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## Phenological Study of Some Herbaceous Species of *Euphorbia* L. (Euphorbiaceae L.) in Nigeria

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**ABSTRACT:** The phenological study provides a detailed comparison of life cycles of six species of *Euphorbia* with a view to enhancing the knowledge of the taxonomic status of the genus. Growth and developmental stages were investigated using pot experiments. Description of six principal growth stages was done and these include leaf development, shoot development, flowering emergence, fruit development, seed ripening and seed dispersal. All the investigations were carried out for 77 days under the same experimental conditions. Germination of all species started 24 hours after sowing indicating that their seeds have physiological capacity to readily germinate owing to their absence of seed dormancy. The life cycles from seed germination to seed dispersal are determined to span between 72-73 days in *E. graminea*, 62-65 days in *E. heterophylla*, 66-68 days in *E. hyssopifolia*, 62-63 days in *E. hirta*, 46-48 days in *E. prostrata* and 70-72 days in *E. thymifolia*. All data were recorded and thereafter subjected to appropriate statistical analysis. Variations are observed in the germination behaviour, seed output and reproductive capacity among the species. Flowering, fruiting and seed production dropped because of the presence of early cyathium abortion which is characterised by enlarged ovaries without seed development, it was high in *E. hirta* and low in *E. thymifolia*. A new identification key to the *Euphorbia* species is included.

**Keywords:** Phenological, *Euphorbia*, taxonomic status, variation

### Introduction

The type of genus of Euphorbiaceae is *Euphorbia* L. which comprises about 2000 species of herbaceous annuals, although some are shrubs or even trees but rarely woody climbers (Olorode, 2012) is rated as the third largest genus of angiosperms after *Astragalus* Linn. (Fabaceae) and *Psychotria* Linn. (Rubiaceae) (Govaerts et al. 2000). Euphorbiaceae, is a large and easily recognized cosmopolitan family with world-wide distribution. It belongs to the sub-class Euphorbidae in the order Euphorbiales, tribe Euphorbieae, and sub-tribe Euphorbiinae (Webster, 1975). It is known commonly as the spurge family which has 300 genera, 52 tribes in five sub-families and over 8,000 recognized species (Webster, 1975; Wurdack et al., 2004). The larger genera in the family include *Euphorbia*, 2000 species; *Croton* Linn. are about 700 species; *Phyllanthus* Linn. consists of 500 species; *Acalypha* Linn. up to 430 species; *Jatropha* Linn. up to 175 species, *Manihot* Mill. 170 species (Judd et al., 1999; Aworinde et al., 2009); *Glochidion* J.R. Forst. & G. Forst. about 300 species, *Macaranga* Thouras (250 species); *Antidesma* consist of 150 species and *Tragia* Linn. 150 species. The five sub-families of Euphorbiaceae include Acalyphoideae (e.g. *Acalypha* Linn. , *Alchornea* SW., *Tragia* Linn., *Ricinus* Linn. and

relatives), Crotonoideae (*Croton*, *Manihot*, *Jatropha*, *Codiaeum* A. Juss., *Aleurites* J.R. Forst. & G. Forst., *Cnidoscopus* Pohl. etc), Euphorbiodeae (*Hippomane* Linn., *Hura* Linn., *Euphorbia*, *Gymnanthes* S.W., *Stillingia* Garden ex. L., *Sapium* Jacq. etc), Phyllanthoideae, Oldfieldioideae. The Acalyphoideae, Crotonoideae and Euphorbioidae are recognised as uni-ovulate sub-families while the bi-ovulate ones are Phyllanthoideae and Oldfieldioideae (Takhtajan, 2009). Milky latex is typical in the sub-families Euphorbiodeae and Crotonoideae. The milky latex is poisonous in the Euphorbiodeae whereas it is innocuous in the Crotonoideae (Charles *et al.*, 2007). Euphorbiaceae is readily distinguished by the highly characteristic trilocular fruits ovary of three carpels maturing into a schizocarpic capsule splitting septicidally separate into separate carpels; female flowers are hypogynous i.e. with superior ovaries usually unisexual, with the male and female occurring on the same inflorescence, monoecious or dioecious, androecium may number from one to numerous. The fruit is usually a schizocarp but sometimes a drupe: the seeds eject explosively. The leaves are alternate, rarely opposite with stipules. Stipules may be in form of hairs, glands, spines or may be absent. The leaves are largely simple but where compound, they are palmate and not pinnate (Heywood, 1978; Judd *et al.*, 2000; Takhtajan, 2009). Majority of Euphorbiaceae, spurge family are herbaceous although some are shrubs or even trees but rarely woody climbers. Many plants in the spurge family are of considerable economic importance as weeds, ornamentals, medicinal plants and green vegetables (Olorode, 2012). The family occurs throughout tropical and temperate regions with greatest diversity in Indo-Malaya and tropical America (Charles *et al.*, 2007).

The morphological, micromorphological, and anatomical features of *Euphorbia* has been studied by researchers like Charles *et al.* (2007); Pahlevani *et al.* (2020); Sanusi *et al.* (2023); Althobaiti (2023); Rabizadeh and Nasiri (2023); Sanusi *et al.* (2023). The significance of phenological observations has been variously emphasized in a number of studies by many workers such as Levin (2006); Park *et al.* (2018); Valdez-Torres *et al.* (2012); Mackenzie *et al.* (2020); Park *et al.* (2021); Salazar-Villa *et al.* (2022); Twining *et al.* (2022) and Vázquez *et al.*, (2023). Based on their comprehensive studies, phenology is reflected by a set of events which include leaf development, shoot development, flowering emergence, fruit development, seed ripening and seed dispersal. According to these authors, phenological study reveals the pattern of growth and development as well as the effects of environmental factors on flowering and fruiting behaviours of plant taxa. Patterns of phenological events are variously used for characterization of the six selected species and evaluation of characters not ordinarily accessible to herbarium taxonomists was addressed in this study. This study therefore aims at analyzing the phenological characters for easy identification and delimitation and also indicating relationships among species.

## Materials and methods

The fresh specimens (of *E. graminea* Jacq., *E. heterophylla* Linn., *E. hirta* Linn., *E. prostrata* Ait., *E. hyssopifolia* Linn. and *E. thymifolia* Linn.) were collected from South Western part of Nigeria and were pressed with the aid of plant press. Identification and authentication were done appropriately at Forestry Research Institute of Nigeria (FRI) and the specimens were deposited both at FRI, Elikaf Herbarium of Olabisi Onabanjo University, Ago-Iwoye, Ogun State and Obafemi Awolowo University, Ile-Ife herbarium (IFE). The species used for this study are Matured capsules of each species studied from different populations were harvested and stored in paper envelopes under ambient temperature. Dried seeds for each species of *Euphorbia* were planted separately in plastic bowls. Planted seeds were checked every day for seedling emergence.

Records of the different growth stages were taken, these include leaf development, shoot development, flower emergence, fruit development, seed ripening and seed dispersal. Seedling growth until flowering and seed shed in the adult plant were monitored daily. Measurements were taken with a metric ruler and a measuring tape. The mean values and standard errors were also calculated for each variable. Significant stages were photographed using Infinix Hot 8 lite phone camera. These photographs were useful for identification and differentiation of morphological features. The description of juvenile plant character as well as floral and fruit characters followed that of Ramakrishnan (1965); Gomez and Espadaler (1997); Narbona *et al.* (2007); Aigbokhan and Ekutu (2012); Faboyede (2015).

## Results

*Phenology of Euphorbia graminea* Jacq.: The germination of seeds started 24 hours after planting and seedlings with two cotyledons are raised above the soil 6 – 9 days after planting (DAP). The first two leaves are sub-circular and green in colour. The next pair of leaves emerged 10 – 15 DAP. These four leaves are elliptic in

shape. First true leaves which are ovate are developed 17 – 20 DAP. The series of leaf development and plant growth continued gradually until an optimal assemblage of leaves and maximum shoot height have been achieved. Finally, at 52 DAP, 20 pair of leaves have been produced and shoot height of 22.6cm has been attained. After 52 DAP, the leaves started falling off and leaf size decreases gradually. Mean of leaf size,  $30.12 \pm 2.11$  mm  $\times$   $20.72 \pm 1.60$  mm, shoot height  $139.63 \pm 11.37$  mm high. Flowering started between 53-57 DAP and continues until 70 DAP. Fruiting commenced between 62-66 DAP and continues for about 72 DAP. Flower production drops at 70 DAP and seed dispersal started 72 DAP. Flowers up to 24 were produced but only 15 matured into fruit. The life cycle spanned between 72-73 DAP from the period of seed germination to the period of seed dispersal.

*Phenology of Euphorbia heterophylla* Linn.: Germination occurs, usually 24 hours after planting. The first two leaves emerged above the ground level 5 days after planting (DAP), colour green or reddish-green, shape elliptic. The four leaves developed 7 – 8 DAP and they are ovate. The 6-leaf (or true leaves), usually green, obovate to lanceolate developed 11-15 DAP. The sequence of leaf development and plant growth continued progressively until maximum aggregation of leaves have been produced and highest shoot height has been reached. At 58 DAP, 19 pairs of leaves have been developed and shoot height is 40.50 cm high. After 58 DAP, the leaves started shedding off and leaf size decreases. The size of leaf is  $52.58 \pm 3.39$  mm  $\times$   $23.12 \pm 1.34$  mm and shoot are  $314.83 \pm 38.51$  mm high respectively. Flowering commenced between 25-27 DAP and continues till 51 DAP. Fruit production starts between 35-37 DAP and ended 62 DAP. Out of 26 flowers produced, only 15 developed into mature fruits. After 51 DAP, the number of flowers decrease and seed dispersal commenced after 62 DAP. The life span of *E. heterophylla* is between 62-65 DAP from the period of seed germination to the period of seed dispersal.

*Phenology of Euphorbia hyssopifolia* Linn: Germination of seeds occurs immediately after planting and germinated seedlings of 2- leaf (or first two leaves) emerged 7-9 days after planting (DAP). The 2- leaf is green in colour, oblong shape. The four leaves developed 10-11 DAP, colour green: shape oblanceovate. The 6- leaf (or six leaves) emerged 12-15 DAP; they are the first true leaves; colour green; shape obliquely obovate-oblong or oblong. The series of leaf production continued sequentially until an optimal assemblage of leaves have been formed. Plant height increased gradually with age till highest shoot height was attained. At 66 DAP, 61 pairs of leaves have been produced and plant height is 60.10 cm high. After 66 DAP, the leaves started falling off and leaf size decreases. Leaf size  $27.15 \pm 1.97$  mm  $\times$   $10.31 \pm 0.88$  mm; shoot height  $337.81 \pm 45.58$  mm high. Flowering commenced between 28-30 DAP and continues for about 66 DAP. After 66 DAP, flowering production stopped. Out of total 89 number of flowers formed, only 50 matured into fruits. Fruiting commenced between 35-37 DAP and continually till 66 DAP, at this point seed started ejecting. Days sent to complete life cycle spanned between 66-68 DAP.

*Phenology of Euphorbia hirta* Linn.: Germination of seeds commenced 24 hours after planting. Germinated seedlings of two cotyledonous leaves (or two leaves) emerged 7-9 DAP; usually rounded-oblong in shape; colour dark green blotched with purple. The second pair of leaves (or four leaves) later developed forming the 4-leaf after 10-11 DAP, colour dark green blotched with purple shape obovate. The true shape, lanceolate-oblong, developed fully at 6- leaf stage between 12-15 DAP. The sequence of leaf development and plant growth proceed until maximum assemblage of leaves and highest shoot height have been reached. After 58 DAP, 12 pair of leaves have been developed and plant height of 290 mm has been attained. At this stage, leaves started shedding off and leaf size decreases. At the end of the experiment, mean and standard error was calculated for leaf length, leaf breadth and shoot height. Leaf size is  $29.39 \pm 2.40$  mm  $\times$   $11.49 \pm 0.94$  mm and shoot height is  $203.30 \pm 21.87$  mm. Flower started emerging between 20-25 DAP and continued till 47 DAP. Fruit started developing between 30-32 DAP and fruiting stopped at 47 DAP and seed dispersal start at this stage. Flowers up to 126 are formed but only fruits up to 92 in number were produced. Days spent to complete life cycle is between 62-63 DAP from seed germination to seed ejection.

*Phenology of Euphorbia prostrata* Aiton: Seed germination started immediately after planting and seedlings with two cotyledons emerged above ground level 3-4 DAP. The first two leaves are green or tinged purple and rounded-oblong in shape. The four leaves were developed after 6-7 DAP; colour green or tinged purple; shape obcordate. The 6- leaf (or true leaves) are developed 7-8 DAP, colour green: shape ovate. The series of leaf development and plants growth continued progressively until an optimal aggregation of leaves and highest shoot height have been reached. After 46 DAP, 91 pairs of leaves have been produced and plant height of 8.9 cm high has been attained. At this stage, leaves started falling and leaf size also reduced gradually. at the end of the experiment, mean  $\pm$  s.e. were recorded for leaf size ( $6.81 \pm 0.34$  mm  $\times$   $3.78 \pm 0.12$  mm) and shoot height is  $57.23 \pm 6.59$ . Flowering commenced 8-10 DAP and continue till 40 DAP. Fruiting started from 15-17 DAP till 40 DAP. Seed started ejecting 40 DAP. Flowers are usually 100 in total, but only 50 bear fruits. The life cycle spanned between 46-48 DAP.

*Phenology of Euphorbia thymifolia* Linn: Germination of seeds commenced readily after planting and seedlings with two primary leaves emerged 7 – 9 DAP; shape rounded-oblong, colour green or tinted red. A second pair of

leaves later developed giving rise to 4-leaf after 10-11 DAP, colour green or tinted red, shape obovate. The true leaves (6 – leaf) developed 15-17 DAP with regular shape ovate and they are similar in colour with 2– leaf and 4– leaf. The sequence of leaf development and plant growth continue progressively until optimal assemblage of leaves and maximum shoot heights have been achieved. After 56 DAP, 73 pair of leaves have been developed and shoot height of 87 mm has been reached. At this stage, leaves started falling off and leaf size decreases. At the end of the experiment, mean and standard error were calculated for leaf length, leaf breadth and shoot height. Leaf size is  $9.90 \pm 0.83$  mm  $\times$   $5.67 \pm 0.28$  mm and shoot height is  $55.75 \pm 7.87$  mm high. Flowering commenced 21-25 DAP and stopped at 70 DAP. Fruit production commenced 28-30 DAP and finished at 70 DAP. After 70 DAP, seed dispersal commenced. Out of 135 flowers produced, only 120 fruits were produced. Days spent to complete life cycle after planting 70-72 DAP.

Artificial keys for the identification of six *Euphorbia* species using phenological characters:

- 1a Primary leaves rounded-oblong or elliptic ..... (2)
- 1b Primary leaves sub-orbicular ..... *E. graminea*
- 2a Days of seedling emergence 7-9 DAP; second pair of leaves obovate or oblanceovate or orbicordate ..... (3)
- 2b Days of seedling emergence 5 DAP; second pair of leaves elliptic..... *E. heterophylla*
- 3a Matured ovary peduncle elongate upward or slightly bent up to 1-2 cm in length ... (4)
- 3b Matured ovary peduncle elongation not visible ..... *E. thymifolia*
- 4a Plant height very tall ..... *E. hyssopifolia*
- 4b Plant height moderately tall to tall ..... (5)
- 5a Fruiting and seed production start 30-32 DAP and end 47 DAP; flower emergence 20-25 DAP till 47 DAP; total number of fruits 92; seed dispersal 47 DAP; Life cycle 66-68 DAP ..... *E. hirta*
- 5b Fruiting and seed production start 15-17 DAP and end 40 DAP; flower emergence 8-10 DAP; total number of fruits 50; seed dispersal 40 DAP; life cycle 46-48 DAP ..... *E. prostrata*

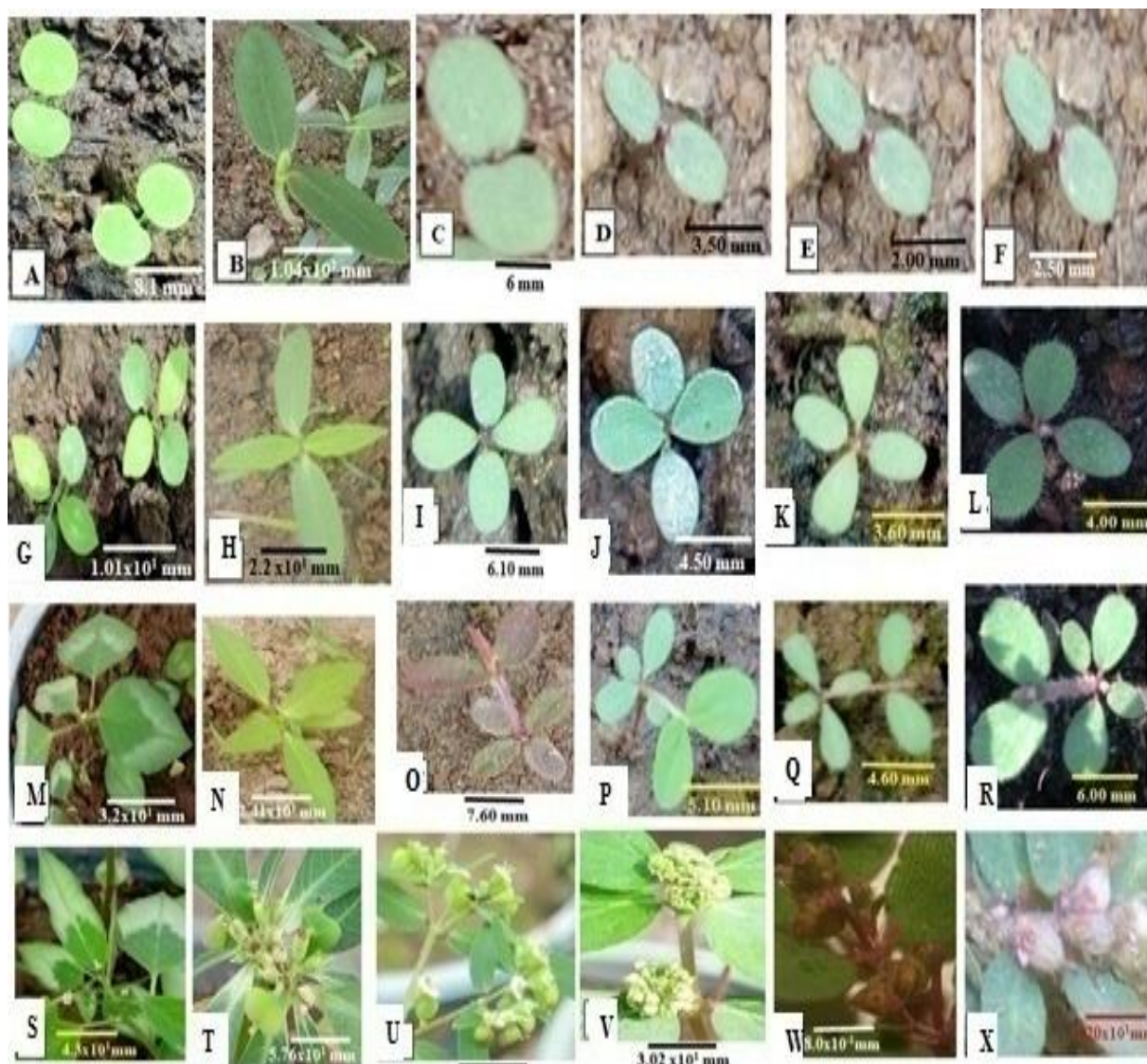
**Table 1:** Phenological characteristics of the species of *Euphorbia* examined

Species	<i>E. graminea</i>	<i>E. heterophylla</i>	<i>E. hyssopifolia</i>	<i>E. hirta</i>	<i>E. prostrata</i>	<i>E. thymifolia</i>
Days of seedling emergence (DAP)	6 – 9	5	7 – 9	7 – 9	3 – 4	7 – 9
Days of 4-leaf emergence (DAP)	10 – 15	7 – 8	10 – 11	10 – 11	6 – 7	10 – 11
Days of 6-leaf emergence (DAP)	17 – 20	11 – 15	12 – 15	12 – 15	7 – 8	15 – 17
Days to flower initiation after planting (DAP)	53 – 57	25 – 27	28 – 30	20 – 25	8 – 10	20-25
Days to fruit initiation after planting (DAP)	62 – 66	35 – 37	35 – 37	30 – 32	15 – 17	28 – 30
Shape of 2-leaf	Sub-circular	Elliptic	Rounded-oblong	Rounded-oblong	Rounded-oblong	Rounded-oblong
Shape of 4-leaf	Elliptic	Ovate	Oblance-ovate	Obovate	Obcordate	Obovate
Shape of 6-leaf	Ovate	Ovate	Lanceolate-oblong	Lanceolate-oblong	Ovate	Ovate
Leaf length (mm)	30.12±2.11	52.58±3.39	27.15±1.97	29.39±2.40	6.81±0.34	9.90±0.0
Leaf breadth (mm)	20.72±1.60	23.12±1.34	10.31±0.88	11.49±0.94	3.78±0.12	5.67±0.0
Plant height (mm)	139.63±11.37	314.83±38.51	337.81±45.58	203.30±21.87	57.23±6.59	55.75±0.0
ONo of leaves (pairs)	20	19	61	12	91	73
No of flower	24	26	89	126	100	135
No of fruit	15	15	50	92	50	120
No of aborted fruits	5	6	10	33	6	4
Days of seed dispersal (DAP)	72	62	66	47	40	70
Days spent to complete life cycle	72 – 73	62 – 65	66 – 68	62 – 63	46 – 48	66 – 68

Measurements represent mean ±standard error

All units in mm

DAP ⇒ Days after planting



**Plate 1:** Synthetic principal growth stages of *Euphorbia* species

A, B, C, D, E and F – Seedling at 2-leaf of *E. graminea*, *E. heterophylla*, *E. hyssopifolia*, *E. hirta*, *E. prostrata*; and *E. thymifolia* respectively

G, H, I, J, K and L – Seedling at 4-leaf of *E. graminea*, *E. heterophylla*, *E. hyssopifolia*, *E. hirta*, *E. prostrata*; and *E. thymifolia* respectively

M, N, O, P, Q and R – Seedling at 4-leaf of *E. graminea*, *E. heterophylla*, *E. hyssopifolia*, *E. hirta*, *E. prostrata*; and *E. thymifolia* respectively

S, T, U, V, W and X – flowering and fruiting initiation stages of *E. graminea*, *E. heterophylla*, *E. hyssopifolia*, *E. hirta*, *E. prostrata*; and *E. thymifolia* respectively

## Discussion

The data presented here provide detailed comparison of the life cycles of six species of *Euphorbia*. A detailed description of six principal *Euphorbia* stages was done. These include leaf development, shoot development, flowering emergence, fruit development, seed ripening and seed dispersal. All the investigations were carried out 77 days under the same experimental conditions. The life cycle from seed germination to seed dispersal in *E. graminea* spanned between 72-73 DAP, 62-65 DAP in *E. heterophylla*, 66-68 DAP in *E. hyssopifolia*, 62-63 DAP in *E. hirta*, 46-48 DAP in *E. prostrata* and in *E. thymifolia*, it is 70-72 DAP. The life cycle was completed when the leaves started drying up and falling off. The period of life span of *E. graminea* is higher while *E. prostrata* has a shorter life span. All the studied species are perennials except for *E. heterophylla* and *E. prostrata* which show annual growing habit. Germination of all species started 24 hours after sowing indicating

that seeds have physiological capacity to readily germinate owing to their absence of seed dormancy. Similar observations have been made from seeds of *E. caducifolia*, *E. characias*, *E. nicaeensis*, *E. thymifolia* and *E. graminea* (Ramakrishnan, 1965; Gomez and Espadaler, 1997; Narbona *et al.*, 2007; Aigbokhan and Ekutu, 2012; Faboyede, 2015). The *Euphorbia* selected presented a range of leaf morphologies from broad flattened to small composite leaves. In these samples of species, *E. heterophylla* exhibits the greatest leaf size in both leaf length and breadth whereas *E. thymifolia* have smallest leaf size. In contrast, *E. thymifolia* display the highest number of leaves while the least number of leaves was obtained from *E. graminea* followed by *E. heterophylla*. Consequently, leaf production could have been counteracted by leaf size (Narbona *et al.*, 2007). Differences have also been observed in seed germination, seed production and reproductive capacity. The observed variation in vegetative plant growth amongst the examined species revealed that *E. hyssopifolia* gave the best mean plant height, followed by *E. heterophylla* whereas *E. thymifolia* shows the least plant height. Fluctuations that involve increase and decrease in plant growth and fruit production were observed in all the species throughout the experiment. The fluctuations can be partly explained by the climatic conditions during the cycle. Decreases in plant height and fruit production are common during period of lower temperature and lower rainfall but if plants are subjected to higher temperature and higher rainfall, plant growth and fruit production increases. The effects of climatic conditions, such as light on the germination of seed of *Euphorbia thymifolia* has been previous described (Ramakrishnan, 1965). For reproductive development, the stages of flower emergence, fruit development, fruit ripening with colour change and seed dispersal display an interesting variations among the examined species. During flowering, protogyny was observed with maturation of female flower before the male flower. Flower presented a single female which is surrounded by many staminate flowers within a cuplike structure called cyathium. At fruit maturation, the fruit colour changes and the peduncle of matured fruit appear as a protuberance from the involucre. In *E. graminea*, the peduncle of matured ovary elongates in the form of a bend whereas in *E. heterophylla*, *E. hyssopifolia*, *E. prostrata*, they are straight or slightly bend in *E. thymifolia*. The matured ovaries are able to eject their seeds, shooting them several centimeters in all the species studied, dispersal occurs by an explosive mechanism of the capsule. The seeds have capacity to germinate readily immediately after dispersal from the plant if the environment conditions are favourable.

Variations have been observed in the germination behavior, seed output and reproductive capacity among the six examined species. *E. hirta* and *E. thymifolia* have the highest reproductive capacity and seed output. Seed germination and seedling morphology of *E. hirta* and *E. thymifolia* are very alike. Similar observation was made by Ramakrishnan (1965) who mentioned that the seedlings of *E. thymifolia* and *E. hirta* are difficult to differentiate up to 4-leaf stage and differences are shown only in the leaves that emerged later. Although, the germination of seeds and the two primary leaves of *E. hirta*, *E. thymifolia*, *E. hyssopifolia* and *E. graminea* are similar, differences are marked at 4-leaf stage except for *E. hirta* and *E. thymifolia* that are similar up to 4-leaf stage. During flowering, fruiting and seed production dropped because of the presence of early fruit abortion which is characterized by enlarged ovaries without seed development. According to Geerts *et al.* (2002), embryo abortion might result from early nutrition barrier which prevent embryonic development. Number of fruits aborted was very high in *E. hirta* and least in *E. thymifolia*.

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