INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 1, p. 311-321, 2019

RESEARCH PAPER OPEN ACCESS

Seed ecology of *Ecbolium ligustrinum* (Vahl) Vollesen, an important medicinal plant of Asiatic tropics

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Key words: *Ecbolium ligustrinum*, Conservation, Jaculator, Seed germination, viability.

http://dx.doi.org/10.12692/ijb/15.1.311-321 Article published on July 18, 2019

Abstract

Ecbolium ligustrinum (Vahl) Vollesen [Acanthaceae] is a regionally threatened widely used medicinally important plant. For the sustenance and formulation of conservation strategy, a thorough study regarding different seed ecological aspects of the plant species *viz*., seed production, seed-ovule ratio, seed-set percentage, seed dispersal and seed structure along with their germination, dormancy, scarification and moisture content have been studied. Morpho-anatomical structures of the seed have also been studied in detail with their specialized dispersive organ jaculator. The exotestally derived seed coat bears short, rigid hairs and some scattered mucilaginous deposits which performs significant role in moisture regulation during their storage. Germination experiments were carried out as per the rule of International Seed Testing Association. For scarification methods Copeland and McDonald's technique has been employed. The seeds of the species showed a coat-imposed primary dormancy which can be successfully broken through acid scarification using 36(N) and 24(N) H_2SO_4. Gradual losses of moisture content of the seeds were associated with their loss of viability.

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Introduction

Seed is the sexually derived fertilized mature ovule, an important input for the conservation and cultivation of the species. Aspects of seed ecology assume one of the very effective parts in the life cycle of flowering plant. The knowledge about the seed ecology is too much essential for the formulation of strategies regarding the successful conservation and sustenance of a species. It was reported that all the ovules in case of a multi-ovulate gynoecium is not necessarily developed into mature seeds (Weins, 1984; Fenner and Thompson, 2005). So, the seed-ovule ratio and percentage of seed-set is an important index for the evaluation of reproductive destiny of a species. Seed coat is the protective layer of the seed. In addition to giving protection, it also supports various physiological functions related to water uptake, moisture content, dispersal, dormancy and germinability of the seed (Werker *et al.*,1979; Egley, 1989; Gutterman, 1993). Tough and impermeable seed coat is often responsible for seed dormancy. Sometimes this dormancy which is also the temporary phase of suspension of germination may help the species to overcome the unfavorable environmental situation (Vleeshowers *et al.,* 1995; Baskin and Baskin, 1998). Seed viability (the ability of a seed to germinate) of a species varies from a short period to a longer duration. A seed with longer viability period provides the better opportunity for storage. Among many other factors, moisture content of a seed has direct impact on its viability (Leopold *et al.*, 1988; Foley, 1994). Therefore, events of seed ecology include seed production, seed set percentage, seed structure, dispersal mechanism, germination status, dormancy, moisture contents and duration of viability, which are again very important to study for achieving the method of sustenance and conservation of a species.

Ecbolium ligustrinum (Vahl) Vollesen is an important medicinal plant belonging to the family Acanthaceae commonly known as “neelkantha”in Bengali vernacular. The different parts of the plant were used traditionally from ancient times in Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications (Kirtikar and Basu, 1987). Roots and leaves of the plant are used as anti-microbial, anti-cancerous, anti-inflammatory, hepatoprotective, free-radical scavengers; the plant is also a rich source of positive cardio-vascular effect producing substances (Chaudhuri *et al.*, 2011; Ashoka Babu, 2011; J.J. Narayanan, 2012). In Ayurvedic medicine, the different parts of the plant is used for the treatment of jaundice, menorrhoea, rheumatism, gout and dysuria by the local people of India and several other countries of tropical Asia (Chopra *et al.*,1956; Lalitha and Sethuraman, 2010; Sharma and Sharma, 2010). Such effects of the plant is due to the presence of alkaloids, Ecbolin-A, orientin, vitexin, isoorientin, isovitexin, glycoflavones and some other flavones (Venkataraman and Gopalkrishnan, 2002; Cecilia *et al.*, 2012).The species is a perennial one and flowers during June to December once in a year. In each day the individual plant bears1-14 flowers per inflorescence throughout the flowering season. As the species allow a few flowers for a longer period of time, the flowering pattern is of “Steady-State” type as per Gentry, 1974.

E. ligustrinum grows on wastelands and gardens in southern and eastern part of India. It is native to India (Hooker, 1872; Golam-Sarwar, 2015). The plant is also distributed in the Arabian Peninsula, Somalia, Kenya and the countries of tropical Asia. In West Bengal the species is distributed throughout the southern part of the state (Prain, 1903).

The plant propagates in nature by seeds and thrives sometimes by their perennial rootstocks. Propagation through seeds is the best way for their greater survivality. In recent time, due to large scale habitat destruction, the species is going to be threatened in near feature. The present survival status of the species demands proper conservation. Despite, the immense medicinal properties of the species no attention has been given on its seed ecology. For the formulation of strategies regarding the successful *in-situ* conservation of the species, studies in its seed ecology was therefore undertaken.

Material and methods

Seed production

The plant produces an ovoid compressed capsule from syncarpous superior ovary. Each mature fruit contains 2-flattened seeds (rarely 4). Mature fruits were collected randomly from the wild habitat of Monteswar block of Burdwan District, Chandannagar area of Hoogly District and Midnapore sadar block of Paschim Medinipur District, West Bengal (Fig. 1). Seeds were isolated from the fruits and were sun-dried (35±2°C) for 5 hours each for two consecutive days. Healthy seeds were divided into a number of seed lots of 100 seeds each and stored in Borosil glass vial with loosely fitted Bakelite caps in laboratory condition (27±2°C).

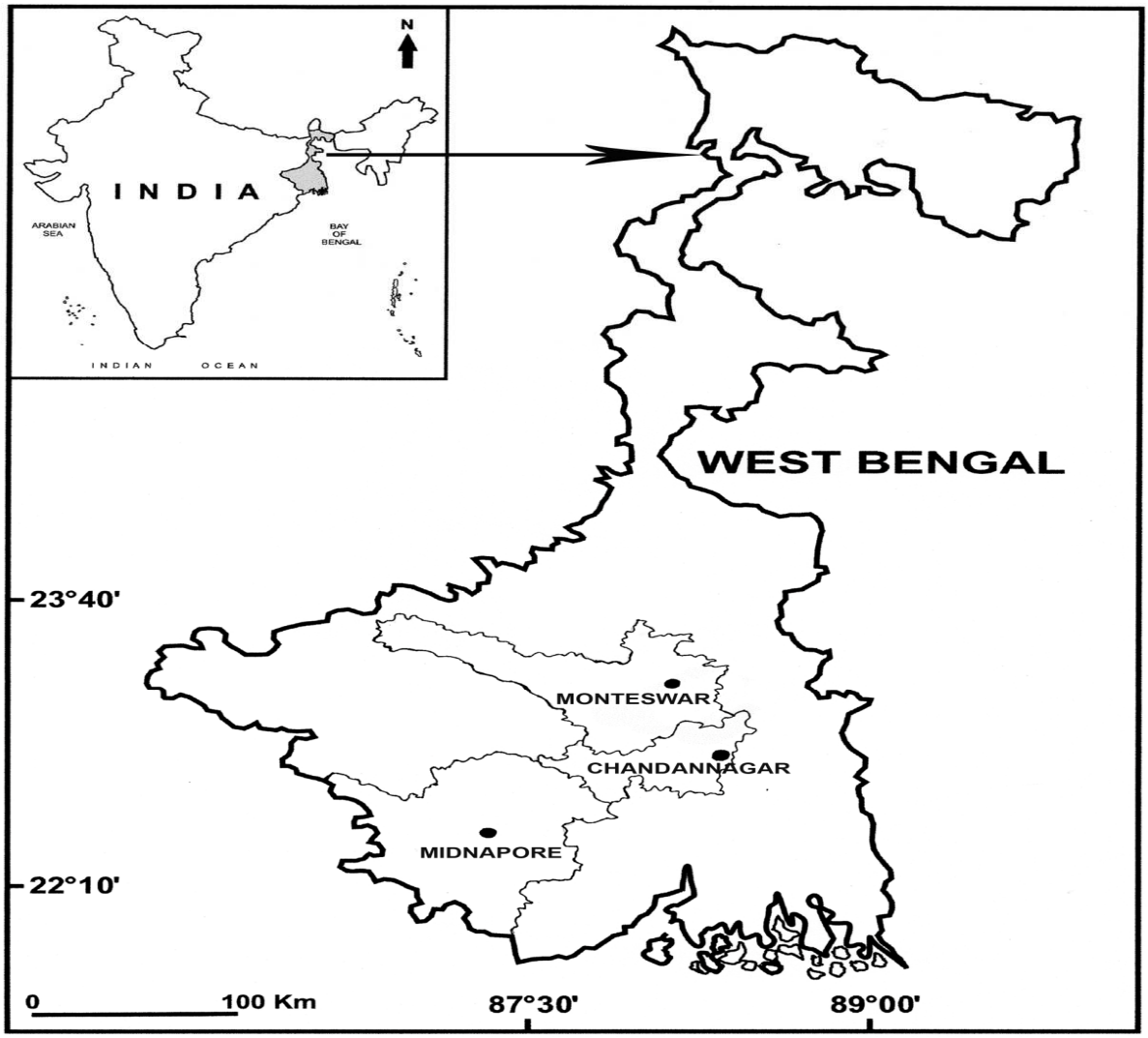

Fig. 1. Map showing the study areas of our research work [Monteswar of Burdwan District, Chandannagar of Hoogly District and Midnpore of Paschim Medinipur District; West Bengal, India].

Seed-ovule ratio and seed set percentage

Seed production of the plant was studied in every month by random collection of 100 fruits in each month during flowering season. Seed weight was determined based on seed lots, each with 100 seeds. To determine the seed-ovule ratio, number of ovules per flower was observed based on randomly collected flowers (20 in number) in each month. The percentage of seed set was obtained by multiplying the seed-ovule ratio with 100.

Seed structure

Structural detail of the seed was worked-out both morphologically (prior to and after removing seed

coat) under a WILDMBB steriobinoculor microscope and anatomically by sectioning the seed using Leica Cryo-Microtome, USIC section of Vidyasagar University, followed by the study under a Leica DMLB compound bright field microscope. Photomicrographs were taken by a Leica DFC 295 digital camera attachment.

Seed germination, dormancy and scarification

Germination experiments were carried out as per the rule of International Seed Testing Association (1996). Prior to seed germination, seeds were rinsed with 0.1% $Hgcl_2$ solution for 90 seconds for surface sterilization and then washed thoroughly with double distilled water to remove traces of $Hgcl_2$, if any. The surface sterilized seeds were kept in double distilled water overnight for imbibition. For germination, imbibed seeds were sowed on moistened filter paper by double distilled water in sterilized Petri dishes. The Petri dishes were placed under diffused natural light at normal room temperature (27±2°C) and germination was recorded day to day. Each experiment was repeated three times during the entire study.

The best-known scarification methods such as hot water treatment, alternating hot-cold temperature treatment and acid scarification treatment were employed (Copeland and McDonald, 2001). For hot water treatment seeds were subjected to hot water (50°C, 55°C, 60°C, 65°C, 70°C, 75°C and 80°C) for 5 minutes and 10 minutes and for alternating temperature treatment the above said hot water treated seeds immediately followed by an exposure to cold water (5°C) for another 5 minutes and 10 minutes.

For acid scarification technique, different concentrations of sulphuric acid [20(N), 24(N), 30(N) and 36(N)] were prepared by diluting the concentrated H_2SO_4 [36(N)] with double distilled water. Optimum scarification effect was deduced by applying those concentrations of H_2SO_4 solutions for variable time durations (5, 7, 10, 12, 15, 20 and 22 min).

Moisture content of seeds under storage

Moisture content of seeds was determined from the difference in weight between the freshly harvested seeds and dry seeds. Seeds were made totally moisture free by keeping the seeds in a LABARD Hot Air Oven for consecutive 2 days at 80°C. Percentage of seed moisture contents with respect to fresh weight of seeds were determined by the formula given below:

$$\frac{\text{Weight of freshly harvested seeds - Weiht of dry seeds)}}{\text{Weiht of freshlly harvest seeds (before drying)}} \times 100 = \%\ \text{of moisture content with respect to fresh weight}$$

The moisture contents of stored seeds were measured at 90 days intervals since storage and the percentage of moisture content was plotted in a histogram (Fig. 3).

Viability of seeds under storage

Viability of seeds under storage conditions was studied at 90 days intervals by using TTC (2,3,5-Triphenyl tetrazolium chloride) test and also by performing germination experiments after optimum scarification. Percentages of germination through time were plotted in a histogram (Fig. 4).

Results

Seed production

Generally the number of seeds produced in a fruit is two, rarely one and extremely rare with four seeds (Figs. 2B, 2E, 2G), three seeds never found, having a mean value of 1.81. The weight of each seed varies from 12.1 to 12.9 mg (mean value 12.45 mg).

The size of fruit ranges from 1.6 cm × 0.7 cm to 1.9 cm × 0.8 cm (mean value 1.78 cm × 0.74 cm) (Fig. 2A) and size of the individual seed is 0.8 cm × 0.6 cm - 0.9 cm × 0.6 cm (mean value 0.82 cm × 0.6 cm) (Fig. 2H).

Seed-ovule ratio and seed set percentage

The number of ovules per ovary in *E. ligustrinum* is four. The average number of seeds produced by a flower is 1.81 (Figs. 2B, 2E). So, the seed-ovule ratio of a flower is 181:400 and the seed set percentage is 45.25.

Seed dispersal

The fruit of *E. ligustrinum* is derived from a bilocular ovary, each locule with two ovules in axile placentation. A young fruit is deep green in colour. During the course of its maturity, initially the young fruit turns light brown and finally dark brown at its fully mature state (Fig. 2A). In most of the cases the mature fruit consists of a leathery pericarp enclosing two seeds, one seed in each chamber (Fig. 2B). Each mature seed is yellowish-brown, attached through funiculus. Jaculator, a slender extension of the funiculus (±0.45 cm long) remains appressed laterally (Figs. 2B, 2C). The jaculator is responsible for ballistic dispersal of mature seeds.

Table 1. Percentage of germination of freshly harvested unscarified seeds of *E. ligustrinum*.

Seed lot	Seed germination (%) in days						
	1-10	11-20	21-30	31-40	41-50	51-60	61-70
Set 1	0	0	0	5.0	5.0	12.60	12.60
Set 2	0	0	0	0	6.5	6.5	6.5
Set 3	0	0	0	6.6	6.6	13.33	13.33
Mean (±SE)	0	0	0	3.87 (±1.62)	6.033 (±0.42)	10.81 (±1.77)	10.81 (±1.77)

SE: Standard Error.

In Acanthaceae, the jaculation mechanisms of seeds are mainly of two types viz. hydrostatic or xerostatic. In case of *E. ligustrinum* it is strictly xerostatic. The jaculation occurs after complete maturation at highest dehydrated condition of the fruit that facilitates the degeneration of cementing tissues of the fruit wall (Figs. 2D, 2F).The jaculation of seeds takes place with a cracking sound when the vertical walls of cementing tissue become degenerated and the valves cannot holds the pressure of the jaculators anymore.

Table 2. Cumulative germination percentage of freshly harvested seeds of *Ecbolium ligustrinum* after scarification using different concentrations of H_2SO_4 for different durations.

Treatment		Days					
		1-5	6-10	11-15	16-20	21-25	26-30
20(N)	5min	0	0	0	0	0	0
	10min	0	0	0	3.33	6.66	10.00
	15min	0	0	0	10.00	26.66	36.66
	20min	0	0	20.00	43.33	66.33	66.33
	22min	0	0	0	16.66	33.33	46.66
24(N)	5min	0	0	0	6.66	13.33	23.33
	10min	0	0	0	16.66	30.00	46.66
	15min	0	0	26.66	53.33	80.00	80.00
	20min	0	30.00	60.00	90.00	90.00	90.00
	22min	0	0	0	20.00	36.66	56.66
30(N)	5min	0	0	0	13.33	26.66	43.33
	7min	0	0	0	20.00	33.33	53.33
	10min	0	0	0	23.33	43.33	63.33
	12min	0	0	0	16.66	36.66	50.00
	15min	0	0	0	10.00	23.33	33.33
	20min	0	0	0	3.33	6.66	13.33
36(N)	2min	0	0	23.33	53.33	73.33	73.33
	5min	0	33.33	63.33	90.00	90.00	90.00
	10min	0	0	16.66	36.66	56.66	56.66
	15min	0	0	0	3.33	6.66	6.66
	20min	0	0	0	0	0	0

The seeds are flattened, light weight and aerodynamically streamlined. Therefore, the force generated by the jaculator able to dispersed the seeds up to 10 ft from the mother plant.

Seed structure

Seeds develop from anatropous, unitegmic and tenuinucellate ovules. Mature seeds are yellowish-brown in colour, 0.82 cm × 0.6 cm, flattened, cordate with chalazal end more or less pointed and micropylar end is deeply notched with the deeply grooved hilum (Fig. 2H). Seed surface is finely elevated with rigid small hairs. Sectional view of the seed is biconvex, with more or less centrally situated embryo (Fig. 2J).

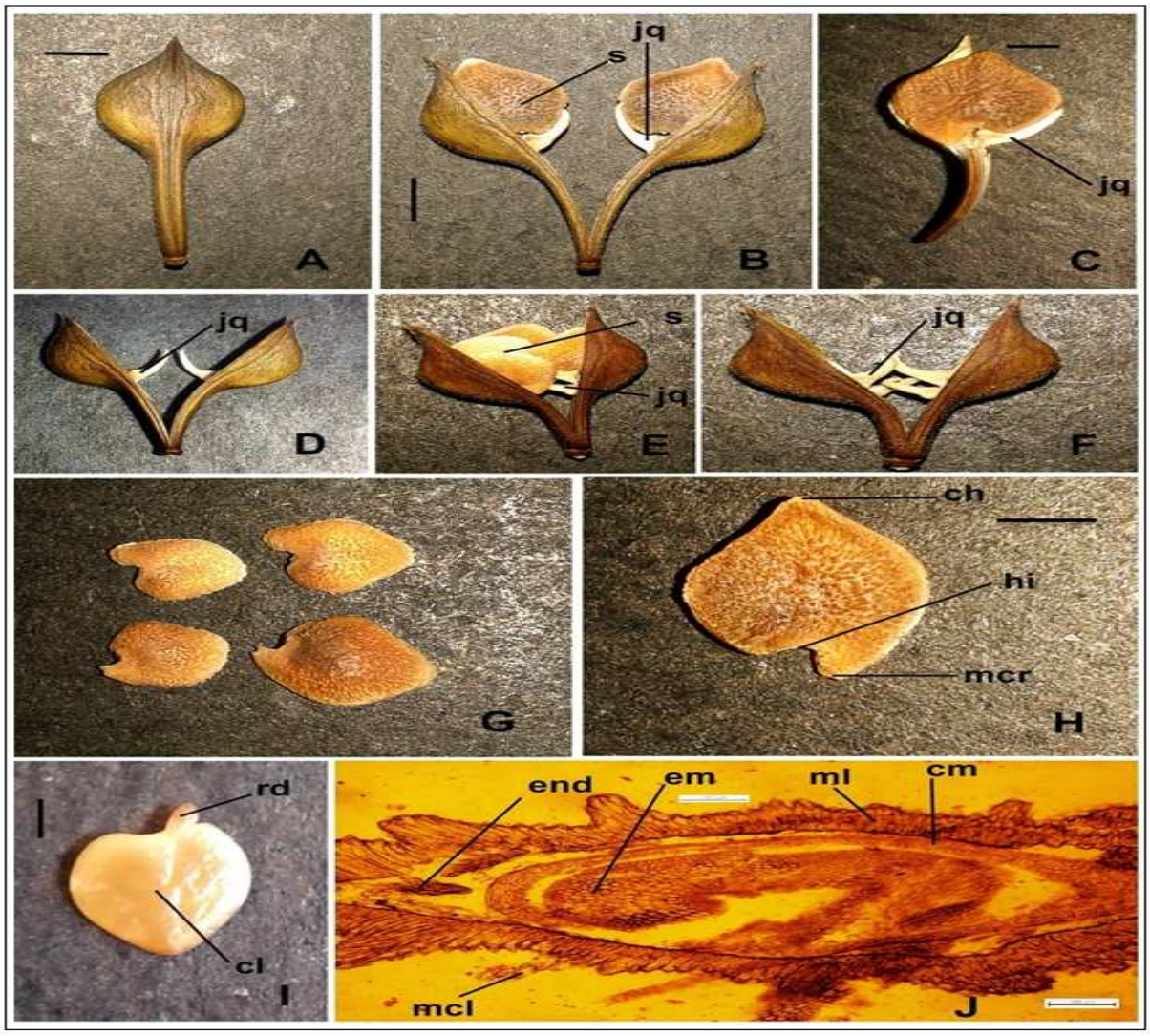

Fig. 2. Structural details of seed of *E. ligusrinum*. A: Mature fruit. B: Dehisced fruit. C: Arrangment of a seed within the fruit and showing the position of jaculator. D: An empty 2-seeded fruit case showing the arrangements of jaculators. E: A dehisced 4-seeded fruit. F: An empty 4-seeded fruit case showing relative arrangements of jaculators. G: All four seeds of a 4-seeded fruit. H: Morphology of a seed with somewhat notched micropylar end and relatively pointed chalazal end and also a grooved hilum, surface showing fine elevation. I: A seed without the seed coat showing its radicle and cotyledons. J: Part of a seed in transverse section showing seed coat with mechanical layer followed by crushed mesophyll, centrally cituated embryo, remnants of endosperm and small amount of dryed mucillage adhere in the seed coat. [s = seed, jq = jaculator, mcr = micropyle, ch = chalaza, hi = hilum, rd = radicle, cl = cotyledones, ml = mechanical layer, cm = crushed mesophyll, em = embryo, mcl = mucilage, end = endosperm]. Scale bar: A = 4 mm; B = 5 mm; C = 2 mm; H = 2.5 mm; I = 2 mm; J = 100 μm.

Seeds are albuminous and exarillate. Anatomically, the seed coat derived from outer epidermis of the testa is represented by a layer of mechanical cells. The mechanical tissue is consists of a layer of thick walled palisade like cells, often bearing rigid hairs (Fig. 2J). The mesophyll and inner epidermis is crushed. The endosperm is cellular and thin walled. Embryo is straight bearing a pair of cotyledons (Fig. 2I).

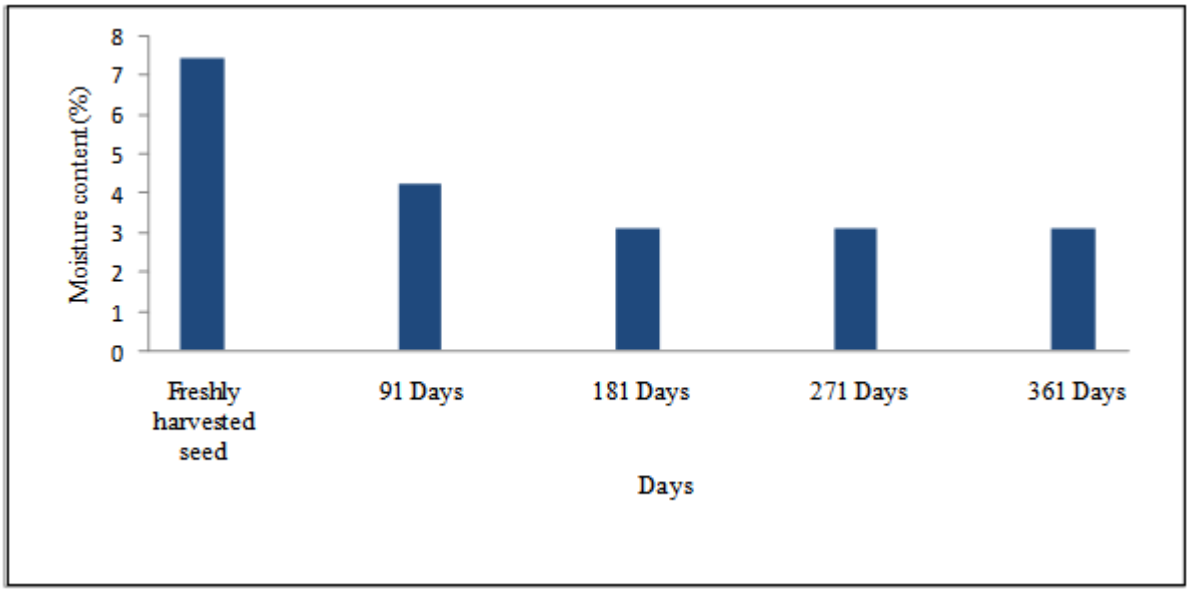

Fig. 3. Percentages of moisture content of *E. ligustrinum* seeds through progressive durations of storage.

Seed germination

The seed coat of freshly harvested seeds of *E. ligustinum* is very hard and become more harden after sun drying. Such sundried seeds can germinate in distilled water in a very low percentage (6.5-13.33%) and also requires long time for commencement (34 days) and completion of germination (51-60 days). In average, the maximum germination percentage is merely 10.81 (Table1).

Dormancy and scarification

In normal condition a maximum 10.81% germination was achieved. TTC (2,3,5-Triphenyl tetrazolium chloride) test imparted positive results in 92% of such freshly harvested seeds clearly indicating the presence of viable embryos even in non-germinated seeds. This observation reveals the existence of a sort of primary dormancy. To overcome the dormancy different scarification experiments were performed. Scarification of seeds by hot water treatments and also hot water-cold water alternate treatments exhibits no more better results as obtained using normal distilled water. Scarification of seeds using concentrated H_2SO_4 gives better result regarding germination. Such scarified seeds imbibed water quite readily. Treatment with 24(N) H_2SO_4 for 20 minutes as well as 36(N) H_2SO_4 for 5 minutes were found to be the most effective showing about 90% seed germination followed by a germination of 80% by 24(N) acid treatment for 15 minutes (Table 2). These scarification treatments most effectively increased the germination percentage and also reduced the inception period (Fig. 5).

Moisture Content of seeds under storage

The moisture content of seeds under storage condition gradually falls up to the sixth month and thereafter no further moisture loss takes place. Freshly harvested sun dried seeds contain 7.42% moisture which after 180 days finally falls to 3.11% during storage. After that the stored seeds exhibit no further moisture loss. The rate of moisture loss with time gradually diminishes after 3 months and continues up to six months. Maximum moisture loss takes place for the first 3 months of storage which was 3.21%. The moisture loss for next 3 months was found 1.1% and after that moisture content remains fixed (Fig. 3).

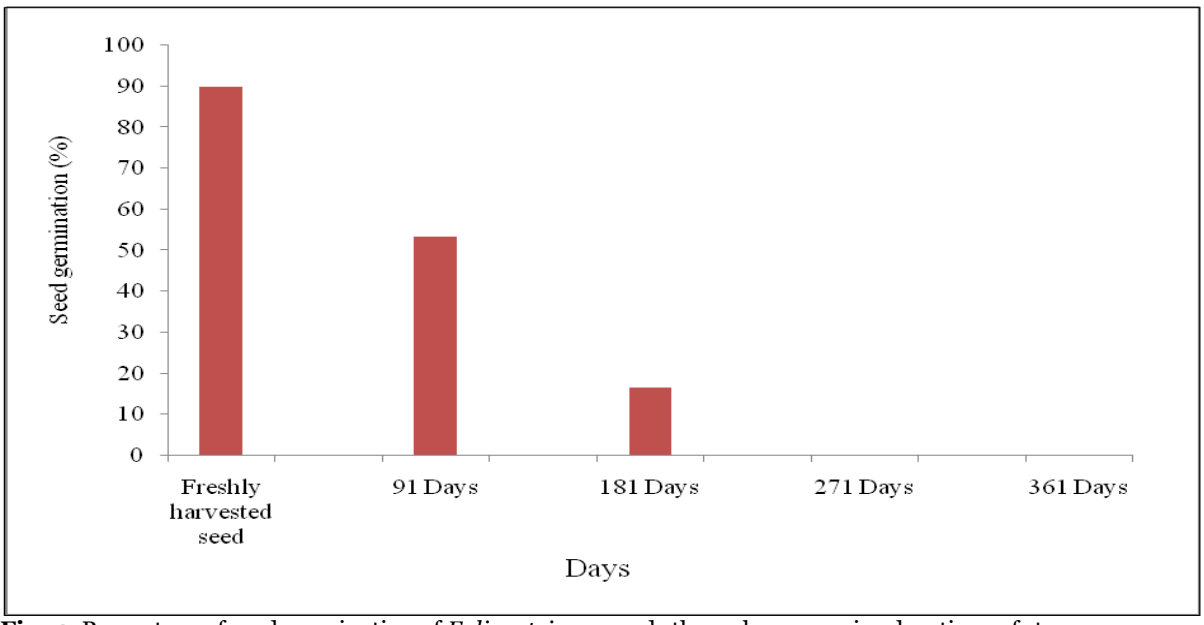

Fig. 4. Percentage of seed germination of *E. ligustrinum* seeds through progressive durations of storage.

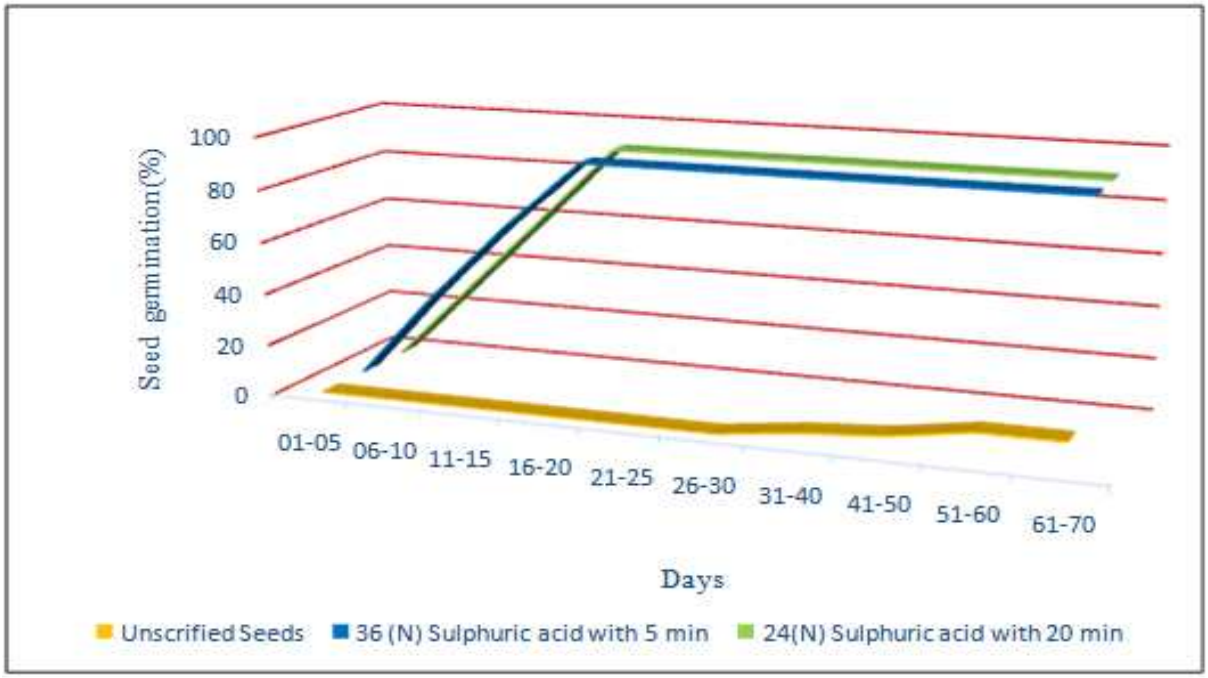

Fig. 5. Graphical representation of cumulative germination percentage of freshly harvested unscarified seeds and after the most effective scarification treatments [36(N) H_2SO_4 with 5 min and 24 (N) of H_2SO_4 with 20 min].

Viability of seeds under storage

Freshly harvested seeds treated with most effective scarification treatment exhibit 90% germination. After 3 months of storage such seeds showed 53.33% germination and after another 3months of storage it became only 16.66%. Finally, after 6 months of storage, seeds exhibit no further germination at all. This record of germination percentage clearly indicates the gradual loss of germinability with time and also the rate of germinability loss steadily increased under storage (Fig. 4). This phenomena is also supported by TTC (2, 3,5-Triphenyl tetrazolium chloride) test.

Discussion

Freshly harvested seeds of *E. ligustrinum* exhibit very low germination percentage (10.81%) in natural condition. However, such seeds with acid scarification

showed maximum 90% germination. So, it is evident that the freshly harvested seeds possess some sort of primary dormancy which is of coat imposed one. It also showed that time period for commencement of germination is much reduced from 51-60 days to 21-25 days through acid scarification. Such a longer period of time (51-60 days) required for seed germination in natural condition is practically not feasible except rainy season. Therefore, this scarification treatment shows a positive effect on seed germination percentage as well as reduced the inception period which may help the species for their better survival by increasing the percentage of germination as well as reducing the dormancy.

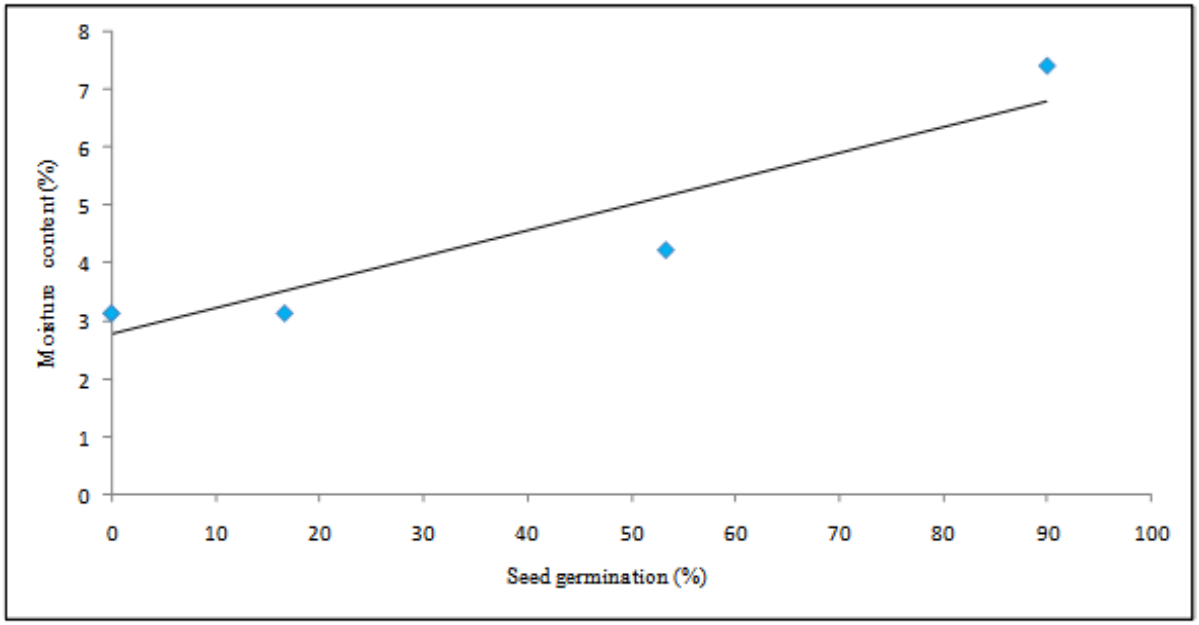

Fig. 6. Relation between the moisture content and percentage of germination of stored seeds of *E. ligustrinum*, showing a positive correlation.

Seeds stored in ambient conditions gradually loose moisture content with time up to 6 months and after that less amount of moisture remain within such seeds. Germination percentage of the seeds also gradually decreases up to 6 months of storage and beyond that no further germination takes place i.e. complete loss of seed viability. Therefore, moisture content and germination percentage the two parallel phenomena of stored seeds show a clear positive correlation between them (Fig. 6).

Conclusion

Ecbolium ligustrinum is used by local people of India including West Bengal for various medicinal purposes.The species is perennial and flowersnearly for six months (mid-June to mid-December) in a steady state pattern with a reasonable fruit set.

The moderate seed-ovule ratio (1:2) with a modest seed set percentage (45%) in wild condition could be a better reproductive strategy of the plant. However, dehiscence of mature seeds require more than a month (33-43 days) since pollination and freshly released seeds exhibit only 10.8 % germination and require nearly two months to complete it. As in south West Bengal, the winter season comes after monsoon and the climate was a dry one with a dearth of water, therefore, to overcome these three to four months water scarcity, the seeds possess some sort of primary dormancy due to hard-impermeable seed coat.

This primary dormancy can be broken by acid scarification which may shorten the germination time period.Our findings provide a better understanding about the viability, germination status and successful strategy for effective germination leading to production of greater number of seedlings of such an extremely important medicinal plant.

Acknowledgement

The authors are thankful to the USIC section, Vidysagar University (Midnapore, India) for providing cryo-microtome during sectioning of seed.Thanks are also due to the authority, The University of Burdwan (Burdwan, India) for providing necessary laboratory and library facilities. The authors are especially indebeted to Late Prof. P. K. Pal, Department of Botany, The University of Burdwan for his invaluable suggestions during the work.

References

Ashoka Babu VL, Arunachalam G, Jayaveera KN, Madhavan V, Banu S. 2011. Free radical scavenging activity of methanolic extract of *Ecbolium viride* (Forrsk). Alston roots. Der PharmaciaLettre **3(4),** 285-288.

Baskin CC, Baskin JM. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego, CA: Academic Press. Appl **8,** 151-164.

Cecilia KF, Ravindharan R, Duraipandiyan V. 2012. Evaluation of antimicrobial efficacy of *Ecbolium viride* (Forssk). Alston root extracts. Asian Journal of Pharmaceutical and Clinical Research **5(3),** 239-241.

Chaudhri D, Pathak AK, Sevanan M. 2011. Phytochemical Screening and antimicrobial activity of extracts from leaves of *Ecbolium linneanum*. Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimatore-641114, India. https://doi.org/10.3329/bjp.v6i2.8732

Chopra RN, Nayar SL, Chopra IC. 1956. Glossary of Indian medicinal plants. New Delhi, CSIR, India.

Corner EJH. 1976. Acanthaceae. In: The seeds of dicotyledons,vol. I. Cambridge University Press, Cambridge, UK.

Egley GH. 1989. Water-impermeable seed coverings as berries to germination. In:Taylorson RB, Ed.Recent Advances in the Development and Germination of Seeds. Plenum Press, New York, USA, 207-223.

Fenner M, Thompson K. 2005.The Ecology of Seeds, Ed 2nd. Cambridge University Press, Cambridge, UK.

Foley ME. 1994. Temperature and water status of seed affect after ripening in wild oat (*Avena fatua*). Weed Science **42(2),** 200-204.

Gentry AH. 1974. Flowering phenology and diversity in tropical Bignoniaceae. Biotropica. **6(1),** 64-68. http://dx.doi.org/10.2307/2989698

Golam-Sarwar AKM. 2015. Medicinal plant genetic resources of Bangladesh-genera represented by single species and their conservation needs. Journal of Medicinal Plants Studies **3(2),** 65-74.

Gutterman Y. 1993. Seed germination in desert plants. Springer-Verlag, Berlin, Heidelberg, New York, USA.

Hooker JD. 1885. The flora of British India,Vol. IV. L.Reeve., London.

International Seed Testing Association. 1996. International Rules for Seed Testing. Seed Science and Technology **24,** 335.

Kirtikar KR, Basu BD. 1987. Indian Medicinal Plants **3,** International Book Publishers, Dehradun, India.

Lalita KG, Sethuraman MG. 2010. Anti-inflammatory activity of roots of *Ecbolium viride* (Forssk) Merril. Journal of Ethanopharmacology **128(1),** 248-250. https://doi.org/10.1016/j.jep.2009.12.030

Leopold CA, Glenister R, Cohn MA. 1988. Relationship between water content and after ripening in red rice. Physiologia Plantarum **74(4),** 659-662. https://doi.org/10.1111/j.1399-3054.1988.tb02032.x

Narayanan JJ, Winka JJ, Rajkumar M, Senthil Kumar KL. 2012. Study of hepatoprotective activity of *Ecbolium viride* (Forssk) Alston. International Organization of Scientific Research Journal of Pharmacy **2(2),** 157-161.

Prain D. 1903. Bengal Plants **1,** Botanical Survey of India, Howrah, India.

Sharma R, Sharma HK. 2010. Ethnomedicine of Sonarpur, Kamrup district, Assam. Indian Journal of Traditional Knowledge **9(1),** 163-165.

Venkataraman R, Gopalakrishnan S. 2002. A lignin from the root of *Ecbolium linneanum* Kurz. Phytochemistry **61(8),** 963-966. https://doi.org/10.1016/S00319422(02)00387-4)

Vleeshouwers LM, Bouwmeester HJ, Karssen CM. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. Journal of Ecology **83,** 1031-1037.

Werker E, Marbach I, Mayer AM. 1979. Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. Annals of Botany **43(6)**, 765-771. http://dx.doi.org/10.1093/oxfordjournals.aob.a085691

Wiens D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia **64(1),** 47-53.