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REPRODUCTIVE BIOLOGY OF FOUR WEEDY *EUPHORBIA* SPECIES FROM ILE-IFE, NIGERIA

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ABSTRACT

Euphorbia heterophylla Linn., *Euphorbia hirta* Linn., *Euphorbia hyssopifolia* Linn. and *Euphorbia prostrata* Linn. are four noxious, yet economically important weeds, useful as phytomedicine in some parts of the world and also in Nigeria. Studies were carried out on their reproductive biology with a view to understanding the mode of their spread. Parameters investigated include number of days to seedling emergence, germination percentage, number of days to flowering, period to anthesis, pollinators, pollen viability, pollen size, floral structure, fruits type, seeds type, average number of fruit per plant, average number of seeds per plant and mode of seed dispersal. Results obtained showed that the seedlings of the four *Euphorbia* species studied emerged within 3 to 5 days; numbers of days to 50% flowering took 44 to 58 days; while flowering and fruiting continued throughout the remaining part of the year; thus exhibited r-strategy, expending more time and allocation of resources on their reproductive phases than their vegetative phases. Also differences existed in their stigma shape, perianth colour, perianth texture, pollen sizes, pollen viability as well as ripened colour of seed which were all significant characters that could be useful in delimiting the species. Furthermore, the study revealed that the four species were essentially insect pollinated and their seeds dispersed by means of explosive mechanism usually characteristic of their schizocarpic fruits. This ability as well as their tendency to exhibit r-strategy could probably account for their ability to spread and invade cultivated fields, waste lands and roadsides.

Keywords: Explosive mechanism, r-Strategy, Seed dispersal, Pollen viability, Seed set

INTRODUCTION

The genus *Euphorbia* consists of about 2000 species and it is the most diverse group of flowering plants on earth (Prenner and Rudall, 2007). There are about 30 species in West Africa out of which about 21 species are well represented in Nigeria (Hutchinson and Dalziel, 1954). Many members of the *Euphorbia* genus belong to a primary system of chromosome number with basic chromosome number of n = 8 and a secondary chromosome number of n = 6, 7, 9 and 10 resulting from aneuploidy and polyploidy (Huang *et al.*, 2012, Bolaji *et al*., 2015*).*

Euphorbia heterophylla Linn., *Euphorbia hirta* Linn., *Euphorbia hyssopifolia* Linn. and *Euphorbia prostrata* Linn. upon which the investigations in this study are based are not only noxious invasive herbaceous weedy *Euphorbia* species that grow on cultivated lands, road sides and waste lands but are also plants of medicinal importance used for treatment of various ailments in many parts of the world, including Nigeria (Herbenger, 1997; Johnson *et al.*, 1999; Falodun and Agbakwuru, 2004; Tabuti, 2008; Sandeep *et al*., 2009; Bolaji *et al*., 2019).

Although there have been reports on various aspects of the morphology, cytology and phylogenetic relationship of some of the members of the genus *Euphorbia*, there is paucity of information on many aspects of their reproductive biology. This investigation becomes necessary because it is an important step to understanding the mode of their spread. This study therefore seeks to address this knowledge gap by reporting many aspects of the reproductive biology of *E. heterophylla*, *E. hirta*, *E. hyssopifolia* and *E. prostrata* that have not been previously reported with a view to providing insightful information that could become very useful in the control of their spread.

MATERIALS AND METHODS

Whole plant collections of *Euphorbia heterophylla*, *E. hyssopifolia* and *E. prostrata* from the wild as well as cultivated forms raised from their seeds were investigated in this study. The

Euphorbia species were obtained from various locations (7°3'9"N, 4°31'34"E; 7°31'7"N, 4°31'28"E; 7°31'8"N, 4°31'35"E) within Ile-Ife, Nigeria. These were identified at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Apart from the characterization of the floral attributes of the four *Euphorbia* species being investigated in this study, other parameters pertaining to their reproductive biology which were documented include: number of days to seedling emergence, germination percentage, number of days to 50% flowering, period to anthesis, percentage pollen viability, average number of fruits per plant, average number of seeds per plant, types of pollinators, colour of ripe and unripe fruit, colour and types of seeds as well as mode of seed dispersal.

The germination study was carried out on 8 cm petri dishes laid with 7cm Whatman filter paper. Thirty (30) seeds were sown in each petri dish in four replicates. Days to emergence of radicle were documented and the percentage of seeds that germinated was also determined using the formula:

$$\text{Percentage germination} = \frac{\text{Number of seeds that germinated}}{\text{Total number of seeds sown}} \times 100$$

The details of the structure of the cyanthia of the four different species studied were investigated with the aid of the dissecting microscope; while the pollen viability study was carried out by harvesting pollen grains from freshly-dehisced anthers onto microscope slides. They were stained with Cotton-Blue-in-Lactophenol for 30 minutes and examined under the light microscope for percentage stainability following the methods of Bolaji and Nwokeocha (2013). The well-formed and deeply stained pollens were considered viable while those with collapsed outline and partially stained or not stained at all were considered to be non-fertile (Faluyi, 1985). One hundred (100) pollens were scored at × 40 magnification for percentage stainability. The pollen size was also measured using ocular micrometer.

RESULTS

The photograph of a typical *Euphorbia heterophylla* studied is shown in figure 1A. The seedlings emerged 5 days after sowing and had 11.6% germination. The number of days to 50% flowering was 48 days and it continued to bloom throughout the year. The inflorescence was cyathium (cyme); flowers bisexual with green pedicel, glabrous perianth, white in colour; ovary superior, green in colour, glabrous, 3-lobed; placentation axile, style single, hollow, green in colour; stigma reddish brown, branched into four; stamen hypogynous, epipetalous; filament green, anther sticky, brown, numerous and attached to the wall of the perianth; fruit schizocarpous, 3-lobed, unripe fruit colour light green, ripe fruit colour green; seed carunculate, unripe seed colour white, ripe seed colour brown (Figure 2). Pollinators that visited the inflorescence were *Calliphora vomitora* (blue-fly) and the mode of seed dispersal was by explosive mechanism. The anthesis took place between 9 am – 11 am in the morning. It had an average pollen size of 3.5 ± 0.04 μm, 83.49% pollen viability; while the mean number of fruit and seed per stand were 28 ± 26.74 and 70 ± 16.88 respectively (Table 1).

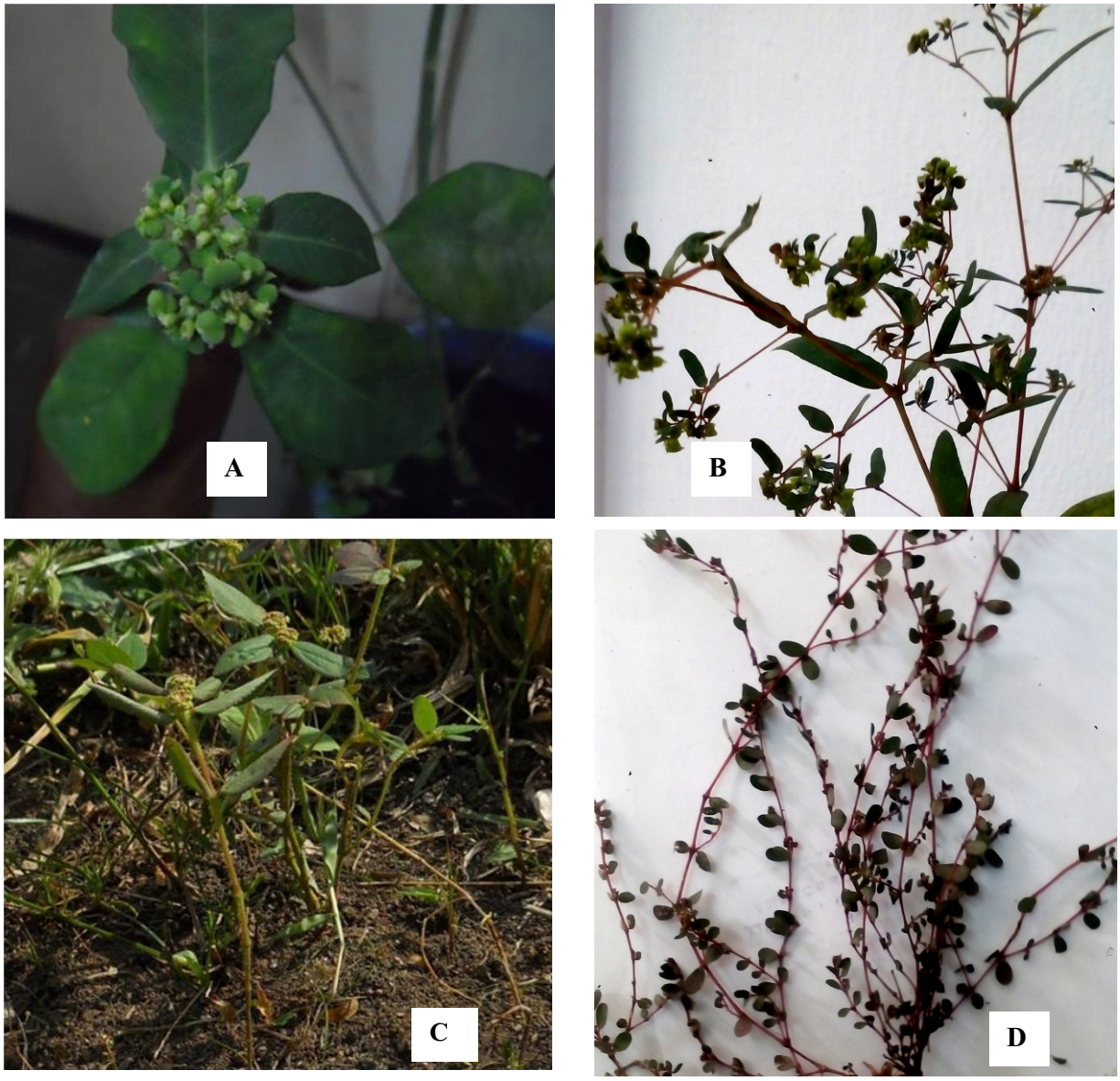

Figure 1: Habit of *Euphorbia* Species Studied

A: *Euphorbia heterophylla;* B: *Euphobia hyssopifolia;* C: *Euphorbia hirta;* D: *Euphorbia prostrata*

Table 1: Comparative Reproductive Biology of the *Euphorbia* Species Studied

Character	*Euphorbia heterophylla*	*Euphorbia hyssopifolia*	*Euphorbia hirta*	*Euphorbia prostrata*
Number of days to seedling emergence	5	5	5	3
Percentage Germination (%)	11.6	42.5	12.5	17.5
Number of days to 50% Flowering	48	47	58	44
Inflorescence type	Cyanthium (cyme), bisexual; pedicel green; perianth white and glabrous	Cyanthium (cyme), bisexual; pedicel green; perianth white and glabrous	cyanthium (cyme), bisexual; pedicel green, perianth green and pubescent	cyanthium (cyme), bisexual; pedicel green; perianth green and pubescent
Pistil	Ovary superior, glabrous, 3-lobed; placentation axile; style single, hollow; stigma reddish brown, branched into four	Ovary superior, glabrous, disc shaped, green in colour; placentation axile, style single, hollow, green; stigma branched into four	Ovary superior, glabrous, cylindrical in shape, green in colour; placentation axile; style single, hollow, green in colour; stigma fused, horse shoe shaped	Ovary superior, highly pubescent, 3-lobed, green in colour; placentation axile; style single, hollow, green in colour; stigma fused, branched into four
Stamen	Hypogynous, epipetalous; filament green; anther sticky, brown, numerous and attached to the wall of the perianth; average pollen size 3.5 ± 0.04 μm; pollen viability 83.49%	Hypogynous, epipetalous; filament green, anther sticky and brown, numerous and also attached to the wall of the perianth; average pollen size of 1.535 ± 0.07μm; pollen viability 62.78%	Hypogynous, epipetalous; filament green, anther sticky, brown, numerous and attached to the wall of the perianth; average pollen size of 1.225 ± 0.02μm; pollen viability 62.14%	hypogynous, epipetalous; filament green, anther sticky, brown, numerous and attached to the wall of the perianth; average pollen size of 1.225 ± 0.02μm; pollen viability 96.04%
Period of anthesis	9 am – 11 am	9 am – 11 am	9 am – 11 am	9 am – 11 am.
Pollinators	*Calliphora vomitora* (Blue-fly)	*Calliphora vomitora* (Blue-fly).	*Anthrenus verbasci* (Carpet bettles) and *Chortoicetes terminifera* (locust)	*Anthrenus verbasci* (Carpet bettles) and *Calliphora vomitora* (Blue-fly).
Fruits	Schizocarpous, 3-lobed, unripe fruit colour light green, ripe fruit colour green; an average of 28 ± 26.74 per plant	Scizocarpous, 3-lobed, unripe fruit colour green, ripe fruit colour lemon green; an average of 56 ± 7.69 per plant	Schizocarpous, 3-lobed, unripe fruit colour light green, ripe fruit colour green; an average of 20 ± 5.76 per plant	Schizocarpous, 3-lobed, unripe fruit colour light green, ripe fruit colour brownish green; an average of 42 ± 5.88 per plant
Seeds	Carunculate, unripe seed colour white, ripe seed colour brown; an average of 70 ± 16.88 per plant	Carunculate, unripe seed colour white, ripe seed colour dark brown; an average of 40 ± 2.62 per plant	Carunculate, unripe seed white, ripe seed red; an average of 36 ± 3.67 per plant	Carunculate, unripe seed white, ripe seed orange; an average of 27 ± 2.90 per plant
Mode of seed dispersal	Explosive mechanism	Explosive mechanism.	Explosive mechanism.	Explosive mechanism.

The photograph of a typical *Euphorbia hyssopifolia* studied is shown in figure 1B. The seedlings emerged 5 days after sowing and had 42.5% germination. The number of days to 50% flowering was 47 days and it continued to bloom throughout the year. The inflorescence was cyathium (cyme), flowers were bisexual with green pedicel, glabrous perianth, white in colour; ovary superior, glabrous, disc shaped, green in colour; placentation axile, style single, hollow, green; stigma branched (four); stamen hypogynous, epipetalous; filament green, anther sticky and brown, numerous and also attached to the wall of the perianth; fruit scizocarpous, 3-lobed, unripe fruit colour green, ripe fruit colour lemon green; seed carunculate, unripe seed colour white, ripe seed colour dark brown (Figure 2). Pollinator that visited the inflorescence was *Calliphora vomitora* (Blue-fly). The mode of seed dispersal was by explosive mechanism. Anthesis took place between 9 am – 11 am. *Euphorbia hyssopifolia* had an average pollen size of 1.535 ± 0.07 μm, 62.78%

pollen viability; while the mean number of fruit and seed per stand were 56 ± 7.69 and 40 ± 2.62 respectively (Table 1).

The photograph of a typical *Euphorbia hirta* studied is shown in figure 1C. The seedlings emerged 5 days after sowing and had 12.5% germination. The number of days to 50% flowering was 58 days and it continued to bloom throughout the year. The inflorescence was cyathium (cyme), flowers bisexual with green pedicel, pubescent perianth, green in colour; ovary superior, glabrous, cylindrical in shape, green in colour; placentation axile; style single, hollow, green in colour; stigma fused, horse shoe shaped; stamen hypogynous, epipetalous; filament green, anther sticky, brown, numerous and attached to the wall of the perianth; fruit schizocarpous, 3-lobed, unripe fruit colour light green, ripe fruit colour green; seed carunculate, unripe seed white, ripe seed red (Figure 2). Pollinators that visited the inflorescence were *Anthrenus verbasci* (Carpet beetles) and *Chortoicetes terminifera* (locust) and the mode of seed dispersal was by explosive mechanism. Anthesis took place between 9 am – 11 am in the morning. *Euphorbia hirta* had an average pollen size of 1.225 ± 0.02 µm and 62.14% pollen viability; while the mean number of fruit and seed per stand were 20 ± 5.76 and 36 ± 3.67 respectively (Table 1).

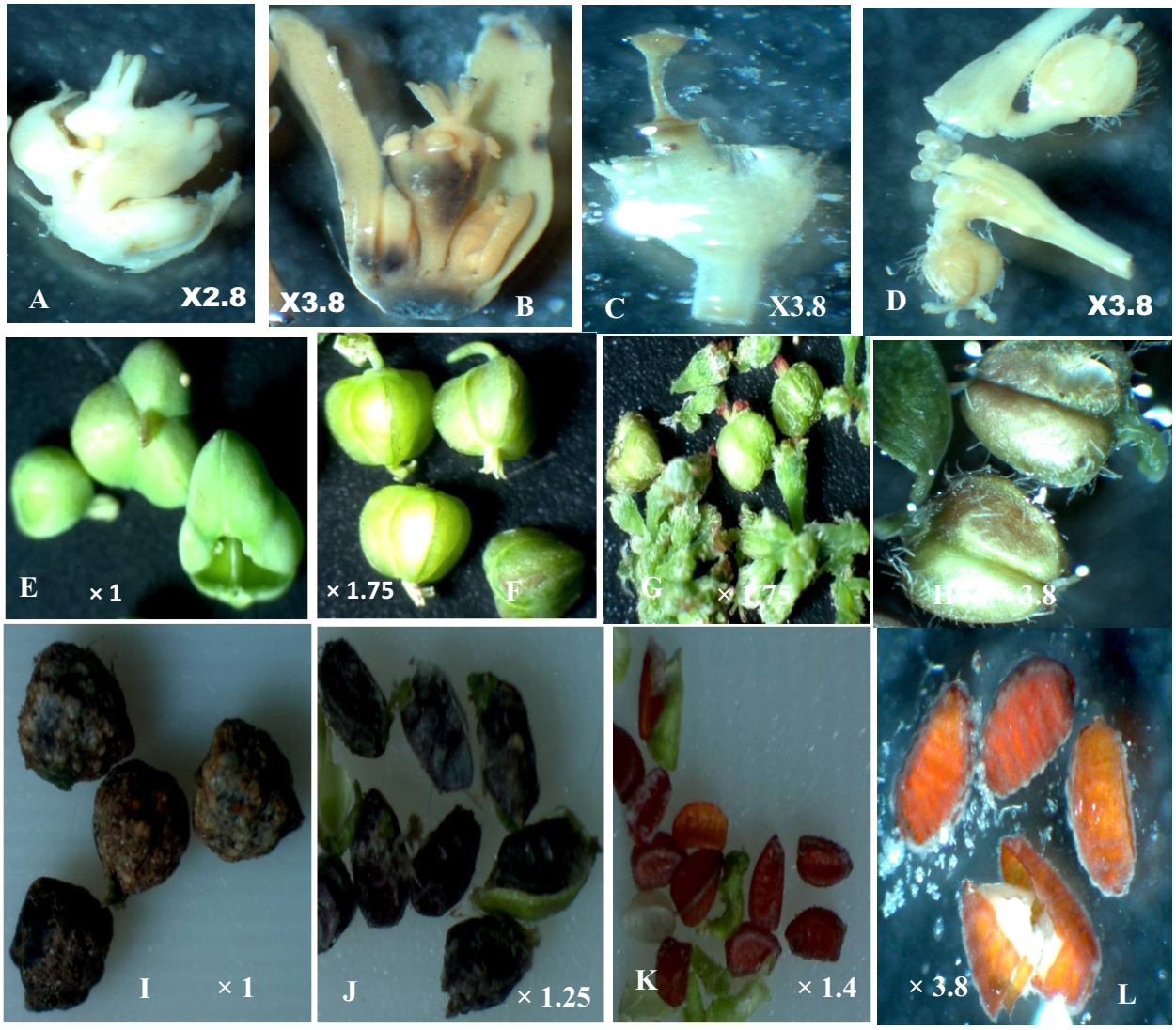

Figure 2: Cyathium, Fruits and Seeds of *Euphorbia* Species Studied

A: Cyathium of *E. heterophylla*; B: Cyathium of *E. hyssopifolia*; C: Cyathium of *E. hirta*; D: Cyathium of *E. prostrata*; E: Friuts of *E. heterophylla*; F: Friuts of *E. hyssopifolia*; G: Friuts of *E. hirta*; H: Friuts of *E. prostrata*; I: Seeds of *E. heterophylla*; J: Seeds of *E. hyssopifolia*; K: Seeds of *E. hirta*; L: Seeds of *E. prostrata*

The photograph of a typical *Euphorbia hirta* studied is shown in figure 1C. The seedlings emerged 5 days after sowing and had 12.5% germination. The number of days to 50% flowering was 58 days and it continued to bloom throughout the year. The inflorescence was cyathium (cyme), flowers bisexual with green pedicel, pubescent perianth, green in colour; ovary superior, glabrous, cylindrical in shape, green in colour; placentation axile; style single, hollow, green in colour; stigma fused, horse shoe shaped; stamen hypogynous, epipetalous; filament green, anther sticky, brown, numerous and attached to the wall of the perianth; fruit schizocarpous, 3-lobed, unripe fruit colour light green, ripe fruit colour green; seed carunculate, unripe seed white, ripe seed red (Figure 2). Pollinators that visited the inflorescence were *Anthrenus verbasci* (Carpet beetles) and *Chortoicetes terminifera* (locust) and the mode of seed dispersal was by explosive mechanism. Anthesis took place between 9 am – 11 am in the morning. *Euphorbia hirta* had an average pollen size of 1.225 ± 0.02 μm and 62.14% pollen viability; while the mean number of fruit and seed per stand were 20 ± 5.76 and 36 ± 3.67 respectively (Table 1).

The photograph of a typical *Euphorbia prostrata* studied is shown in figure 1D. The seedlings emerged 3 days after sowing and had 17.5% germination. The number of days to 50% flowering was 44 days and it continued to bloom throughout the year. The inflorescence was cyathium (cyme), flowers bisexual with green pedicel, pubescent perianth, green in colour; ovary superior, highly pubescent, 3-lobed, green in colour; placentation axile; style single, hollow, green in colour; stigma fused, branched into four; stamen hypogynous, epipetalous; filament green, anther sticky, brown, numerous and attached to the wall of the perianth; fruit schizocarpous, 3-lobed, unripe fruit colour light green, ripe fruit colour brownish green; seed carunculate, unripe seed white, ripe seed orange (Figure 2). Pollinators that visited the inflorescence were *Anthrenus verbasci* (Carpet beetles) and *Calliphora vomitora* (Blue-fly). The mode of seed dispersal was by explosive mechanism. Anthesis took place between 9 am – 11 am. *Euphorbia prostrata* had an average pollen size of 1.225 ± 0.02 μm and the percentage pollen viability was 96.04%. The mean number of fruit and seed per stand were 42 ± 5.88 and 27 ± 2.90 respectively (Table 1).

In the four species studied, there was a very short interval of about 10 days between the flowering period and the fruiting period. Once fruiting commenced, the period of flowering and the period of fruiting overlapped such that the plants continued to produce flowers and fruits concurrently all through the remaining part of the year.

DISCUSSION

Understanding reproductive biology helps to clarify the potential use and value of characters in systematic treatments (Anderson *et al*., 2002). In this study, using floral features to characterize *Euphorbia heterophylla*, *E. hyssopifolia*, *E. hirta* and *E. prostrata* has actually helped in identifying marked differences that could be useful in delimiting the four species. According to Anderson *et al*. (2002) systematics and reproductive biology have been intertwined through botanical history because they both rely on reproductive characters.

The number of days from seedling emergence (3 – 5 days) to 50% flowering (44 – 58 days) was relatively short (Table 1) compared to the period of flowering, fruiting and seed dispersal (continuous throughout the remaining part of the year). This implies that the four species of *Euphorbia* studied exhibited r-strategy, expending more time and allocation of resources on their reproductive phases than their vegetative phases. According to Gadgil and Solbrig (1972) herbaceous plants tend to be r-strategists in comparison to trees.

The movement of anther to the stigma in the four *Euphorbia* species studied was enhanced by the presence of the brown liquid chemical substance on the cyathium as well as the different insects which were probably attracted to the various inflorescences of the species studied due to their floral attributes. According to Harder and Johnson (2009), floral traits like flower size, shape, colour, scents and nectar content can strongly influence pollinator visitation rates, number of

pollen grain transferred between flowers and seed production.

It is also noteworthy that *Calliphora vomitora* (Blue-fly) was found visiting the inflorescence of both *E. heterophylla*, *E. hyssopifolia* and *E. prostrata*; while *Anthrenus verbasci* (Carpet beetles) visited both *E. hirta* and *E. prostrata*. *Chortoicetes terminifera* (locust) was found on only *E. hirta*. According to Ollerton (2017) the relationship between pollinators and plants range from generalist pollinators to highly specialized pollinators; and this relationship can vary depending on changing pollinator abundance during the year.

Pollen viability (Table 1) varied in the four *Euphorbia* species studied ranging from 62.14% in *E. hirta* to 96.04% in *E. prostrata*; and this did not correlate with the pollen sizes. *E. heterophylla* with pollen viability of 83.49% had pollen size of 3.5 ± 0.04 μm; *E. hyssopifolia* with pollen viability of 62.78% had pollen size of 1.535 ± 0.07 μm; *E. hirta* with pollen viability of 62.14% had pollen size of 1.225 ± 0.02 μm; while *E. prostrata* with pollen viability of 96.04% had pollen size of 1.225 ± 0.02 μm. This implies that the pollen sizes of these species did not reveal any significant effect on their viability. According to Oselebe *et al.* (2014) pollen viability determines the ability of pollen grains to effect fertilization, hence fruit set and differences in pollen viability among genotypes are largely of genetic origin.

The seeds of the four species of *Euphorbia* genus studied were dispersed by explosive mechanism due to the schizocarpic nature of the fruit which split open when ripen and dry to disperse the seeds. According to Hufhuis and Hay (2017) explosive seed dispersal is an example of autochory, where seeds are dispersed by a plant's own mechanisms and it provides a valuable opportunity for plants to move from place to place. This mechanism could be responsible for their spread in cultivated fields, gardens, roadsides and waste lands.

CONCLUSION

This study revealed significant floral traits such as differences in perianth texture, perianth colours, ripen fruit colours, ripen fruit texture, ripen seed colour, ripen seed texture and shape of style which could be valuable in delimiting the four *Euphorbia* species investigated. It also showed that the four species of *Euphorbia* studied exhibited r-strategy, expending more time and allocation of resources on their reproductive phases than their vegetative phases while dispersing their seeds through explosive mechanism; thus enhancing their spread and ability to invade cultivated fields, road sides and waste lands.

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