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RESEARCH PAPER

FLOWERING, POST-POLLINATION DEVELOPMENT AND PROPAGATION OF EBOLO (*Crassocephalum crepidioides* (Benth.) S. Moore) IN ILE-IFE, NIGERIA

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ABSTRACT
A study of Crassocephalum crepidioides, a green leafy vegetable mainly collected from the wild in Nigeria and other parts of Africa, was conducted in order to elucidate flowering, post-pollination developments, seed production and germination potential of the plant and investigate the eligibility of other propagation methods. From the flowering studies, it was discovered that peripheral florets opened and were pollinated before central florets. The species had appropriate or good pollination period, however, there might not be sufficient pollens for pollination. The post-pollination period for the maturity of the developing fruits/achenes was observed to be short leading to the production of many immature and malformed fruits or achenes. Partial reflexing and no reflexing of the receptacle were observed to be hindering adequate fruit dispersal. Crassocephalum crepidioides produced up to 768-1152 seeds per plant indicating that the seed production potential of the plant is very high. Germination percentage was not consistent with age and may be influenced by seed maturity. Seeds are photoblastic and germination was promoted by soaking in water. Vegetative propagation was hindered by floral initiation.

Keywords: *Crassocephalum crepidioides, flowering, germination potential, post-pollination development, vegetative propagation*

INTRODUCTION
Crassocephalum crepidioides (Benth.) S. Moore, with the common name fireweed, is an African leafy vegetable that belongs to the family Asteraceae (Compositae). Locally it is called “Ebolo” by the Yoruba people of Southern Nigeria (Adams, 1963). The tender and succulent leaves and stems of ‘Ebolo’ are mucilaginous, and are used in the preparation of soups and stews, especially in West and Central Africa (Burkill, 1985). They are also used for several purposes, medicinal and otherwise in different parts of West, East and South Africa (Ajibesin, 2008).

In Southern Nigeria, the leaves of ‘Ebolo’ are used to treat indigestion and headache. In DR Congo leaf sap is given to treat upset stomach while in Uganda it is used as a treatment for fresh wounds. The Tanzanians use the dried leaf powder as a snuff to stop nose bleeding and smoke it to treat sleeping sickness (Denton, 2004). Tannin found in the roots of the plant is used to treat swollen lips and according to Dairo and Adanlawo (2007), it is a good source of protein in human and animal nutrition. It also possesses antioxidant and cyto-protective properties (Wijaya *et al*., 2011).

According to Lowe and Soladoye, (1990) it is a low priority vegetable to researchers in Africa. The wide genetic variation is yet to be exploited and there are no records of germplasm collections in Africa. Breeding of improved cultivars is needed, as well as research to solve the problem of seed availability that has hitherto limited cultivation (Denton, 2004). Information about its germination and seed production is scanty in literature.

The demands of flowering and fruiting phases interact and as reported by Burtt (1975), the structure and organization of the capitulum of the Asteraceae (or Compositae) must meet the demands of both phases. He also opined that the study of the co-evolution of the flowering and fruiting phases of the life history of plants has been neglected. He therefore called for more studies of these two phases.

Reproductive success is determined by availability of vectors of pollen, degree of self-compatibility or both (Hernandez, 2008). In addition, other factors including structure and lifespan of flowers and environmental conditions are crucial for reproductive success. According to Hernandez (2008), the origin of incompletely developed fruits (seeds immature and seeds mature and empty) could be attributed to environmental, physiological and/or anatomical causes as well as to lack of pollination or to post pollination failures during the early development of embryos (Hernandez and Belles, 2005).

Therefore, the objectives of this study were to;

i. elucidate flowering, post pollination development and seed production

ii. investigate the germination potential of *Crassocephalum crepidioides* and to

iii. assess the suitability of vegetative propagation for this species.

MATERIALS AND METHODS

Fifty (50) seedlings of *Crassocephalum crepidioides* growing in their natural habitat at the staff quarters of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, between latitude: 7°46 N, longitude: 4°56 E were tagged and used for the study. Twenty (20) were transferred to a garden for closer observation and collection of seeds.

Flowering, post-pollination development and seed production

Observations were made daily on the field and garden on 20 plant stands of the species. Flower bud at anthesis, number of days in anthesis, fertilization of florets, number of hours or days from bud opening fully to beginning of bud reclosure, period of flower reclosure and re-opening, appearance of pappus on ripe fruits in the capitulum and dispersal of achenes were studied. Post-pollination events in terms of receptacle reflexing of the capitulum were also studied. Collected achenes were inspected for maturity and immaturity. With the aid of a magnifying lense, matured fruits were further separated into fruits filled with embryo and fruits not filled with embryo.

Germination

The base of a partially opened capitulum was opened and pale/white seeds were observed mostly at the centre (Fig. 3). The pale/white seeds which are immature were not used for germination tests.

Effect of light

Seeds of the same age were used in this experiment. Twenty-five (25) seeds of *C. crepidioides* (per petri-dish) were evenly placed in two 9cm-diameter petri-dishes. Each petri-dish was lined with moistened Whatman's Filter paper no. 1.

For the light treatment, the petri-dish was covered with Petri dish cover and left unwrapped and in natural daylight condition. For the dark treatment, the Petri dish was also covered with Petri dish cover but, wrapped with aluminum foil paper to study seed germination in the dark. Both were kept at room temperature (18-28°C) for observation of germination. A seed was considered germinated when the cotyledons and radicle emerged from the seed coat and final germination percentages were determined when no germination was observed for more than 7 days. Germination percentage was calculated using the formula.

$$Germination\ percentage = \frac{tng}{tnp} * 100$$

Where *tng* = total number of seeds germinated, and *tnp* = total number of seeds planted. Treatments were replicated three times.

Effect of seed age

Germinability of freshly harvested seeds was determined by placing 25 seeds evenly in a 9 cm diameter petri-dish lined with Whatman's Filter paper no. 1 moistened with 2 ml distilled water. Petri dishes were then covered with petri dish cover and kept at room temperature (18-28°C) and in natural daylight conditions. Treatments were replicated eight times. Experiments to determine the effect of seed age on germination was carried out by using seeds of the following age

1, 2 and 3 days old
1, 2, 3, 4, 5, 6 and 7 weeks old and
1, 2, 3, 4, 8 and 12 months old

The same procedure carried out for the germination of freshly harvested seeds above was used for the germination of seeds. Treatments were replicated three times.

Effect of acids and soaking

Germination tests were performed on three sets of fresh, 1, 2, 3, 4, 8 and 12 months old seeds. The growing conditions were the same as those mentioned in effect of age on germination. The first set was soaked in 0.2% H_2SO_4 acid solution for 15 minutes, after which the seeds were rinsed in distilled water twice and planted as earlier mentioned. The second set was soaked in 0.2% HNO_3 acid solution for 15 minutes, rinsed and planted as above. The third set was soaked in water and placed in a dark cupboard for three days after which the seeds were planted in Petri dishes. A control was also set up in which the seeds were not pre-treated before planting. Treatments were replicated three times.

Vegetative propagation

One-nodal and two–nodal cuttings of about 4-6 cms in length were taken from well-developed and "healthy" vegetative stock plants. 24 cuttings had new growth from the nodes while another set of 24 cuttings had all leaves removed. Cuttings were inserted just deep enough to stand in moist soil and were spaced 6 cms apart in bowls (12 cm deep and 23 cm in diameter). The cuttings were firmed into the soil and watered gently but thoroughly to close any remaining air pockets in the propagating substrate and then kept in the shade. The number of cuttings that sprouted within a period of two months was recorded.

Statistical Analysis

Standard error of means was calculated and where necessary, data was subjected to analysis of variance, and means were separated with Duncan's mul-tiple range test (DMRT), using system analysis software (SAS) version 9.2 at the 0.05 level of significance. The results were presented as graphs.

RESULTS

Flowering, post-pollination development and seed production

In bud, the capitulum of *C. crepidioides* is surrounded by a wall of green phyllaries (Fig. 1A). It was observed in all the plant stands that peripheral florets opened and were pollinated before the florets at the centre (Fig. 1B). Post-pollination sign was the withering of the corolla of pollinated florets within 24 hours, that is, one day after pollination followed by a gradual reclosure of the involucre, the diameter of the

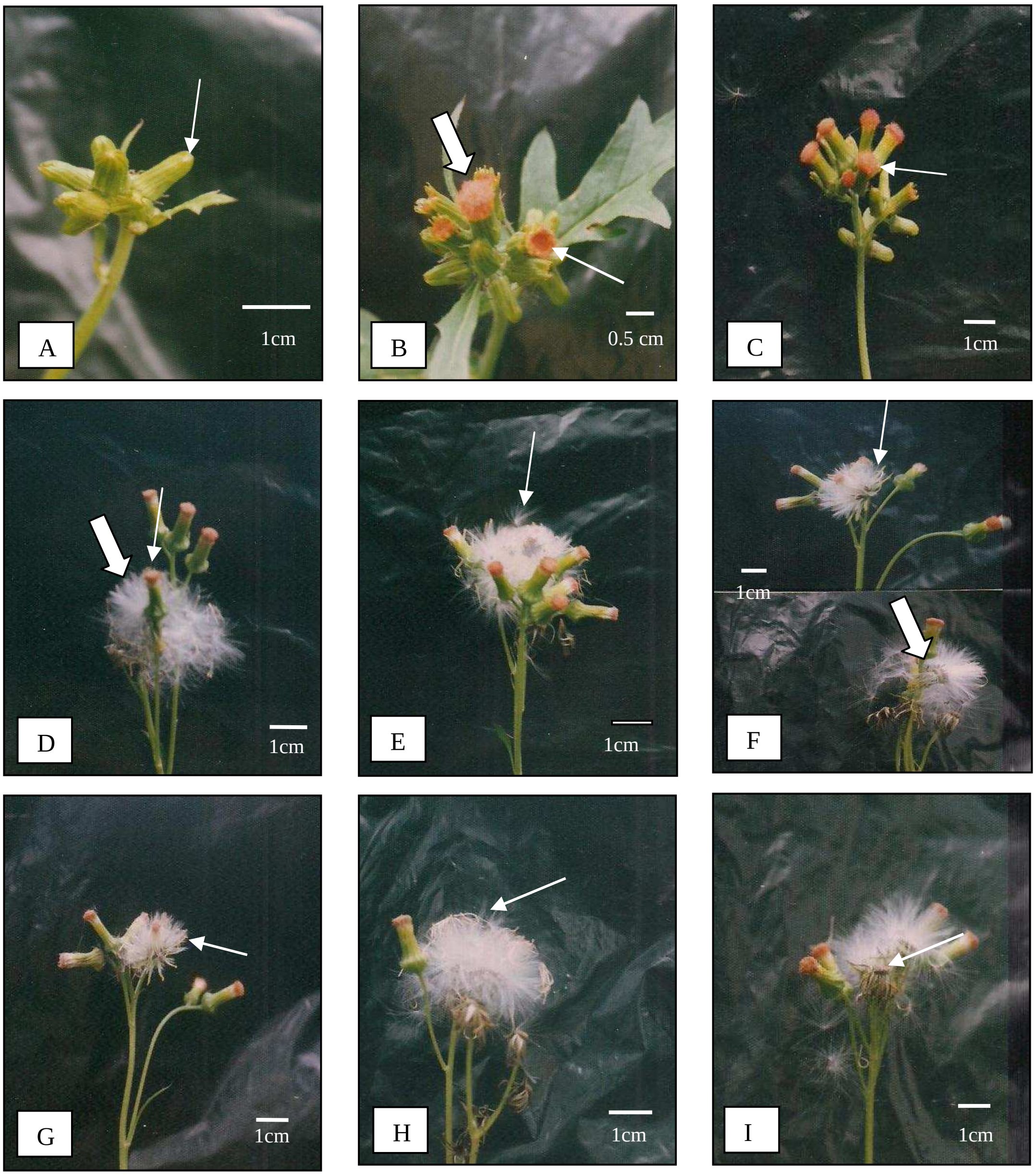

Fig.1: A-I. Pollination and fertilization in *Crassocephalum crepidioides* (Benth.) S. Moore. A: Capitulum in bud (with line arrow); B: Capitulum undergoing pollination and fertilization (with block arrow), Peripheral florets opening first (with line arrow); C: Different capitulum stages on the same branch, Florets completely opened (with line arrow); D: Capitulum re-closure (with line arrow), Pappus opened (with block arrow); E: Capitulum re-closure at different re-closure stages, Seed with attached pappus being dispersed (with line arrow); F: Capitulum with pappus and withered florets on top (line arrow points to receptacle not reflexed), Capitulum with pappus and seeds (with block arrow); G: Pappus opening, withered florets on top, receptacle not reflexing (with line arrow); H: Pappus opened (arrow pointing to withered floret); I: Total receptacle reflexing (with line arrow).

capitulum becoming smaller (Fig. 1D, E).

Flower opening to flower reclosure happened in 3-4 days while flower reclosure to pappus appearance occurred in 5-6 days. After this, the capitulum reopened gradually within 24-36 hours, that is, 1 to 1½ days with the diameter increasing displaying first the pappus of the peripheral matured achenes (Fig. 1 F, G) followed by the pappus of the central matured achenes (Fig. 1H, 2A, B, C). By this time, the phyllaries had dried up and turned brown.

With receptacle reflexing, pappus radiated from matured achenes. Three incidents of capitulum reflexing were observed in this species. Firstly, capitulum receptacle completely reflexed, in which case, it was turned inside-out when dry like a closed umbrella (Fig. 2B, C). Secondly, capitulum receptacle partially reflexed (Fig. 2B, C, D) and thirdly, capitulum receptacle not reflexed at all (Fig. 2 B, D). The most prevalent in occurrence was partially reflexed receptacle followed by totally reflexed receptacle and lastly, receptacle not reflexed at all (Table 1).

After post-pollination, achenes or seeds were observed to fall into three groups, immature, mature filled with embryo, mature and empty (Table 2). The immature achenes were between 0 - 77.94% per capitulum, the mature filled with embryo between 21.53 - 90.3% per capitulum, the mature and empty achenes were between 0 - 32.97% per capitulum.

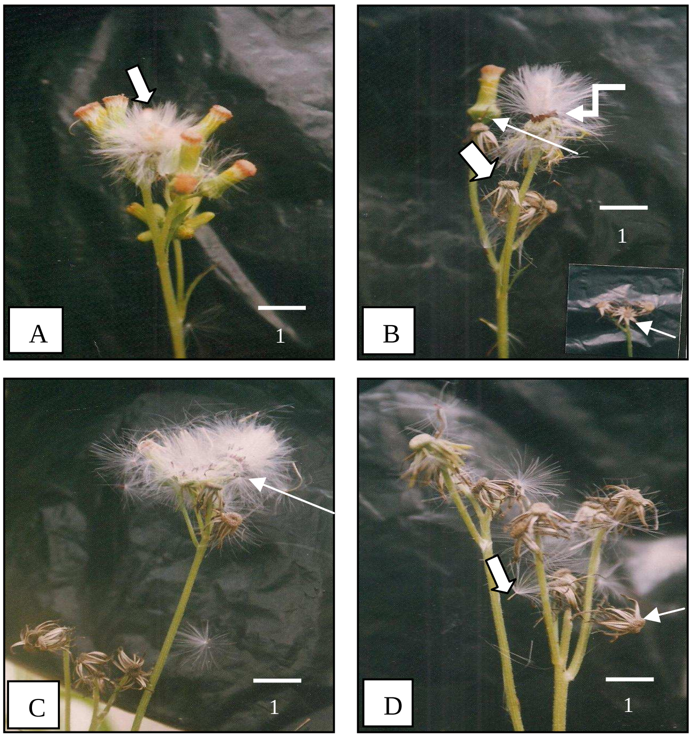

Fig. 2: A-D. Post-pollination developments in *Crassocephalum crepidioides* (Benth.) S. Moore. A: Capitula at different developmental stages (Arrow points to pappus opening); B: Receptacle reflexed showing seeds and pappus (with elbow arrow), Total receptacle reflexing (with line arrow), Partial receptacle reflexing (with block arrow), No receptacle reflexing (inset); C: Pappus and seeds being dispersed (with line arrow); D: Single seed with pappus (with block arrow), Partial receptacle reflexing (with line arrow)

Table 1: Number of Receptacle Reflexing in the *Crassocephalum crepidioides*

S/No	Not Reflexed at all	Partially Reflexed	Totally Reflexed	Total
1	25	45	30	100
2	25	40	35	100
3	20	45	35	100
4	24	50	26	100
5	20	55	25	100
6	18	60	22	100
7	35	32	33	100
8	30	35	35	100
9	29	28	43	100
10	20	55	25	100
Total	**246**	**445**	**309**	**1000**

Crassocephalum crepidioides produced an average of 96 seeds per inflorescence and there were 8 to 12 inflorescences per plant. The average weight of 1000 seeds was 0.176g.

Germination

Two colors of seeds were observed; dark brown and pale/white (Fig. 3) with the dark brown seeds being the matured seeds and the pale/ white seeds immature. The base of a partially opened capitulum was opened and these pale/ white seeds were observed mostly at the centre (Fig. 3). As explained above, seed maturation starts from the peripheral regions and progresses inward as observed here. Fresh seeds of *C. crepidioides* germinated within 8 -10 days and germination percentage ranged from 8% to 89%.

Effect of light

Crassocephalum crepidioides seeds of all ages utilized (naturally dispersed and hand-harvested) germinated under light but not in the dark.

Effect of seed age

The germination percentage of seeds based on age did not follow a regular or predictable pattern (Figs 4A and B). One year old seeds did not germinate at all.

Effect of acids and soaking

All treated seeds germinated. Germination percentage of the untreated seeds was low (21.5 %) and germination percentage of seeds treated with H_2SO_4 was lower (16.4%) than that from the control (untreated seeds). Soaking of seeds for three days in darkness promoted germination of *C. crepidiodes* seeds (Fig. 4C).

Vegetative propagation

Two nodal cuttings were not significantly different from one nodal cuttings in terms of sprouting. Cuttings with or without shoot-buds were also not significantly different from one another in terms of sprouting. There was 100% sprouting in all cases. There was however, no appreciable vegetative growth before the onset of flowering and thereafter senescence.

Table 2: Number of seeds per capitulum: percentage distribution of filled mature seeds, immature seeds and empty mature seeds

S/No	Mature & Filled Seeds		Immature Seeds		Mature & Empty Seeds		Total
	Number	(%)	Number	(%)	Number	(%)	(Number)
1	34	33.70	61	60.40	06	5.94	101
2	30	22.06	106	77.94	00	00	136
3	42	35.60	72	61.02	04	3.39	118
4	28	21.53	79	60.77	23	17.69	130
5	56	61.54	05	5.49	30	32.97	91
6	45	43.69	25	24.27	33	32.04	103
7	80	80.80	02	2.02	17	17.17	99
8	93	90.30	00	00	10	9.71	103
9	132	88.60	02	1.34	15	10.07	149
10	76	56.72	52	38.81	06	4.48	134
11	80	62.50	48	37.50	00	00	128
12	80	80.00	20	20.00	00	00	100
13	72	73.97	26	26.53	00	00	98
14	92	60.93	51	33.77	08	5.29	151
15	99	79.20	24	19.20	02	1.60	125
16	111	85.38	18	13.85	01	0.77	120
17	103	78.03	29	21.97	00	00	132
18	94	73.44	31	24.22	03	2.34	128
19	94	69.12	42	30.88	00	00	136
20	36	26.87	98	73.13	00	00	134
21	103	80.47	23	17.97	02	1.56	128
22	53	40.15	76	57.58	03	2.27	132
23	42	38.89	56	51.85	10	9.26	108
24	95	75.40	29	23.02	02	1.59	126
25	75	69.44	33	30.56	00	00	108

DISCUSSION

Four major factors are necessary to meet demands of both the flowering and fruiting phases. They are the efficiency of pollination, the balance of the breeding system, the protection of the maturing achenes and adequate dispersal of the achenes (Burtt, 1977; Adedeji, 2005). In the *C. crepidioides*, peripheral florets opened first and were pollinated before the florets at the centre. Flower opening to flower reclosure (pollination period) happened between 3-5 days, while flower reclosure to pappus appearance occurred in 5-6 days. Adedeji (2007) in her work on the flowering sequence duration and fruit production in the three species of Emilia, observed flower opening to flower reclosure (pollination period) to be 3-4 days, 4-8 days and 4-5 days in *E. pratermissa, E. coccines and E. sonchifolia* respectively, while flower reclosure to pappus appearance (post-pollination period) occurred in 8-9 days in *E. praetermissa*, 5-6 days in *E. coccinea* and 8-9 days in *E. sonchifolia. E. coccinea* with 5-6 days post pollination period was observed to have the lowest fruit set. Burtt (1977) identified two major factors important to meet the de-

A

B

Fig. 3: A: Seeds of *Crassocephalum crepidioides* (Benth.) S. Moore.; B: capitulum opened at the base showing seeds of *Crassocephalum crepidioides* (Benth.) S. Moore.

mands of the fruiting phase as firstly, the protection of the maturing achenes and secondly, the adequate dispersal of the seeds. In this study, it was observed that *C. crepidioides* had appropriate pollination period for the family as inferred from the study of Adedeji (2005). However, the low number of days for flower reclosure signifies lesser number of days for the development and protection of the maturing achenes before pappus appearance which would lead to the dispersal of many immature and malformed fruits (Ayodele, 1999; Adedeji, 2007).

In this species, the mature seeds are dark brown while the immature seeds are pale/white. The taxon *C. crepidioides* was observed to have immature achenes between 0-77.94% per capitulum, the mature filled with embryo between 21.53-90.3% per capitulum, the mature and empty achenes between 0-32.97% per capitulum. This raises some issues: firstly, the immature achenes and empty achenes observed were probably due to the few number of days that maturing florets had for post-pollination as reported by Adedeji (2007) for the genus *Emilia*. Nakamura and Hossain (2009), in their work on the factors affecting the seed germin-

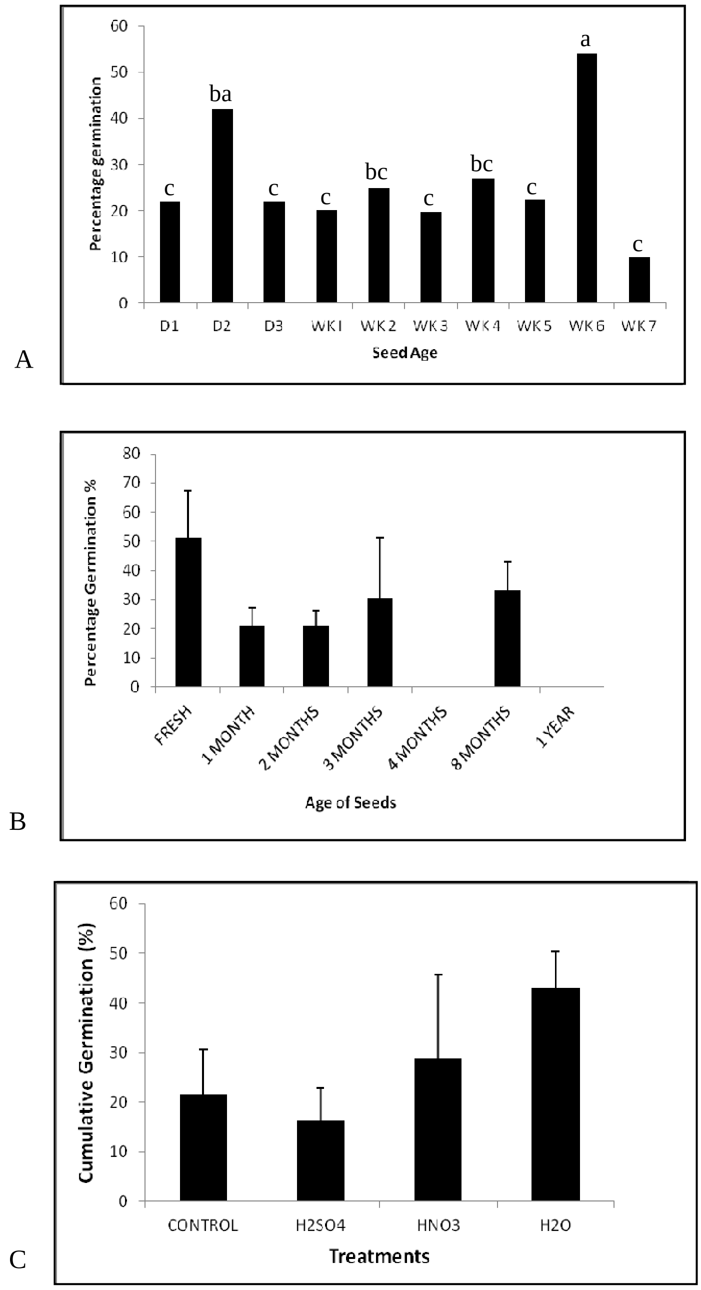

Fig. 4. A: Effect of seed age on germination of *Crassocephalum crepidiodes*. Values with the same superscript letters are not significantly different at P< 0.05; B: Effect of seed age on the germination of *Crassocephalum crepidiodes*. The vertical bars represent the standard deviations of the means. C: Effect of acid and soaking (in water) on the germination of *Crassocephalum crepidioides* seeds. Vertical bars represent standard errors of the means.

ation and seedling emergence of *C. crepidioides* observed that the germination of seeds from opened (mature) capitula was significantly higher than from partially opened or unopened capitula, signifying the importance of longer post-pollination period for maturing florets on the plants, for achene maturity. Secondly, it was observed that *C. crepidioides* do not occur in large populations which could affect accessibility to sufficient pollens for pollination. This is corroborated by Shibata *et al.* (2008), who reported that as the distance from conspecific reproductive adults increase, the percentage of immature and empty seeds increases significantly, indicating higher pollination success with higher local population density and lower pollination success with lower local population density.

The number of achenes/seeds per capitulum was in the range 91-151. According to Denton (2004), *Crassocephalum crepidioides* produces seeds (achenes) profusely and due to the fine silky pappus hairs covering the achenes, they are easily dispersed by wind. However, receptacle reflexing is an essential phenomenon aiding efficient fruit/seed/achene dispersal (Ayodele, 1999; Adedeji, 2005). A combination of lightweight fruits and fully reflexed receptacle resulted in a longer distance of fruit dispersal, even in the absence of wind (Ayodele, 1999). However in this study, partial reflexing occurring more frequently than others and no reflexing of the receptacle were observed to be hindering adequate fruit/seed dispersal. This explains the report of Chen *et al.* (2009), that the seed dispersal ability of *Crassocephalum crepidioides* is limited.

The average number of seeds per inflorescence recorded implies that each plant stand can produce up to 768-1152 seeds per plant indicating that the seed production potential of the plant is very high. This is typical of wind dispersed seeds to ensure survival of the species. The average weight of 1000 seeds recorded in this experiment, corresponds to Denton (2004) who recorded the weight of 1000 seeds as 0.2g. Germination of *C. crepidioides* seeds within 8 - 10 days is consistent with other reports (Denton 2004; Chen *et al*. 2009). Germination under light but not in the dark indicates that these seeds are photoblastic. This suggests that for germination of *Crassocephalum crepidioides* seeds to be successful it requires light. According to Egley and Duke (1985), light requirement for seed germination is common especially in species that have small seeds (Taylorson 1987). An advantage of the light sensitive seeds is that they will not germinate when deeply buried in the soil, which allows seedling emergence only when their establishment is more likely (Lu *et al*., 2006).

Nakamura and Hossain (2009) reported that the seeds of *C. crepidioides* did not emerge from a depth of 1 cm or more because they are very small in size and contain very little food reserves. However, from the results obtained in this study, this could have been due to the effect of light on the germination of these seeds since seeds that were not buried at all and incubated in the dark did not germinate. Chen *et al*. (2009) also reported that seed germination of *C. crepidioides* was inhibited by darkness and that inhibition was reversed in most of the seeds when they were transferred into the 12h light/ 12h dark regime.

The obvious lack of pattern in the germination percentage of seeds based on age under the same light condition may be attributed to the fact that hand-harvested seeds were not allowed to naturally disperse and some of the seeds may not have been fully matured and filled at the time of collection. According to Nakamura and Hossain (2009), seed germination was influenced significantly by seed maturity with seeds collected from completely opened capitula having the highest germination percentage. They reported that germination percentage decreased significantly when seeds were collected from partially opened capitula, and drastically when seeds were collected from unopened capitula. Eight months old seeds were shown to be viable while one year old seeds were not.

Chen *et al*. (2009) reported that the seeds of *C. crepidioides* had no obvious dormancy and retained a high viability after room storage for ten months. Retention of viability for at least ten months is reflected in the ability of the seeds to sprout in the next rainy season. This could be the reason why it is believed that it cannot be planted among the people of the Western part of Nigeria where the plant is known as a vegetable. Nakamura and Hossain (2009) reported that it is thought that the small seed (0.2 mg) fails to maintain a normal physiological condition over one year of storage at ambient temperatures.

The fact that *C. crepidioides* is tolerant to acidic condition has been reported by other authors (Chen *et al* 2009; Nakamura and Hossain, 2009) implying that the seeds can germinate and grow in acidic soil. Soaking in water was found to promote germination than acid treatment. Soaking in water is thought to enhance effects on germination and growth probably, due to hydrolysis of complex sugars into simple sugars that are readily utilized in the synthesis of auxins and proteins. The auxins produced, help to soften cell walls to facilitate growth and the proteins utilized in the production of new tissues (Sabongari and Aliero, 2004). However, in this case, the effect of soaking may be to leach out chemical inhibitors; this is achieved in nature by heavy rains. It has been observed that *C. crepidioides* is abundant in the rainy season when the seeds in the seed bank spring to life. They are also observed in the dry season where there is abundance of water. Seeds of *C. crepidioides* have also been reported to be able to survive and germinate under flood conditions (Nakamura and Hossain, 2009).

Vegetative propagation of leafy vegetables is with an outlook to generate lots of leaves, however, where flowering occurs after rooting and establishment of cutting, that purpose is defeated. Chen *et al*. (2009) reported that *C. crepidioides* is unable to propagate vegetatively. Our observations on the field revealed that branches root at the point of contact with the soil and sprout. However, observations from stem cuttings planted for vegetative propagation revealed the termination of the vegetative stage and beginning of the flowering stage almost immediately after sprouting. The rooting of branches at the point of contact with the soil was apparently a different phenomenon from the ability of the plants to propagate vegetatively with reference to harvest of leaves. The timing of onset of flowering is crucial to plant survival in sexual reproduction but early flowering can be a hindrance to vegetative growth during vegetative propagation. Sachs *et al.* (1967) documented numerous independent reports of gibberellin induced inhibition of flowering. Gibberellins are floral inhibitors in many woody perennials including apple, stone fruit and mango if applied to shoots before floral initiation. Experiments with annual and biennial beet cultivars also showed that considerable stem extension accompanied by axillary bud development was induced by gibberellic acid treatment and the plants did not flower (Schwabe, 1980). The effect of gibberellins on the vegetative propagation of *C. crepidioides* is worth investigating.

In conclusion, studies on the flowering, pollination and post pollination of *Crassocephalum crepidioides* revealed that peripheral florets opened and were pollinated before florets at the middle. This also led to peripheral fruits or achenes maturing before those at the middle and a short post-pollination period lead to the production of many immature and malformed fruits. Three incidents of capitulum reflexing were observed: completely reflexed, partially reflexed and non reflexed receptacles, the last two hindering adequate fruit dispersal.

Crassocephalum crepidioides seeds were photoblastic needing light and very moist soils for optimal germination. Seeds can tolerate acids and germination may be influenced by seed maturity with indications that seeds may not be viable after one year in storage. From the observations of the stem cuttings propagated, slowing down of flower initiation or using younger source plants may enhance vegetative propagation.

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