983). The time or duration of the anthesis of a plant depends on several factors, like anther dehiscence, stigma receptivity, pollen presentation, development of stigmatic pollen load and pollinator activity. For the study of pollination biology and also to the plant breeder the knowledge of anthesis is very important.

Stigma receptivity refers to the ability of the stigma to support germination of viable and compatible pollen. Generally stigma becomes receptive at the time of flower opening which is more or less simultaneous with anther dehiscence. However, in *protogynous* and *protandrous* species, the stigma becomes receptive few hours to a few days before or after anther dehiscence respectively.

Solanum indicum L., Solanaceae, a moderate-size erect shrub, referred to as 'Brihati' in vedic literature, is well-known for its medicinal values as digestive, astringent, anthelmintic, antiasthmatic, analgesic, anti-inflammatory, antipyretic, antivomiting and CNS depressant (Kritikar & Basu 1935, Deb et al. 2014). This taxon is known to be distributed widely in the warmer parts of South East Asia including the

lateritic soil of south-western part of the West Bengal (Hooker 1885, Prain 1963). However, a recent survey carried out in the districts of South West Bengal reveals that the species has become threatened in the region, necessitating its conservation as well as sustainable utilization. For the purpose, anther dehiscence, pollen dispersal, duration of stigma receptivity and anthesis, which was hitherto unknown, was investigated.

Different aspects of pollination biology of a number of other species of *Solanum* have been investigated from time to time. Detailed works on pollination biology of *S. rostratum* has been done by Bowers (1975) whereas pollination biology of *S. palinacanthum*, a Brazilian species was worked out by Coleman & Coleman (1982). Silva et al. (2017) studied the pollination biology of *Solanum lycopersicum* L. (Solanaceae) in open-field of the Southeast of Minas Gerais State, Brazil. Reproductive biology i.e., pollen production, pollen germinability and viability and pollination of the species *S. viarum* , *S. torvum* and *S. sisymbriifolium* have been studied by Saha & Datta (2014; 2017a; 2017b; 2018).



Text-Figure 1. Map of West Bengal showing the location [.] of studied area.

MATERIAL AND METHODS

The plant *Solanum indicum* is a moderate size shrub, 0.8-2.0 meters in height. The present work is based on plants occurring in three wild populations at Kanchannagar (23°13¹57° N: 87°51¹49° E) and Ganja (23°14¹33°N, 87°51¹12° E) villages of Burdwan District (Text-Figure 1) and Tarabagh locality of Burdwan Town (23°14¹18°N:87°51¹39°E) of the state of West Bengal in India, together with those grown in the experimental plot of Botany Department, Burdwan University.

Anther dehiscence: The details of the events of anther dehiscence were recorded in flowers since fully mature bud condition throughout the floral longevity. The degree of dehiscence of anthers was quantified from the extent of the thecal pore. Observations were made by using a 20x hand lens and stereo-binocular microscope.

Pollen dispersal: The calculation of the percentages of pollen dispersal, pollen counts were done at one hour interval from the initiation of anther dehiscence to the end of floral longevity of anthers from flowers in open-pollinated condition. Pollen content of an anther was counted by dilution method as per the methodologies given by Cruden (1977). Determination of mean value was based on 10 anthers collected from 10 flowers belonging to 5 different individuals. The individual anthers were carefully crushed in separate centrifuge tubes and each was diluted to a volume of 10 ml by adding distilled water. Then 0.5 ml of the homogeneous solution from each tube was taken on a slide and covered with a 22 mm x 50 mm cover slip. Pollen grains were counted under the 10x objective of a bright-field microscope. The process was repeated for four times for each of the 10 samples. Then the data obtained from the total of 2 ml (0.5 ml x 4) homogeneous solution was multiplied by 5 to calculate the total number of grains in 10 ml solution. Finally, the calculated mean value based on the 10 anther samples was multiplied by five (as number of anthers are five in a flower) to determine the total number of

pollen grains produced per flower. The process was repeated at regular intervals for three consecutive years. The percentage of pollen dispersal at various intervals through the progress of flower longevity was calculated from the loss of pollen grains from the anthers at that time with respect to the mean value of initial pollen content in an anther as deduced from the study of pollen production. Statistical analysis of the data was done and results were plotted graphically.

Stigma receptivity: Attainment and duration of stigma receptivity were initially ascertained by observing the morphological changes in stigma, followed by confirmation through dehydrogenase activity with Hydrogen Peroxide Test (Zeisler 1933, Galen & Plowright, 1987) and *in vivo* pollen germination experiments. The duration during which pollen deposition over the stigma takes place was determined by examining excised stigmas at two hours interval all through the floral longevity, following the methods of Kearns & Inouye (1993).

Anthesis: The duration of anthesis of the species was deduced by taking into consideration the duration of stigma receptivity, pollen dispersal and pollinator activity.

OBSERVATIONS AND RESULTS

Flowers of *S. indicum* are disc shaped, complete, bisexual, hypogynous.

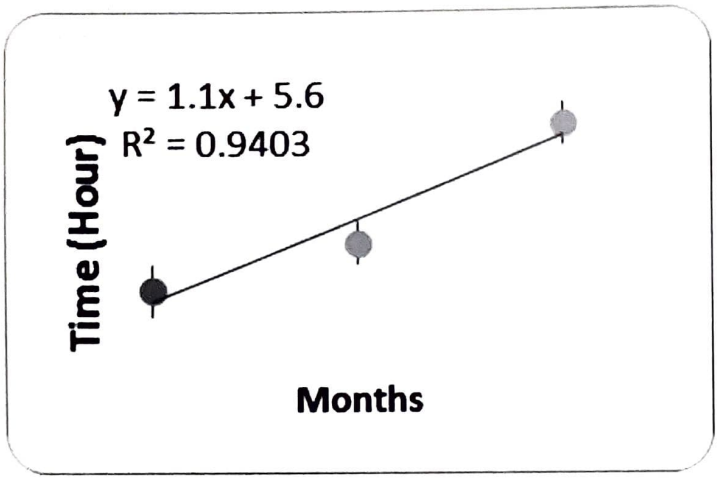
Anther dehiscence: The androecium of the species, comprises of five stamens, is a cone shaped structure forming an androecial column around the style (Plate 1, Fig. A). Dehiscence of the anther commences just after or is simultaneous with the flower opening. The mode of anther dehiscence is gradual by means of terminal pore. Initiation of anther dehiscence by the appearance of a pair of minute terminal pores (one for each theca) (Plate 1, Fig. B). Through time, the thecal pore of dehisced anther gradually increases in diameter. It reaches up to ±0.2 mm after 2-3 hrs from its initiation (Plate 1, Fig. C) and finally into a single wide opening of 0.4-0.5 mm in diameter with a firm necrotic margin (Plate 1, Figs. D, E). Detailed timings of different events of anther dehiscence, i.e., initiation of dehiscence, gradual changes through time and completion of dehiscence in different months are given in the Table. 1 and graphically represented in Text-Figure 2 A-C.

Pollen production and pollen dispersal: The average number of pollen grains produced in a single anther is 30184.7. As the flower contain five anthers, the estimated number of pollen grains produced per flower of *S. indicum* would be 30184.7x5=150923.5. Though the anther dehiscence takes place simultaneously with the flower opening, pollen dispersal starts with the first arrival of the pollinator which varies slightly with seasons. Simultaneous with pollinator activity, pollen grains are released steadily from the dehisced anthers throughout the duration of flower longevity.

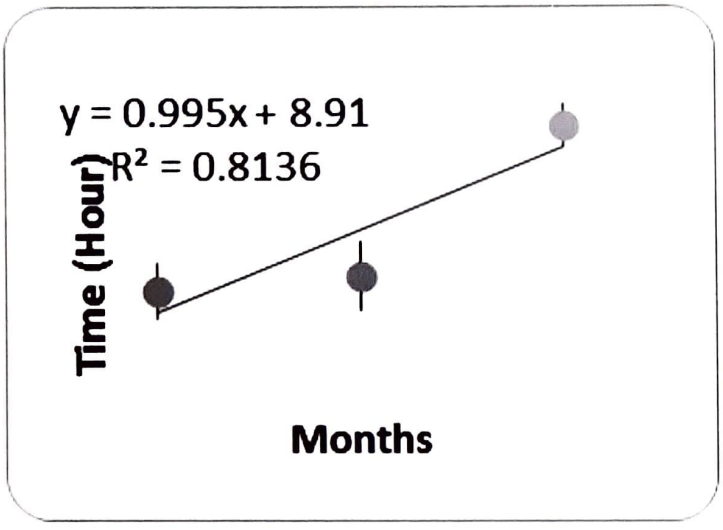
During peak flowering phase (November-March) nearly 10% of the total pollen grains in an anther are released during the initial three hours of flower opening. Maximum pollen dispersal takes place in the next two hours, when 60-70% of the total pollen grains from the anther are released. On the other hand, in April-June,

Table 1. Timings of the major events of anther dehiscence of *S. indicum* in different seasons.

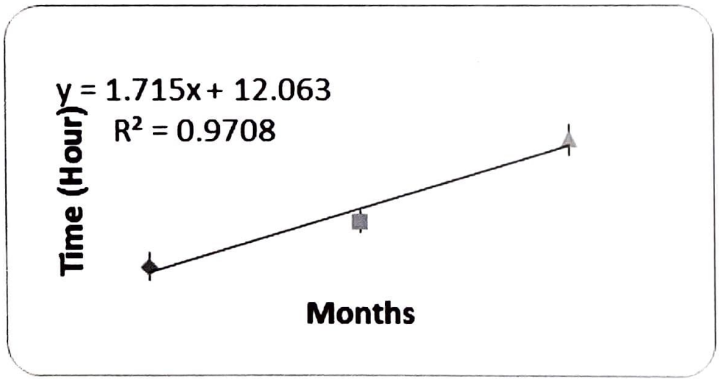
Events	Time in different months		
	April-June [Mean time (±SE)]	**July-October [Mean time (±SE)]**	**November-March [Mean time (±SE)]**
Each of the anther tips of freshly opened flowers showing minute pore at its terminal portion, i.e., the onset of anther dehiscence	5.30-9.30 am [6.48 (±0.36) am]	5.50-10.00 am [7.30 (±0.28) am]	7.00-11.30 am [9. 12 (±0.29) am]
The pore gradually increases in diameter and assumes a diameter of nearly 0.2 mm.	7.00 am-12.00 pm [10.12 (±0.34) am]	7.30 am-12.15 pm [10.21 (±0.40) am]	9.15 am-2.15 pm [12.05 (±0.25) pm]
Ultimately a single, wide pore is formed due to the shrinkage of the partition wall between the thecae and margin of the pore becomes necrotic.	2.00-3.00 pm [1.50 (±0.38) pm]	2.00-3.45 pm [3.08 (±0.28) pm]	3.45-6.30 pm [05.20 (±0.41) pm]



A



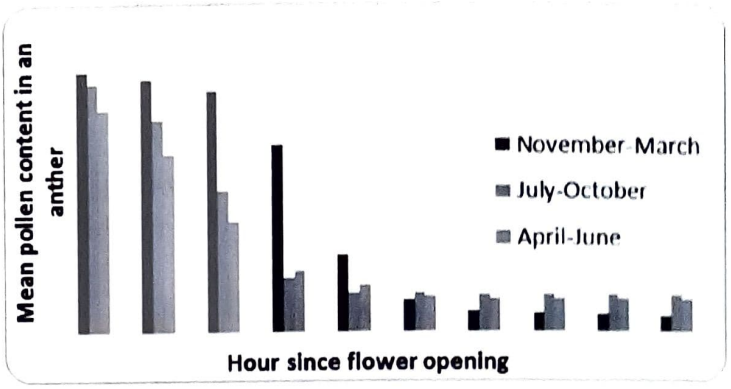
B



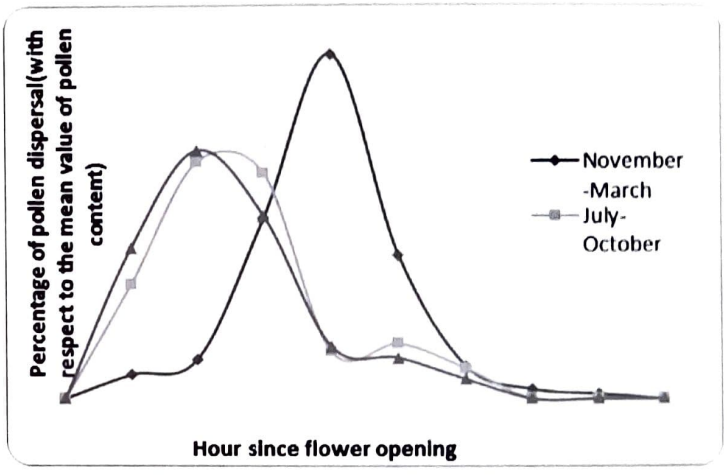
C

Text-Figure 2 A-C: Graphical representations of the timings of the major events of anther dehiscence in *S. indicum* in different months. A. Timings of the initiation of anther dehiscence. B. Timings when the anther pore gradually increases in diameter and assumes a diameter of nearly 0.2 mm. C. Timings of coalescence of the thecal pores and the appearance of necrotic margins.

maximum pollen dispersal takes place during 2nd to 4th hours after flower opening. In the later hours (5th or 6th onwards) pollen dispersal steadily decreases (Text-Figure. 3 & 4). A little amount (1-0.5%) of the total pollen is left within the anthers even at the time of the flower abscission.



Text-Figure 3. Graphical representation of the mean pollen content in an anther of *S. indicum* through time since flower opening in different seasons.



Text-Figure 4. Graphical representation of the percentage of pollen dispersal of *S. indicum* through time since flower opening in different seasons.

Stigma receptivity: The nature of stigma of *S. indicum* is of wet type and appears juicy and quite shiny at its receptive phase (Plate 1, Fig. F).

The stigma assumes receptivity 15-20 minutes before the flower opening, when it exhibits the sticky and lustrous look, emit bubbles in H_2O_2 solutions and supports *in vivo* pollen germination (Plate 1, Fig. K).

The time and duration of stigma receptivity varies with seasonal changes, from summer to winter. After complete opening of the flower the stigma becomes dark green in colour, the style tends to curve outwardly and soon after flower opening, the style together with the stigma assumes the configuration of a snake's hood, piercing the anther cone laterally (Plate 1, Figs F-H). The receptivity of the stigma is maintained through the flower longevity up to nearly 6th -7th hour from flower

Explanation of Plate 1:

A. The undehisced anthers of a freshly opened flower. B. Dehisced anthers of an open flower. C. Each theca of the anther of a freshly opened flower shows an apical pore of nearly 0.2 mm in diameter. D- E. Anthers of later stage, the two pores of an anther have coalesced, giving rise to a relatively wide (0.4-0.5 mm) single pore with markedly necrotic margin. F. Stigma showing the physiological receptivity by its somewhat sticky and lustrous look. F- G. Outwardly curved styles assuming the configuration of a snake's hood. H. Magnified view of the receptive stigma. I- J. Stigma loses its receptivity by losing its sticky and lustrous look and change in colouration. K. *In-vivo* pollen germination on the stigma of a flower.

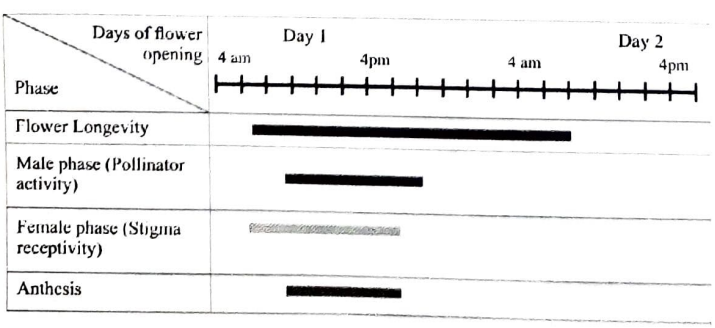
Table 2. Details of the stigmatic events of *S. indicum* through the floral longevity in different months.

Months Stigmatic events through flower longevity	April-June [Mean time (±SE)]	July-October [Mean time (±SE)]	November-March [Mean time (±SE)]
Stigma projecting out of the corolla of a closed flower (mature bud), which is sticky and lustrous supporting pollen germination and exhibiting dehydrogenase activity.	5.00-9.10 am [6.15 (±0.37) am]	5.30- 9.50 am [7.00 (±0.34) am]	6.15-10.10 am [8.50 (±0.58) am]
At the time of complete opening of the corolla disc, stigma is green in colour, sticky and lustrous, supporting pollen germination and exhibiting dehydrogenase activity.	5.15-9.30 am [6.35 (±0.27) am]	5.40-10.30 am [7.30 (±0.50) am]	7.00-11.10 am [9.20 (±0.61) am]
Onset of the loss of stigma receptivity marked by its pale, somewhat dry look.	2.30-3.30 pm [3.05 (±0.47) pm]	3.55-4.45 pm [4.20 (±0.52) pm]	5.40-6.45 pm [6.15 (±0.67) pm]

opening. After that dehydration of the receptive part of the stigma, as deduced from the loss of its juicy appearance and gradual assumption of dull look, marks the commencement of the loss of its receptivity (Plate 1, Figs I, J). Details of the stigmatic events through the floral longevity in different months of the year are given in the Table. 2.

Anthesis: Though, the stigma becomes receptive 15-20 min before the flower opening, anther dehiscence takes place simultaneously with the flower opening. However, pollen dispersal from dehisced anther begins only with the commencement of pollinator activity. In November-March, during the peak flowering time, the time of the first arrival of the pollinators is at 9.10 (±0.18) am, in July to October at 8.30 (±0.12) am and in April to June at 7.40 (±0.08) am.

The longest duration of anthesis, ±9 hours, ranging from 9.10 (±0.18) am with the first arrival of pollinators to 6.15 (±0.67) pm with the loss of stigma receptivity, is observed during winter, when the plant remains at its peak flowering phase. On the other hand, in summer (April-June) the duration of anthesis spans for ±7.5 hours, ranging from 7.40 (±0.08) am with the first arrival of pollinators to 3.05 (±0.47) pm with the loss of stigma receptivity.



Text-Figure 5. Graphical representation of the durations of floral longevity, pollen (male) phase, stigmatic (female) phase and anthesis of *S. indicum* during peak flowering season.

CONCLUSIONS

The events of anther dehiscence to some extent are delayed from summer through winter. Also, the duration of total time taken by an anther since initiation to completion of dehiscence has been found to increase gradually from summer through winter. Through time, the thecal pore of dehisced anther gradually increases in diameter and the partition wall between the two thecae shrinks leading to the coalescence of two thecal pores to a single wide opening.

The stigma of *S. indicm*, becomes receptive 15-20 minutes prior to flower opening and anther dehiscence. Thus the species is to be regarded as protogynous. However, during that initial phase of stigma receptivity, pollination cannot be affected because of non-availability of pollinators in nature. The pollen dispersal from dehisced anther can take place only when the pollinator activity begins. Therefore, such a physiological protogyny of the species has no significance in its reproductive biology. From functional point of view, the species may be regarded as homogamous.

Though, pollinator activity continues beyond the loss of receptivity of stigma, it has no role in pollination, because of the non-availability of receptive stigma in nature. Thus, the duration of anthesis of the species commences with the first arrival of pollinators and lasts till the stigma retains its receptivity. The overall duration of anthesis in the species diminishes gradually from winter (November- March) through summer (April-June) in a year.

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