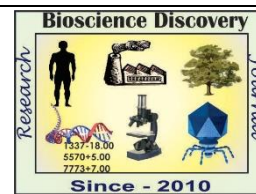


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Research Article



Seedling phenology of *Ecbolium ligustrinum* exhibiting epigeal phanerocotylar type of germination

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Abstract

Ecbolium ligustrinum (Vahl) Vollesen (Acanthaceae) is characterized by some special features in its seedling phenology and germination. After suitable scarification seed takes 7-8 days for inception of germination. The hypocotyls exhibit rapid growth leads to lifting of seed coats with emerging cotyledons indicate epigeal pattern of germination. These cotyledons at 13-14 DARE, are completely emerged out and seed coats are fall off from the germinating seeds show signs of the phanerocotylar type of seed germination. Thus, the germination is of epigeal phanerocotylar type. The cotyledons are green, fleshy, photosynthetic and considered as paracotyledons. At 16-17 DARE emergence of plumule is recognized which is again marked as the completion of germination phase and onset of seedling phase. The 2nd and 3rd pair of foliage leaves emerges at 25-26 and 36-37 DARE respectively. These three (1st, 2nd and 3rd) pairs of foliage leaves are quite different in their shape, size, margins and venation pattern from the adult leaves, indicates the occurrence of eophylls. The seedling phase is completed by 154-168 DARE with the formation of 12th node with 11th pair of foliage leaves and by the abscission of those three pairs of eophylls. Though the epigeal phanerocotylar with foliaceous cotyledons (EPF) type of seedling shown by the species is quite common among dicotyledons, the seedling of the species is characterized by some notable morphological features like thickened hypocotyl, a pair of phanerocotylar epigeal cotyledons and three pairs of eophylls in opposite decussate phyllotaxy. Individual eophyll is ovate in shape with slightly dentate margins and acute to sub-acuminate apex. The eophylls are easily distinguishable from the foliage leaves of adult individual by their shape, size and venation pattern. Such specific features are very much helpful for identification of a species at its juvenile stage before flowering and fruiting. Thus, phenological studies of the seedling of widely used medicinally important plant species provide the great opportunities for their successful conservation in natural habitat.

INTRODUCTION

The seedling stage is arguably the busiest phase in plant's lifetime (Farnsworth, 2008). Emergence of seedlings and their establishment are crucial early plant life cycle stages (Harper, 1977; Angevine and Chabot, 1979; Silvertown *et al.*, 1993). After

germination, the seedling depends on its own phenological and physiological attributes such as size, function, position of cotyledons and amount of seed reserves which may be crucial to capture resources to cope with various factors threatening its sustenance in the wild habitat (Fenner, 1985).

Seedling morphology is nothing but constituted by this suite of attributes. Apart from its size, the seedling of a species differs in many aspects from its adult individual. In vast majority of the species, the seedling above their cotyledonary leaves, give rise to one or more cataphylls, which differ architecturally from the typical foliage leaves. Those are referred to as eophylls (Duke, 1965). A typical dicotyledonous seedling comprises a primary root with associated secondary and tertiary branches, a hypocotyl, distinct collet, a pair of cotyledonary leaves and a plumular bud with subsequent internodes and eophylls. Seed leaves, seed lobes or cotyledons in the broad classical meaning are the first leaves of the juvenile plant (de Candolle, 1825). The cotyledon occurs in three different manifestations. They may be either food-storing (fleshy), haustorial or photosynthetic at the moment of germination. The photosynthetic cotyledons are referred to as paracotyledons (Vogel, 1980). The emergence of plumule indicates the completion of seed germination as well as the commencement of seedling phase of a plant. The seedling phase extends upto the young plant assumes the miniature form of an adult individual. Phenological patterns of seedlings of every individual have been ascribed to genetic factors and often are species specific (Holdsworth *et al.*, 2001; Ackerly, 2004; Wang *et al.*, 2009). Seedling establishment in its own habitat represents the final hurdle in process of fruitful regeneration.

After seeds, seedling typically suffers the highest mortality rate of any life history stage and, therefore, is important in selection and evolution of the species. Moreover, the seedlings of many taxa, particularly herbs are so small as to escape attention in their natural habitats (Canne, 1983; Kakati and Barthakur, 2017). Developmental processes of the seedlings are critical for their establishment and thereby for the survival of the species in a constantly changing environment (Oyelana, 2011). The phenological pattern of seedlings in each species is therefore of great significance in understanding the reproductive ecology of this sexually reproducing species. It is now emphasized that seedling phenology should be thoroughly investigated for better conception of germination, establishment and juvenile growth during the natural regeneration of vegetation (Troup, 1921; Jackson, 1974). Also, the features of seedling are very much helpful in identifying the individuals of a species at their

juvenile stages much before the flowering and fruiting.

Ecbolium ligustrinum (Vahl) Vollesen (Acanthaceae) is a perennial woody herb with erect branches. The root, stem and leaf of the plant are used for various medicinal purposes. Roots of the plants are used for jaundice, menorrhagia and rheumatism (Kirtikar and Basu, 1987; Elumalai *et al.*, 2011). As per the ethnobotanical survey conducted in east Godavari district of Andhra Pradesh, it is found that the roots of the plants used in folk medicine for their diuretic properties (Sridhar *et al.*, 2014). Leaves are used in gout and dysuria, decoction of leaves for liver troubles and against tumors (Khare, 2007; Yusuf *et al.*, 2009). Ethanolic (50%) extract of the plant possesses positive cardiovascular effects (Asolkar *et al.*, 1992). It also possesses several pharmacological properties such as anti-microbial, anti-diarrheal, hepatoprotective and antioxidant activity. Leaves, roots and flowers of the plant contain glycoflavones; Orientin, Vitexin, Isoorientin, Isovitexin and other flavones (Ghani, 2009). The plant extract contains several organic compounds which are mostly heterocyclic aromatics and anti-oxidants. The ethyl acetate root extracts contain a novel hetero furanoid compound; 4-methoxy-5-[4-(4-methoxy-1,3-benzodioxole-5-yl)perhydro-1H,3H-furo [3,4-c]-furan-1-yl]-1,3-benzodioxole (Ezhilmuthu *et al.*, 2008).

The species is known to distribute in the Arabian Peninsula, Somalia, Kenya and countries of tropical Asia including India often in isolated patches (Hooker, 1885). In recent time the species became going to be rarer in the plains of Bengal. The seedling phenology of *E. ligustrinum*, which was hitherto unknown, has been studied in detail.

MATERIALS AND METHODS

The phenological changes in seedlings of *Ecbolium ligustrinum* were primarily studied from specimens growing in three wild habitats viz. Chandannagar in Hooghly District; Vidysagar University campus, Midnapore in Paschim Midnapur District and Monteswar village of Burdwan District, West Bengal. Further details for the day-to-day developmental changes that take place from the day after radicle emergence (DARE) were recorded from naturally grown seedlings in the research plot of the Botany and Forestry Department, Vidysagar University campus, Midnapore. Observations were based on a total of 240 seedlings for three consecutive years (2013-2015).

Individual seedlings were numbered and tagged properly for detail observational records day-to-day regarding their morphological changes. Architectural details of the seedlings, specifically the leaves have been described as per the terminologies of Hickey (1973), Vogel (1980) and LAWG (1999). Illustrations of important stages were drawn (Figure 1. A-D) and photographs were taken using a Nikon D 5000 Digital Camera (Japan).

RESULTS AND DISCUSSION

Ecobolim ligustrinum produced one to four-seeded dry, leathery, loculicidal, glabrous, anterior-posteriorly flattened more or less ovate capsule of 17-21mm × 6-9mm in diameter. Individual seeds were broadly ovate, acute, unequally cordate, compressed and 6-9mm × 5-6mm in size. A fully imbibed seed after scarification in water takes 7-8 days for inception of germination, when the radicle is seen to just emerge out of the seed coat (Figure 2A). The germination was epigeal type. The hypocotyl exhibited quite rapid growth, leads to the gradual lifting of the cotyledons above the ground level and the cotyledons are still remaining within the seed coat. The hypocotyl is quite thickened, creamish white in colour, glabrous in appearance and form a bending structure. Radical is whitish with somewhat cottonish look.

After an interval of 3-4 days since inception (i.e. Day of radicle emergence) when the hypocotyl attains a height of ± 4mm and the embryonic root grows upto a length of ± 6mm, the greenish cotyledons (± 3×1mm) were started to come out from seed coat through the lateral side of the seed towards the micropylar end. The hypocotyl was greenish in colour and forms a prominent hook like curvature (Figure 2B). At 7-8 days after radical emergence (DARE), 60% of the cotyledones (± 4×5 mm, at that time) were came out from the seed coat and two cotyledons individually also started to separate from each other and form a loop-like configuration. At that time the hypocotyl reached up to ± 9mm in length and the embryonic root assumes a length of ± 12mm with four to six sub-branches (Figure 2C). At 10-11 DARE, the hypocotyls (± 15 mm) became almost straight and lifted the cotyledons from ground level up to ± 20 mm, and till the seed coat remain attached at the tip of the green cotyledons (Figure 2D). At 13-14 DARE the cotyledons are completely came out from seed coat and the seed coats completely shedded off from the germinating seeds. As the cotyledons were come out from the seed coat

during germination, the seedling is to be regarded as a phanerocotylar type. Again, the cotyledons were fleshy, green in colour and photosynthetic in nature. Hence, the structure was a paracotyledon. At this stage the cotyledons are ± 9 × 7 mm in size, hypocotyls nearly 20 mm in length and the main root reached up to 15-20mm in length with five to seven sub-branches. The plumule was recognizable as a minute projection (± 1mm) at the junction of the cotyledons (Figure 2E). Emergence of plumular bud marked the completion of the germination phase and the onset of seedling phase.

At 16-17 DARE, when the hypocotyl attained a length of ± 24 mm, formation of the first internode takes place by differentiation of the second node marked by the emergence of the first pair of foliage leaves as a minute projection (± 3mm) (Figures 1A, 2F) and tap root (primary root) was increased in size up to ± 30 mm in length with eight to ten sub-branches (secondary roots).

At 22-23 DARE, each of the first pair of juvenile foliage leaves reached ± 20 × 10 mm. Leaves were arranged in opposite-decussate pattern, light green in colour, differentiated into short petiole (<4mm), ovate lamina with slightly dentate margins, obtuse-acute base, acute apices, surface glabrous, semi-caspedodromous venation (3-4 lateral veins on each side arranged alternately). In the axils of this first pair of foliage leaves the shoot apex was recognizable as a minute projection of ± 3 mm in length. The paracotyledons reached to its maximum size, ± 22 × 18 mm, though hypocotyl, epicotyl, foliage leaves as well as total seedling irrespective of its shoot and root continued to elongate (Figure 2G).

At 25-26 DARE, the vegetative bud from the axil of the first pair of foliage leaves assumed the shape of a pair of miniature foliage leaves (light green in colour) and started to separate from each other. The third nodes became apparent through the exposure of a second pair of foliage leaves as small projections. At 28-29 DARE, the second pair of foliage leaves reached ± 25 × 12 mm and exhibited the similar architecture as described earlier for the first pair of foliage leaves (Figure 2H). This second pair of foliage leaves arranged oppositely in decussate manner with the first pair. The first internode between the two (1st and 2nd) pairs of foliage leaves assumed a length of ± 2 mm. The hypocotyl reached to its maximum length i.e. ± 27 mm and epicotyls (± 15 mm) till continued to elongate. At this stage the first pair of foliage leaves

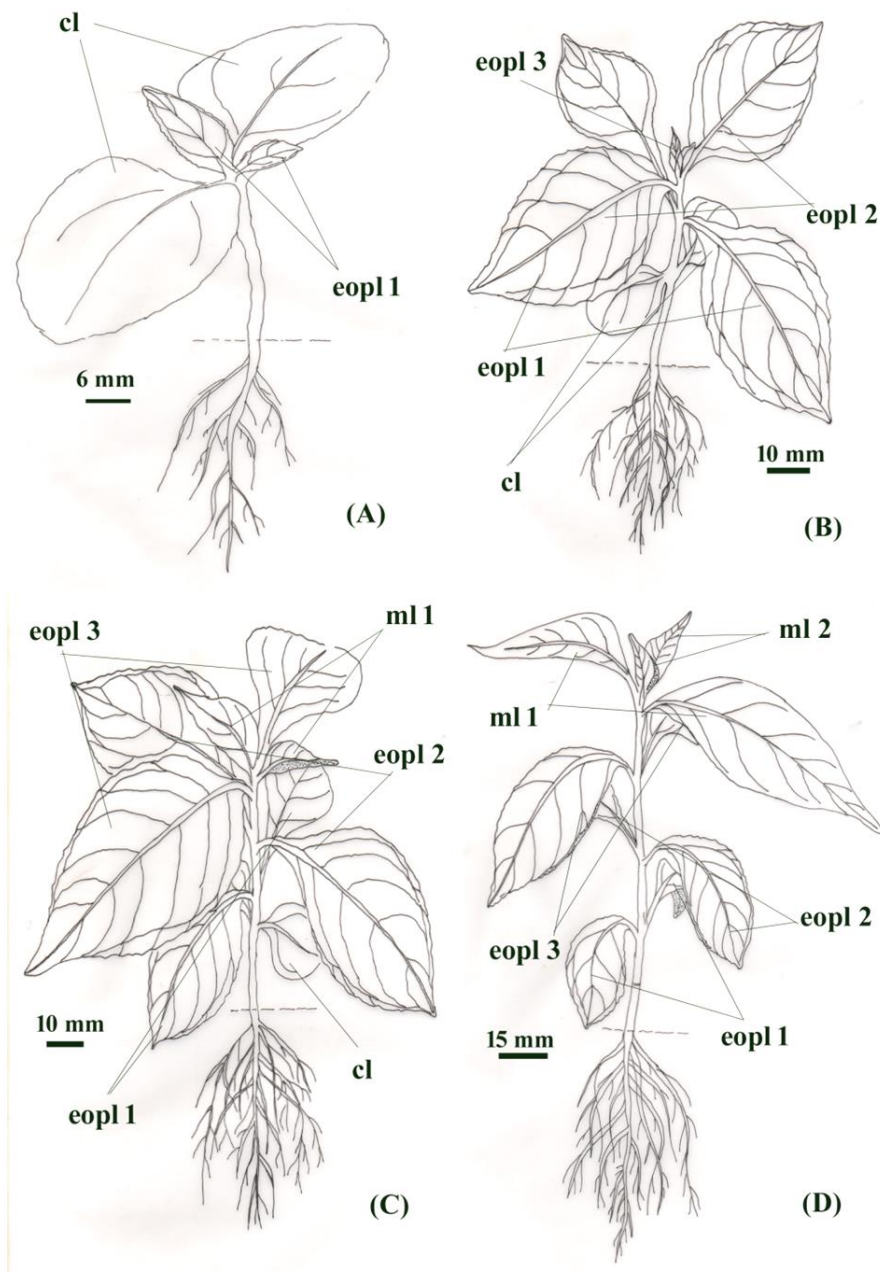


Figure 1. Line drawings of the major stages of the seedling of *Ecbolium ligustrinum*. (A) Seedling showing the first pair of eophylls with ovate lamina, slightly dentate margins, and acute apices, with a pair of fleshy green, photosynthetic phanerocotyledons. (B) Seedling showing the opposite decussate disposition of the three pairs of eophylls with semi-caspedodromous venation. (C) Seedling exhibiting the first pair of mature leaves with lanceolate lamina, entire margins, acuminate apices and unicostate reticulate venation. (D) Seedling showing the first and second pair of mature leaves with abscised phanerocotyledons. (cl, cotyledon; eopl 1 to 3, first to third pair of eophylls; ml 1, 2, first and second pair of mature leaves).

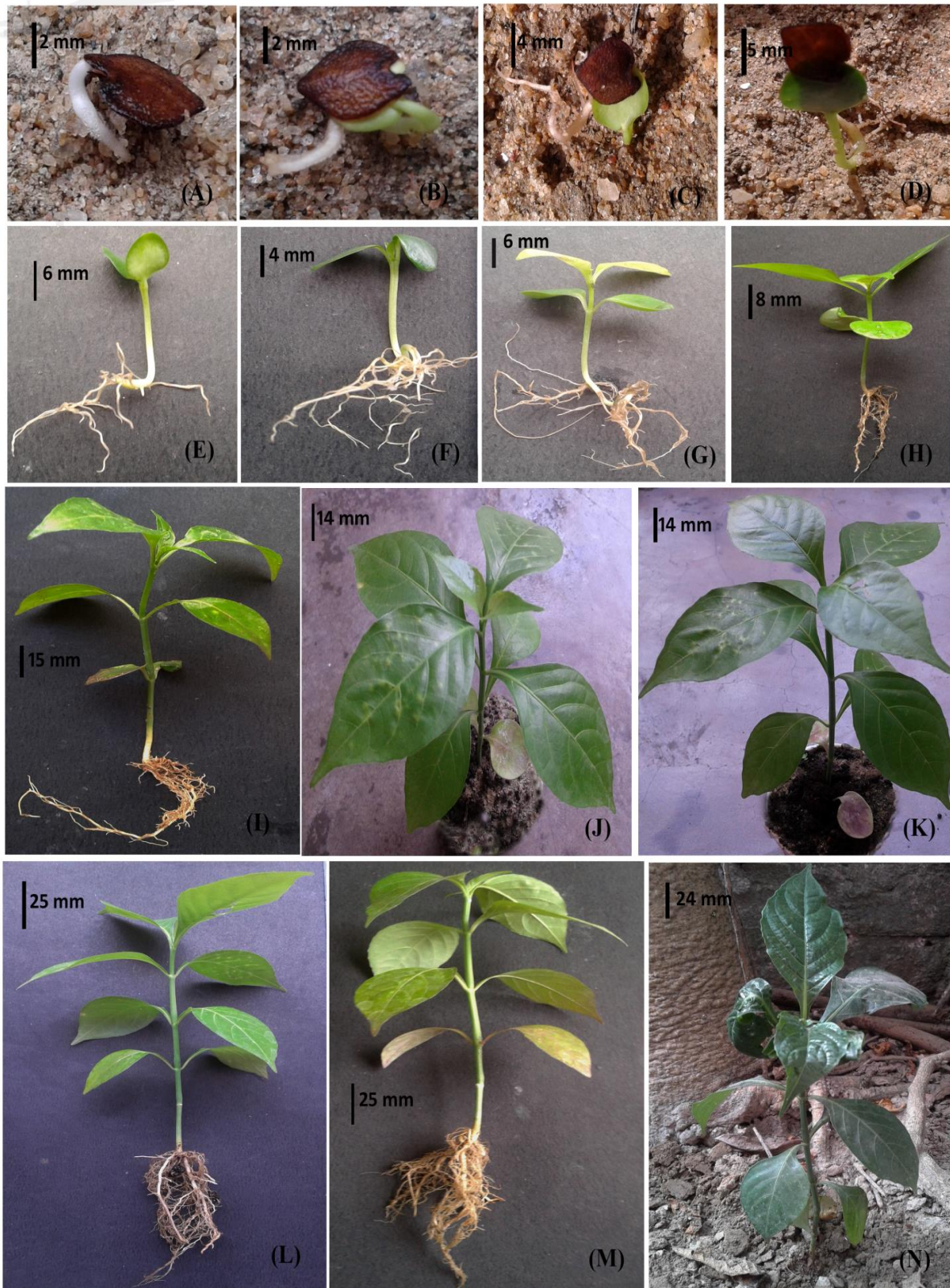


Figure 2. Photographs of the major events of seedling phenology of *Ecbolium ligustrinum* through the 'days after radical emergence' [DARE]. (A) Seed soon after germination, showing the growing radical. (B) DARE 3-4, Paracotyledons just started to come out. (C) DARE 7-8, Major portion of paracotyledons come out. (D)

DARE 10-11, Seed showing typical hypogeal germination. (E) DARE 13-14, Young seedling, plumule is seen as a minute projection. (F) DARE 16-17, Emergence of first pair of eophylls. (G) DARE 22-23, Paracotyledons attain its maximum size. (H) DARE 28-29, Seedling showing differentiation of second pair of eophylls. (I) DARE 36-37, Seedling with the emergence of third pair of eophylls. (J) DARE 49-50, Seedling showing the emergence of fourth pair of foliage leaves (First typical adult leaves). (K) DARE 61-62, Seedling showing the abscission of one of the paracotyledons with the exposure of three pairs of eophylls and a single pair of typical adult foliage leaves. (L) DARE 67-68, Remaining paracotyledon is abscised and differentiation of sixth node by the emergence of miniature fifth pair of foliage leaf bud. (M) DARE 84-90, A seedling exhibiting the emergence of sixth pair of foliage leaves in a condition of abscised of first pair of foliage leaves (First pair of eophylls). (N) DARE 105-110, A seedling showing the emergence of seventh pair of foliage leaves, in which seedling bares two pairs of remaining eophylls and four pairs of typical adult foliage leaves.

attained a size of $\pm 48 \times 23$ mm, and tap root in length of ± 36 mm with ten to twelve secondary roots. At 36-37 DARE, the shoot attained a height of 70-75 mm with simultaneous enlargement of the foliage leaves. At this stage the emergence of third pair of foliage leaves took place, in opposite decussate disposition with those of the second pair (Figures 1B, 2I). Architecturally, the third pair of foliage leaves was intermediate type between juvenile leaves (1st and 2nd pair of foliage leaves) and adult leaves (4th pair of foliage leaves and so on). The third pair of foliage leaves was green in colour, nearly ovate lamina with slightly dentate margins, quite elongated obtuse-acute base, acute to sub-acuminate apex, surface glabrous and semi-caspedodromous venation with 6-7 lateral veins in each side, arranged alternately. The epicotyl reached to its maximum length which is ± 20 mm; first and second internodes were about ± 15 mm and ± 4 mm respectively. The cotyledons started to senescence, distal part of the structure imparts a yellowish-brown necrotic look. The tap root system comprises the primary root in length of ± 60 mm with twelve to fifteen secondary roots and profuse tertiary and quaternary branches.

At 49-50 DARE, the fifth node was formed by the emergence of the fourth pair of foliage leaves (Figures 1C, 2J). The shoot and root of the seedling elongated further with simultaneous enlargement of leaves, differentiation of opposite-decussate leaves at successive nodes continued. At 61-62 DARE, one of the two paracotyledons abscised from the cotyledonary node. At this stage the shoot attained a height of 100-105 mm with simultaneous enlargement of fourth pair of foliage leaves having a size of $\pm 56 \times 27$ mm with the height of 2nd, 3rd and 4th internodes are ± 25 mm, ± 25 mm and ± 5 mm respectively (Figure 1k).

A striking phenomenon occurred with the formation of this fourth pair of foliage leaves. These leaves were

unlike to those of previous nodes, with devoid of dentations along their margins. A leaf of the fifth node (fourth pair foliage leaf) at its early stage of development differs from those of the adult plant by its nearly ovate shape, and sub-acuminate apex, but through further growth it became lanceolate with an acuminate apex, uncostate-pinnately reticulate venation, and thereby architecturally approaches those of the mature plant. However, the first three pairs (1st, 2nd and 3rd) of foliage leaves produced earlier on the second to fourth nodes maintained their ovate shape, slightly dentate margins and acute apices throughout their lives until abscission. As these initially produced three pairs of foliage leaves were strikingly differ in size and form from the typical foliage leaves of adult plants, those to be regarded as eophylls.

At 67-68 DARE, the sixth node is differentiated with the emergence of fifth pair of foliage leaves. The remaining paracotyledon abscised from the cotyledonary node. At this stage the shoot length reached in 125-130 mm and tap root was about 70-75 mm with profuse numbers of secondary, tertiary and quaternary branches (Figures 1D, 2L).

At 84-90 DARE, the seventh node is differentiated by the emergence of the sixth pair of foliage leaves, associated with the abscission of first pair of foliage leaves. The shoot attained a height of 160-165 mm and tap root is 80-85 mm in length with profuse branching, formed a bushy appearance. The fourth and fifth pair of foliage leaves became $\pm 80 \times 34$ mm and $\pm 82 \times 32$ mm in size respectively at this stage. The height of the 5th and 6th internodes were ± 26 mm and ± 04 mm accordingly (Figure 1M).

From 105-110 DARE, with the formation of the eighth node, seventh pair of foliage leaves emerged attaining a shoot height of 182-195 mm. Foliage leaves emerged from sixth node onwards is lanceolate with acuminate apex and entire to slightly irregular margins (Figure 1N). At 154-168 DARE,

with the formation of 11th to 12th nodes and by the abscission of the third pair of eophylls from the fourth node, the plantlet appeared as a miniature of an adult plant. This actually denoted the completion of the seedling phase and onset of adulthood. Seedling phenology of the species *E. ligustrinum* was very much distinctive from its adult one exhibiting thick, fleshy, greenish paracotyledons. Paracotyledons were foliaceous, several times thicker than the adult foliage leaves and truly isocotylar in nature. These cotyledonary leaves were cordate in shape with entire margins, surface glabrous, moderate sized ($\pm 22 \times 18$ mm) and retained for long time nearly ± 61 -68 DARE. These features clearly ascribed that the paracotyledons played a crucial role for the survival of the seedlings until becoming dependent on photosynthesis. The species showed three pairs of eophylls and were morphologically distinguishable from true foliage leaves by their ovate lamina with slightly dentate margins, obtuse-acute base and acute apices and semi-caspedodromous venation. Such observations clearly believed the heteroblastic development of seedling species. According to Mundhra and Paria (2009) the phanerocotylar epigeal germination was generally considered as an advanced characteristic feature which was again exhibited by this plant species. Phanerocotylar epigeal seedlings generally grow faster than seedlings with other types when they were exposed to increased light (Popma and Bongers, 1988). Species with EPF seedlings exhibit a wide array of morphophysiological features to survive with distinct understory light system (Kitajima, 1992). In spite of its association with gap-dependent species (Martínez-Ramos and Samper, 1998), EPF seedlings like *E. ligustrinum* also establish and grow in the shaded understory as in some taxa of Melastomataceae, Moraceae, Rubiaceae and Lamiaceae (Ellison *et al.*, 1993; Metcalfe and Grubb, 1995; Rani and Datta, 2017). So, the present study on seedling phenology not only provide the strategies regarding conservation, forestry research, weed interactions, reintroduction of plants in natural habitats i.e. management of phytodiversity but also indicate the advanced adaptive nature of the species.

CONCLUSION

Thus, in *Ecbolium ligustrinum* the cotyledons were seen to come out during 3-4 days since inception and during 13-14 DARE emergence of plumule was recognized which is again marked as the completion of germination phase and onset of seedling phase.

The seedling phase is completed by 154-168 DARE with the formation of 12th node with 11th pair of foliage leaves and ± 195 mm shoot height. Though the epigeal phanerocotylar with foliaceous cotyledons (EPF) type of seedling shown by the species is quite common among dicotyledons, however, three pairs of eophylls and morphologically distinguishable leaves from true foliage leaves by their ovate lamina with slightly dentate margins, acute apices and semi-caspedodromous venation depicted as the unique seedling morphotype of the plant species.

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