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# SEED ECOLOGY OF THE IMPORTANT MEDICINAL SHRUB *SOLANUM INDICUM* L.

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*Key words: Solanum indicum,* Diaspore, Seed, Ecology

## Abstract

Various aspects of seed ecology of the regionally threatened medicinally important plant *Solanum indicum* L. *viz.,* seed production, seed-ovule ratio and seed-set percentage, seed dispersal, seed structure, germination, dormancy and scarification, moisture content, viability under storage, have been investigated. Besides light microscopic observations, anatomical details of seed have been studied under scanning electron microscope. The dispersal units of the seeds of the species, being invested in a film of dehydrated mucilage derived from the fruit pulp, are to be regarded as diaspores. The exotestally derived seed coat is specially device for adherence of a mucilaginous coating. The diaspore mucilage performs significant roles in anchorage and moisture regulations of germinating seeds. The seeds of the species are characterized by a sort of coat-imposed primary dormancy which can successfully be overcome by a treatment with 24(N) sulphuric acid. The dormancy helps the seed to bypass the unfavourable condition of summer.

## Introduction

Seeds, the sexually derived propagules of flowering plants, carry the precious embryos of future individuals. Ecological success of seeds is the most critical for reproductive success of a species and thereby of immense importance in formulating strategies for conservation and cultivation. Percentage of seed-set, overall seed production and structure, dispersal, germination, dormancy, loss of moisture through time and duration of viability of seeds of a species are fundamental ecological parameters (Fenner and Thompson 2005).

*Solanum indicum* L., Solanaceae, a moderate-sized 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 and Basu 1935, Deb *et al.* 2014). A number of bioactive compounds, *viz.*, alkaloids, solanine, solanidine, N-(p-transcoumaroyl) tyramine and N-trans-feruloyltyramine and steroidal glycosides, indiosides A-E and scopoletin have been detected in different parts of the plant (Chopra *et al.* 1958, Syu *et al.* 2001). The plant is not known to be cultivated anywhere in the world and is utilized exclusively from wild source. The species 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 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, seed ecology of the species, which was hitherto unknown, was investigated.

Different aspects of seed ecology of a number of other species of *Solanum* have been investigated from time to time. Groth (1989) described the seed morphology of *S. aculeatissimum*, *S. americanum*, *S. ciliatum*, *S. sisymbriifolium*, *S. sordidum* and *S. viarum*. Karihaloo and Malik (1996) worked out the structure, development and histochemistry of the seed epidermis of *S. melongena* and *S. violaceum.* Seed dormancy and germination, sometimes using $GA_3$

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pretreatment, of *S. sisymbriifolium*, *S. torvum*, *S. sanitwongsei*, *S. indicum*, *S. integrifolium*, S. *khasianum, S. surattense, S. insanum* were worked out by Ibrahim *et al*. (2001). Bithell (2002) and Taab and Andersson (2009) studied the germination requirements of laboratory stored seeds of *S. nigrum* and *S. physalifolium*. Kopcińska *et al*. (2004) traced the seed development in *S. muricatum*. Junlakitjawat *et al*. (2010) described the micromorphology of the seed coat of nine species of *Solanum* in Thailand, *viz*., *S. aculeatissimum* Jacq., *S. erianthum* D. Don, *S. violaceum* Ortega. *S. mammosum* L., *S. nigrum* L., *S. sanitwongsei* Craib., *S. spirale* Roxb., *S. stramoni-folium* Jacq. and *S. trilobatum* L., Pal and Pal (2011) studied the dormancy, scarification, invigoration and storage of *S. surattense* seeds.

## Materials and Methods

Seeds of *Solanum indicum* are produced in globose berries. Mature fruits were randomly collected from individuals growing in and around Burdwan [23.2333° N, 87.8667° E], West Bengal, India (Fig. 1). Seeds, after isolating from fruits followed by sun-drying (35 ± 2°C) for 5 hrs each for 2 - 3 consecutive days, were divided into sets of 100 seeds each and stored in Borosil glass vials with loosely fitted Bakelite caps under laboratory conditions (27 ± 2°C) in ± 11 hrs diffused natural light.

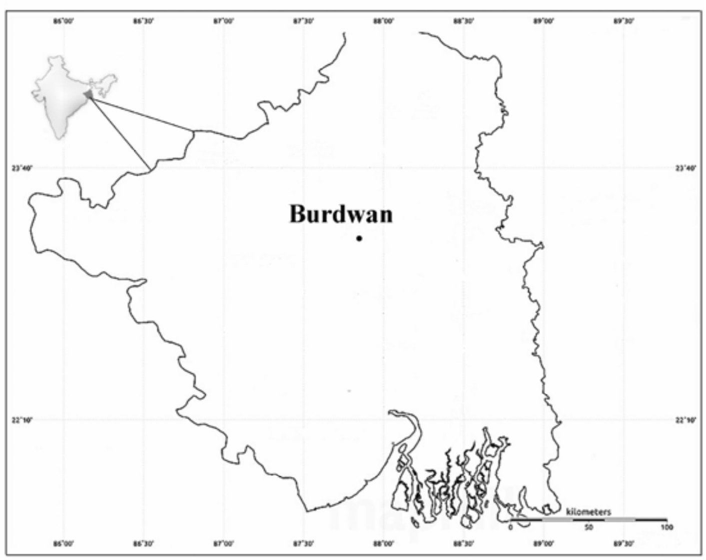


Fig. 1. Map of South West Bengal showing the location (●) of collection sites.

Seed production was studied by random collection of 50 fruits in each month. Determination of numbers of ovules per flower was based on 10 flowers collected in each month and the percentage of seed set was obtained by multiplying the seed-ovule ratio with 100. Measurement of seed weight was based on 10 seeds lots, each with 100 seeds.

Structural details of seeds were worked out by splitting as well as sectioning followed by observations under a WILD (Switzerland) M3B Stereo-binocular microscope and a Leica (Germany) DMLB compound bright field microscope with a Leica DFC 295 digital camera attachment. For scanning electron microscopy, intact specimens of dry seeds for surface studies and cracked specimens for anatomical observations were fixed on brass stubs and coated with gold by an Eiko Engineering (Japan) Ion Coater and observed using a Hitachi (Japan) S-530 SEM aided with a digital photomicrographic system.

Germination experiments were carried out as per the rules of International Seed Testing Association (1996). Each experiment was repeated four times. Seeds were rinsed with 0.1% $HgCl_2$ solution for 90 sec, washed thoroughly with double distilled water and kept overnight in double distilled water for imbibitions. Fully imbibed seeds were allowed to germinate in distilled water on a Petri dish (Borosil, 10 cm in diameter) under diffused natural light at room temperature (27± 2°C). For scarification, both alternate temperature treatment and acid scarification were employed (Copeland and Mc Donald 2001). Dry seeds were subjected to hot water (50, 55, 60, 65, 70 and 75°C) for 10 min, immediately followed by an exposure to cold water (15°C) for another 10 min. Acid scarification was optimized by using different concentrations of sulphuric acid [18(N), 24(N), 30(N) and 36(N)] for variable durations (1, 2, 4, 8 and 15 min).

To determine the moisture content, seeds were made totally moisture-free by keeping in a LABARD Hot Air Oven for seven consecutive days at 50$^{oo}$C and the percentage of seed moisture content was calculated with respect to fresh weight of seeds. Viability of seeds under storage was studied at 30 days intervals by germination experiments after optimum scarification.

## Results and Discussion

The number of seeds produced in a fruit of *Solanum indicum* varies from 20 to 36 (mean value of 28.04 ± 0.94). The size of a fruit depends, to some extent, on the number of seeds it contains. The weight of each seed is 2.6 - 2.8 mg (mean value 2.74 mg ± 0.03). The number of ovules per ovary in a flower varies from 32 - 46, with an average of 37.3 ± 4.36. The average number of seeds produced by a flower is 28.04 ± 0.94. Therefore, the seed-ovule ratio of the species and the seed-set percentage are 280 : 373 and 75.06, respectively.

The fruit of *S. indicum* is derived from a bilocular, multiovulate ovary with axile placenta bearing relatively small, anatropous, unitegmic and tenuinucellate ovules (Figs 2A, B). A young fruit is deep green in colour. The seeds initially remain attached to the placenta and subsequently become detached and freed within the locular cavities. In mature fruit the placental tissue and partition wall are dissolved into a mucilaginous pulp. The green young fruit gradually turns light orange, then bright orange and finally dark brown at its fully mature state (Figs. 2C-G). The mature fruit consists of a fleshy pericarp which encloses the mucilaginous pulp within which a large number of seeds remain embedded. By 2 - 3 days, such a mature fruit, after being dried up while still on the plant, is shed on to the ground. Seeds are liberated by gradual decay of the fruit wall over the soil. At this stage individual seeds remain enclosed within a thin film of firmly adhered dehydrated mucilage derived from the fruit pulp. Fahn and Werker (1972) envisaged that a seed as dispersal unit when possesses an additive structure is to be referred to as 'diaspore' rather than as 'seed'. In view of that, the mature seed of *S. indicum*, being dispersed with a firm layer of dehydrated mucilage as an additive structure, are preferably be referred to as 'diaspore' (Fig. 2H).

Mature seed diaspores are pale yellow in colour, relatively small, flattened, biconvex in sectional view and obliquely obovate in outline with the micropylar end relatively pointed and the chalazal end broad (Figs 2I-K). The raphe is faintly discernible as a slightly elevated structure. Seed surface is finely reticulate with parallel series of minute irregular pits (Figs 2J-L). Seeds are albuminous and exarillate. Anatomically, the seed coat, derived from outer epidermis, is represented by a layer of mechanical cells. The mesophyll and inner epidermis are crushed (Fig. 2M). The mechanical layer is made up of a compact layer of cells with undulate facets (Fig. 2N).

The inner and radial walls of the cells are strongly thickened and freed by the dissolution of the outer thin walls giving rise to the reticulate ornamentation with the thickened edges as hairs (Figs 2N, O). The endosperm is cellular and thin walled. Embryo is filiform and curved. Hypocotyl is longer than the folded cotyledons (Fig. 2P).

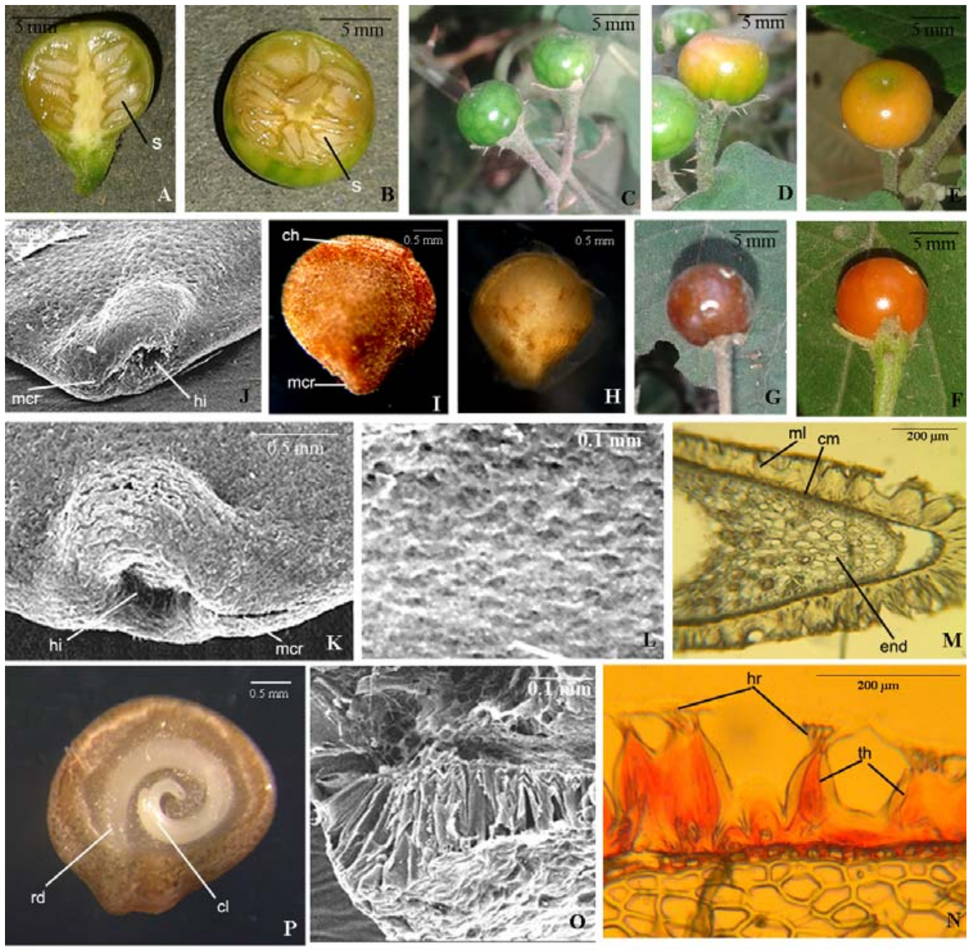


Fig. 2. Structural details of seed (diaspore) of *Solanum indicum*; A and B: Longitudinally and transversely chopped developing fruit, respectively showing seeds attached to axile placenta of bilocular ovary. C-G: Successive stages of fruit maturity, initially being deep green (C), turning gradually to yellow (D), orange (E), red (F) and finally dark brown (G) at full maturity. H: Water soaked diaspore showing the swelled mucilage around the seed coat. I: Sun dried diaspore exhibiting obliquely ovate outline with micropylar end somewhat pointed and chalazal end relatively broad, J-K: SEM photographs of micropylar end of diaspores showing hilum and two-lipped micropyle. L: SEM photograph of diaspore surface showing reticulate sculpturings. M: Part of diaspore in transverse section showing seed coat with outer mechanical layer followed by crushed mesophyll and inner epidermis and centrally the cellular endosperm, N: Magnified view of part of the seed coat in transverse section stained with safranin showing the cells of the mechanical layer with thickenings of inner and radial walls which are freed by dissolution of outer wall and the thickened edges as hairs. O: SEM photograph of mechanical layer of seed coat of a fractured diaspore. P: Inner view of seed by splitting up of seed coat showing the central filiform embryo with radical and folded cotyledons. [ch = chalazal end, cl = cotyledons, cm = crushed mesophyll, end = endosperm, hi = hilum, hr = hairs, mcl = mucilage, mcr = micropylar end, ml = mechanical layer, rd = radical, s = seeds, th = thickening].

Seeds of *S. indicum* follow the structure of typical solanoid seeds (Edmonds 1983, Karihaloo and Malik 1996). As the mechanical layer of the seed coat develops from the outer epidermis of the integument, the seeds are exotestal in construction (Corner 1976). Reticulate ornamentation with the thickened edges as hairs of the seed coat functions as the repository of diaspore mucilage. The small, discoid diaspores with specific gravity more than 1 (diaspores sink readily in water) are in no way suitable for long distance dispersal by wind or water. Nevertheless, torrential rain or cyclonic wind might have some role in the dispersal of the seeds, as the plants generally grow along the slopes of undulated terrains. However, such dispersal, if at all, would be of quite limited extent. Because of such limited seed dispersal, individuals of *S. indicum* are seen to grow in close proximities in their natural habitats. The diaspore mucilage plays several vital roles in reproductive ecology of the species: (i) the mucilaginous coating protects the seed from extreme desiccation, (ii) the plant grows in laterite terrains; the mucilage of the dispersed diaspores, on rehydration in rain water of the monsoon, helps the seeds to adhere to the ground soil of sloping terrains and checks their further migration, (iii) during germination, the hydrated mucilage around the germinating seed prevents its desiccation and functions as a prolonged source of moisture supply.

**Table 2. Cumulative percentage of germination through days of freshly harvested seeds of *Solanum indicum* after scarification by different concentrations of sulphuric acid for different durations.**

| Days | 1 - 5 | 6 - 10 | 11 - 15 | 15 - 20 |
|---|---|---|---|---|
| Treatment | | | | |
| 18(N) | | | | |
| 4 min | 0 | 25 | 66.66 | - |
| 8 " | 0 | 50 | 75 | 83.33 |
| 15 " | 0 | 41.66 | 58.33 | 58.33 |
| 24(N) | | | | |
| 1 min | 0 | 41.66 | 58.33 | 58.33 |
| 2 " | 10 | 58.33 | 75 | 83.33 |
| 4 " | 10 | 66.66 | 83.33 | 91.23 |
| 8 " | 10 | 50 | 58.33 | 66.66 |
| 30(N) | | | | |
| 2 min | 0 | 33.33 | 33.33 | 33.33 |
| 4 " | 0 | 25 | 25 | 25 |
| 36(N) | | | | |
| 1 min | 0 | 25 | 41.33 | 41.33 |
| 2 " | 0 | 25 | 25 | 25 |

Freshly harvested seeds of *S. indicum* possess relatively soft seed coats which become a little hardened after sun drying. Such sun-dried seeds can germinate in distilled water but the time taken for the germination is too long and the percentage of germination is also quite low. At least 22 days are required for the commencement of germination and during a course of 31 - 40 days a maximum of 55.55% germination is obtained. However, tetrazolium test reveals the existence of viable embryos in 90% of such seeds. The observation indicates the existence a sort of primary dormancy. In view of that scarification experiments were performed. Scarification of seeds by hot water treatment yielded the same results as obtained with normal distilled water, thereby proved to be ineffective. On the other hand, scarification in $H_2SO_4$ imparted fast as well as enhanced percentage of germination. Scarified seeds imbibed water quite readily and treatment with 24(N) acid for 4 min was found to be the most effective, bringing about the germination of 90% of seeds, followed by a germination of 80% by 24(N) acid for 2 min and 18(N) acid for 8 min (Table 2). Increase in germination from 55 to 90% by acid scarification reveals the existence of a sort of coat-imposed primary dormancy in freshly harvested seeds. For germination under natural conditions, the seed is required to remain in water-soaked condition for nearly one and a half months in the moist soil, which is not possible except in the rainy season. Thus, the dormancy prevents germination of seeds under unfavourable conditions when seedlings would not be able to survive.

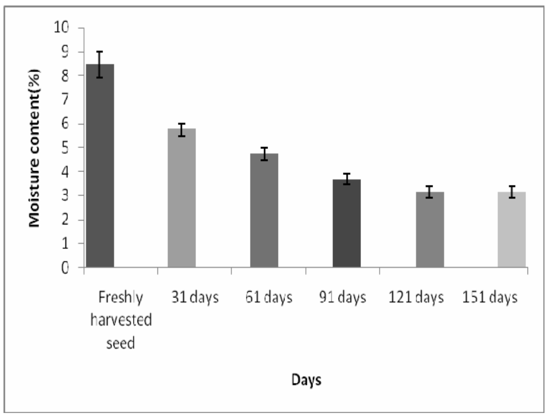


Fig. 3. Percentages of moisture content of seeds of *Solanum indicum* through progressive durations of storage.

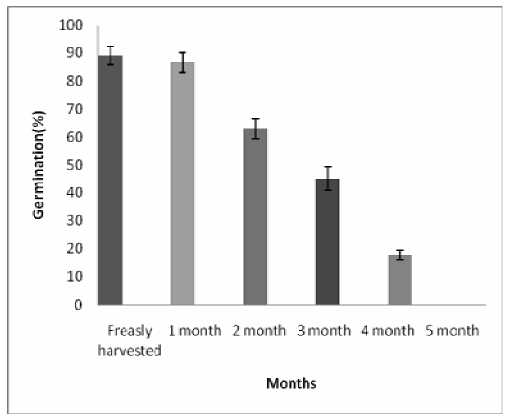


Fig. 4. Percentages of germination of seeds of *Solanum indicum* through progressive durations of storage.

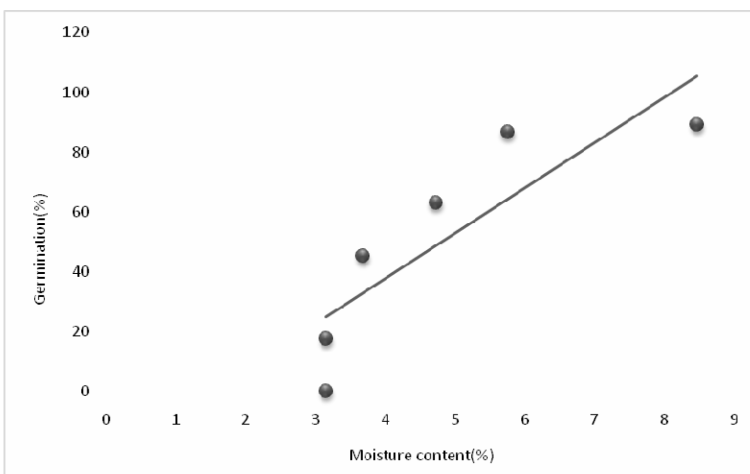


Fig. 5. Relation between the moisture content and viability of stored seeds of *Solanum indicum*.

The moisture content of seeds under storage steadily falls with time. Freshly harvested sun dried seeds contain 8.46% moisture, which falls finally to 3.14% after an interval of 120 days of storage. After that the stored seeds exhibit no further moisture loss. Also, the rate of moisture loss during the first four months of storage gradually diminishes with time. Maximum loss of moisture, i.e. 2.71%, was noticed during the first month of storage. The losses moisture in the 2nd, 3rd and 4th months of storage were found to be 1.00, 1.05 and 0.53%, respectively (Fig. 3). Freshly harvested seeds exhibit 89.02% germination. After one month of storage (i.e., in the 2nd month) germination of seeds falls to 86.58%. Percentage of germination of seeds steadily diminishes through time. After duration of four months of storage, merely 17.45% germination was obtained and subsequently the stored seeds exhibit no germination at all (Fig. 4).

Also, germinability of stored seeds exhibits a positive correlation with their moisture content. When the percentages of germination were plotted against the respective moisture contents, a linear relation was obtained (Fig. 5).

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