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Research Article

Establishing optimal conditions for nursery production and domestication of *Crassocephalum crepidioides* (Benth.) S. Moore.

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Abstract

Crassocephalum crepidioides (Benth.) S. Moore is a plant consumed as a green leaf vegetable in several regions of Benin. But the species is still not domesticated and is harvested from wild according to the seasons. The plant remains as an undervalued food plant in Benin. This study aims to find the optimal conditions for nursery production of this species for its better valorization and domestication in order to contribute towards reducing food insecurity. We tested seed germination capacity and seedling growth of *C. crepidioides* in a nursery. We used cow dung and poultry manure to fertilize the transplanting board before transplanting seedlings from the seedbed whereas control plots were kept without fertilization. In addition, we tested shade impact on seedlings considering two variants (under shade and out of shade). Seedlings were transplanted in the following three spacings (20x20 cm, 30x30 cm and 40x40 cm). We used two water doses (22 liters/day and 44 liters/day) to water each 3.30 m^2 seedbed. The freshly harvested seeds showed about 19 day's latency with a germination rate to 15.10 % while conserved seeds showed a latency time to 5 days with a germination rate to 12.70 %. Fertilization and shade influenced significantly the growth of *C. crepidioides*. Cow dung and poultry manure are suitable for soil fertilization for better plant development. The dose of water supplied to the seedbeds and transplanting spacings during the dry season also significantly influenced the growth of *C. crepidioides*. We found that that the 20x20 cm transplanting spacing reduces evaporation and transpiration, which is better for plant development.

Keywords: greens vegetable, ecological requirements, gbolo, *Crassocephalum crepidioides* Benin

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Introduction

Of the 3,000 plant species inventoried in Benin's forest ecosystems (MEHU, 2011), 162 species are consumed by local populations as food plants (Codjia et al., 2003). Of these, *C. crepidioides* occupies a prominent place given its nutritional and medicinal importance. It is an annual plant that grows spontaneously on newly cleared fields. The leaves of this plant are sold in local markets in almost all regions of Benin, and consumed as a green leaf vegetable during the period of the plant's occurrence (Akoegninou et al., 2006). Its leaves are nutritionally very rich in vitamin C and minerals (100 g of fresh leaves contain 9.17 mg of vitamin C, 308.45 Kcal and 1012 mg of calcium) (Adjatin et al., 2013). Vitamin C is prescribed in most of our hospitals, for the synthesis of collagen and inter-cellular; a substance that gives contraction to muscles, vascular tissue, bones, tendons and ligaments (Olayinka et al., 2012). Thus the amount of vitamin C contained in the fresh leaves of *C. crepidioides* helps with health problems. The hot extracts of the leaves also play an antibacterial role (Omotayo et al., 2015). *C. crepidioides* also contains a large amount of satisfactory protein and a large amount of essential amino acids that constitute an alternative source of energy when the carbohydrate metabolism is weakened via gluconeogenesis contributing to stunting in children and lack of development during pregnancy (Iheanacho et al., 2009).

Despite all these qualities, consumers do not plant the species. Its exploitation remains in the form of collection from the wild during the period of the occurrence on newly cleared farms. It thus urged to domesticate C. *crepidioides*. However, the search for horticultural conditions of production is an alternative that could contribute to the domestication of this species. In this study, we did a germination test of *C. crepidioides* seeds that were not preserved and those that were preserved. We also compared the growth and morphology of *C. crepidioides* according to two animal dung (cow dung and poultry manure) under the shade and without shade. Similarly, the growth and morphology of *C. crepidioides* is studied according to different spacings and doses of water. We discuss the implications of our results for the enhancement of *C. crepidioides* and also help the population to obtain information related to the production of the species.

Materials and Methods

Study area

The study was conducted in the village of Adakplamè, Kétou district which is located about 150 km from Cotonou, the economic capital of Benin. The district

of Kétou is located at the northern end of Plateau department between 7°10 and 7°41'17" north latitude and between 2°24'24" and 2°47'40" East and covers an area of 1,775 square kilometers (INSAE/RGPH3, 2002). The village of Adakplamè which is the largest one in the district of Kétou is distanced from the town centre by about 12 km and is surrounded by two forest reserves which are Dogo classified forest and Kétou classified forest. The climate in this zone is tropical, Guinean type with bimodal rainfall with two rainy seasons and two dry seasons. The long rainy season covers the period of March to July and the short dry season covers the month of August. The short rainy season covers the period of September to October and the long dry season covers the period of November to February. The average annual rainfall varies from 1,200 to 1,300 mm. The annual average temperature varies from 25 °C to 29 °C and the average relative humidity varies from 68 % to 85 %. Annual evapotranspiration varies from 89.19 mm to 144.13 mm.

Plant material

C. crepidioides seedlings are obtained from germination of seeds in the nursery. The common name of the species is "Crassocephale" or *faux crépide* in French and vernacular names are Gbolo (Yoruba and Adja), Akogbolo or Hôwounhôgui (Mahi), or Terikiagborou (Bariba), Wankadjobrou (Ditamari), This herbaceous annual plant of up to 1 m height (Akoegninou et al., 2006) belongs to the Asteraceae family. The leaves are simple, lobed form with toothed edge, surrounded by fine hairs. The flower is attached on a peduncle with a set of long sepals with tube shape containing a hundred white stamens (setaceous) bearing each, a seed at the base and showing red colour at the maximum petals height (Figure 1). The seeds are particularly very small and their average weight is 0.15 mg/seed.

Figure 1. Seeds, flowers and C. crepidioides

a. Data collection methods

i. Seed germination tests

C. crepidioides seeds were collected from identified local individuals in the farms at Adakplamè (municipality of Kétou). Two germination tests have been performed. The first germination test focused on freshly harvested seeds, dried and then sown two days after harvest and the second germination test was carried out on dried seeds that were stored in a perforated box for ventilation for 7 months. In the first test 1,800 seeds of *C. crepidioides* were sown on a straw seedbed straw of which 96 plants were replicated. In the second test 3,000 seeds of *C. crepidioides* were conserved for 7 months then sowed in the same conditions of which 232 plants were replicated. Seeds were sowed to the flight on two traditional seedbeds (1 m x 1 m). The seedbeds were watered two times per day (2 litres in the morning and evening). For the tests, the number of seeds germinated were counted daily for two months and seed germination rate was determined as follows:

$$\mathrm{Tg} = \frac{\text{Number of germinated seeds}}{\text{Number of sowed seeds}} \times 100$$

ii. Transplanting seedlings

❖ **Effect of soil fertilization and shade on the growth of *C. crepidioides*.**

This trial was conducted during the rainy season from August to October 2016. Six weeks after sowing, seedlings obtained from the first seed germination test were transplanted at a spacing of 60x60 cm on 12 planting boards of 3 m x 1.10

m fertilized with cow dung and poultry manure. Two factors were studied; three level of fertilization (cow dung (**cd**), poultry manure (**pm**) and control (**c**)) and two level of shade (under shade and out of shade). The experimental design is a randomized block design with two replications (Figure 2). Each block is composed of six planting boards with two planting boards per treatment. Twelve (12) kilogrammes of fertilizer were provided to each fertilized board.

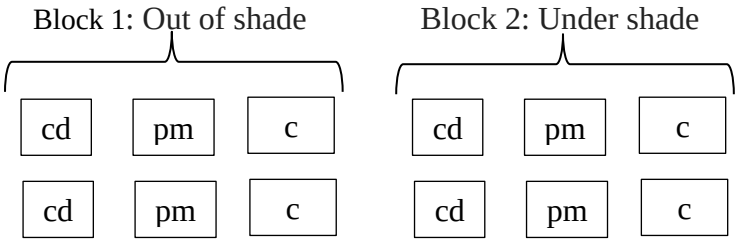

Figure 2. Experiment design of first trial of rainy season.

❖ **Effect of transplanting spacing and amount of water on growth of *C. crepidioides*.**

This trial was carried out during the dry season, from January to March 2017. Two factors were studied; spacing between plants and water doses. Six weeks after sowing, seedlings resulting from seed germination at the second germination test were transplanted on six boards of 3x1.10 m, regularly watered twice a day. The boards were fertilized with cow dung. The experimental design is a randomized bloc design with two replications (Figure 3). Two treatments were applied which are water dose: 2.68 L/m^2/day either 22 liters of water a day, with 11 liters in the morning and 11 liters in the evening; 5.36 L/m^2/day either 44 liters of water a day (22 liters morning and 22 liters evening) and transplanting spacing (20x20 cm (**S1**), 30x30 cm (**S2**) and 40x40 cm (**S3**)).

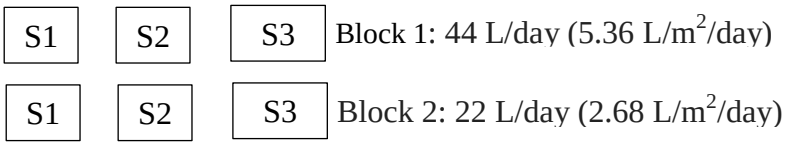

Figure 3. Experimental design of second trial of the dry season.

b. Measurement and data analysis

Quantitative variables such as plant size (Ht), number of leaves (Nf), length (Lf) and width (lf) of limb, number of internodes (Nen), Leaf weight (Pf) and number of flowers (Nfr) were measured on 5 plants per planting board. Data was collected weekly starting from the third week after transplanting. Roots weight (Pr), fresh leaves weight and dry leaves weight were measured on the seventh week after transplanting. We used Kruskal-Wallis test (non-parametric equivalent of ANOVA one-way) to test the effect of fertilization on Ht, Lf, lf, Pf, Pr and Ps. Generalized linear model (GLM) was also used to test the effect of fertilization on Nf, Nen, and Nfr and Wilcoxon test was used for comparison of means. We generated a correlation matrix using all measured variables. Analyses were performed using R.3.2.4 (R Core Team, 2016) software.

Results

Germination rate of C. crepidioides

Figure 4 shows germination rates of freshly harvested seeds and stored seeds for 9 months respectively. The analysis of this figure revealed that the freshly harvested seeds have a latency period of 19 days and a germination rate of 15.10 % in 47 days after sowing while stored seeds have a latency time of 5 days and a germination rate of 12.70 % in 19 days.

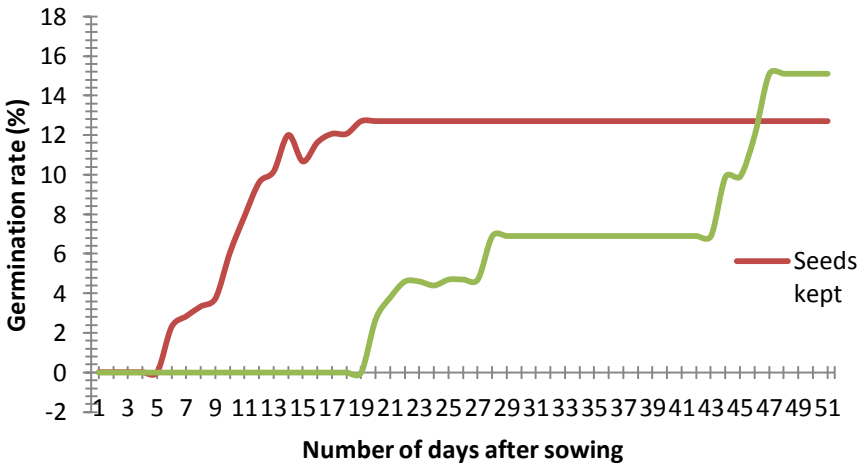

Figure 4. Germination rate of *C. crepidioides*.

Influence of soil fertilization on the growth of C. crepidioides

Table 1 shows the effect of soil fertilization on plant height, leaves size and roots weight. Soil fertilization statistically has a significant effect (Prob. <0.1 %) on plant height, leaves length and roots weight. However, means comparison test shows that *C. crepidioides* apparently has uniformed growth, whether the soil is fertilized with cow dung or poultry manure. In contrast, plants grown on soils without fertilization (controls) showed lower values of growth parameters (Table 1) with a highly significant difference.

Table 1. Effect of fertilization on the growth of *C. crepidioides*.

Variables	Fertilizers	chi-sq	Prob	Means
Plant height (Ht in cm)	Cow dung	21.22	0.000***	22.85[a]
	Poultry droppings			23.33[a]
	Control			11.50[b]
Length of leaves (Lf in cm)	Cow dung	19.46	0.000***	19.90[a]
	Poultry droppings			20.52[a]
	Control			13.18[b]
Width of Leaves (lf in cm)	Cow dung	20.38	0.000***	7.19[a]
	Poultry droppings			7.24[a]
	Control			4.85[b]
Weight of Roots (Pr in g)	Cow dung	13.93	0.000***	0.09[a]
	Poultry droppings			0.07[ab]
	Control			0.05[b]
Weight of Fresh leaves (Pf in g)	Cow dung	18.39	0.000***	0.19[a]
	Poultry droppings			0.15[a]
	Control			0.09[b]

There are strong positive correlations between plant height, leaves length (r = 0.87) and width (r = 0.87) leaves and between length and width of leaves (r = 0.95) (Table 2). Thus, *C. crepidioides* leaves sizes (length and width) are proportional to plant height. Table 3 shows the effect of soil fertilization on number of leaves and number of internodes. Soil fertilization has a highly positive statistically effect (prob. <1 %) on number of leaves and number of internodes of *C. crepidioides*. Fertilization of the soil with cow dung or poultry droppings increased the number of leaves produced by *C. crepidioides*. Mean number of leaves produced per plant varied from 21.80 to 26.70 on fertilized soils whereas number of leaves was 12.25 on the control.

Table 2. Matrix of correlations between growth parameters.

Variables	Ht	Nf	Lf	Lf	Nen	Pf	Pr
Ht	1						
Nf	0.54	1					
Lf	0.87	0.72	1				
lf	0.87	0.65	0.95	1			
Nen	0.28	0.83	0.54	0.43	1		
Pf	0.69	0.76	0.78	0.75	0.66	1	
Pr	0.71	0.49	0.74	0.71	0.33	0.74	1

Ht: plant height; Nf: number of leaves; Lf: length of leaves; lf: width of leaves; Nen: number of internodes. >0.70: strongly correlated; 0.30 to 0.70: moderately correlated; < 0.30: weakly correlated.

Table 3. Effect of fertilization on number of leaves and number of internodes.

Variables	Fertilizers	Coefficient	Standard error	t value	means	Prob
Number of leaves (Nf)	Cow dung	0.779	0.148	5.23	26.70	0.000***
	Poultry droppings	0.576	0.154	3.74	21.80	0.000***
Number of internodes (Nen)	Cow dung	1.115	0.341	3.26	9.15	0.001**
	Poultry droppings	0.993	0.347	2.861	8.10	0.005**

Influence of the shade on the growth of *C. crepidioides*

Table 4 shows the effect of the shade on the growth of *C. crepidioides*. Plant size, length and width of leaves, weight of fresh leaves and weight of roots are higher under the shade. In contrast, the number of leaves and number of internodes are higher when out of shade. A significant statistically effect of shade is noted on plant size (Prob. <0.1 %), length (prob. <5 %) and width (p <1%) of leaves, number of internodes (Prob. <0.1 %) and weight of roots (Prob. <1 %). Figure 5 (a and b) shows pictures of *C. crepidioides* plants under the shade and out of the shade, respectively.

Table 4. Effect of shade on growth of *C. crepidioides*.

Variables	V	Prob.	means
Size (Ht in cm)	370	0.001**	23.7 14.75
Number of leaves (Nf)	146	0.124	18 22.5
Length of leaves (Lf in cm)	341.5	0.025*	19.28 16.45
Width of leaves (lf in cm)	384	0.001**	7.04 5.82
Number of internodes (Nen)	38.8	0.000***	3.73 9.77
Weight of roots (Pr in g)	191	0.7	0.15 0.14
Weight of Fresh leaves (Pf in g)	205.5	0.001**	0.09 0.06

Figure 5. Under the shade plants (a) and out of the shade plants (b) on fertilized soil.

Influence of amount of water on growth of *C. crepidioides*.

The dose of water used to irrigate the seedbed has significant effect (Prob <5%) on the number of internodes, number of leaves and the dry weight of the leaves. The total height, width, length and wet weight of the leaves are not influenced by the dose of irrigating water. The seedbeds watered with 44 litres of water per day, produced highly grown plants (11.05 cm and 18.91 cm height; 35 and 48.44 leaves respectively for 22 litres and 44 litres). Table 5 presents the mean values of growth parameters of *C. crepidioides* according to the water doses used to irrigate the seed beds.

Table 5. Effect of water dose on the growth of *C. crepidioides*.

Variables	P value	22 liters	44 liters
Total height	0.49	11.05	18.91
Leaf width	0.49	2.97	4.37
Leaf length	0.84	8.58	12.53
Number of internode (Nen)	0.02	4.27	7.55
Number of leaves	0.02	35	48.44
Number of flowers	0.00***	1.77	11.38
Fresh leaf weight	0.06	25.5	48.94
Dry leaf weight	0.005	2.23	5.27

Influence of spacing on growth of *C. crepidioides*.

The average values of growth parameters of *C. crepidioides* are higher for 20x20 cm planting spacing (Table 6) compared to 30 x 30 cm and 40 x 40 cm. The variance analysis probabilities indicate that transplant space have significant effects (Prob <5 %) on number of internodes, number of leaves, and leaf dry weight, while the dose of water used to irrigate does not influence the total height, width, length and wet weight of the leaves (Table 5).

Table 6. Effect of transplanting spacings on the growth of *C. crepidioides*.

Variables	P value	20cm x 20cm	30cm x 30cm	40cm x 40cm
Total height	0.48	16.58±5.12	14.87±7.94	13.50±5.11
Leaf width	0.49	4.09±0.95	3.20±1.17	3.71±0.61
Leaf length	0.84	11.86±2.37	9.57±3.22	10.23±2.02
Number of internode (Nen)	0.02	6.83±2.83	4.41±5.40	5.50±2.64
Number of leaves	0.02	42.66±20.91	38.58±26.54	43.91±15.12
Number of flowers	0.00***	6.83±9.68	10.91±21.09	2.00±3.93
Fresh leaf weight	0.06	39.50±19.92	34.33±30.06	37.83±20.24
Dry leaf weight	0.005	4.16±2.08	3.61±3.33	3.47±2.26

Discussion

Germination of *C. crepidioides* seeds

The germination rates of *C. crepidioides* is low; this could be due to high density of seeds sowed within a small space as seeds were so small and were sprayed without counting. Indeed, according to (Sounon et al., 2009), the high density of seeds sowed within a restricted space could reduce the space available for germination of each seed and then induce competitive effects between seeds; which can slow down their germination. Much lower germination rates could also result from infertility of some seeds in fruits. There are 30 to 50 seeds in a fruit of *C. crepidioides*. The long periodof germination (from the 19th to 47th day) of

freshly harvested seeds could be a result of embryonic dormancy of seeds, due to the presence of inhibiting substances in the pericarp; which slow down the metabolic reactions of seed germination (Wédjangnon et al., 2016). Compared to these freshly harvested seeds, those which have been dried for days then stored for months have a very short germination time which starts from the 5th to 19th day after sowing. The long dry period and the long duration of conservation would have allowed better drying of seeds and reduction of inhibiting substance that would have consequently reduced the duration of latency and germination time.

Effect of soil fertilization and shade on growth of *C. crepidioides*

Soil fertilization with cow dung and poultry manure improved the growth of *C. crepidioides* plants. This growth is apparently uniformed on soils fertilized with cow dung and poultry manure. However, a statistically significant difference is observed between all plant growth parameters on fertilized soils compared to controls (Table 1). These results could be explained by the physicochemical properties of both fertilizers. Cow dung and poultry manure are organic fertilizers rich in organic matter, nitrogen, potash and phosphorus, which allow for the best growth of seedlings (Charbonnier et al., 2012; Lehmann et al., 2012).

Figure 6. Plants produced with soil fertilization (a) and without soil fertilization (b).

Plants produced out of shade have more leaves than those produced under shade. This could be explained by significant photosynthesis out of shade, which causes large branching and consequently a large number of internodes. The height growth of plants leaves length and width under shade can be explained by the phototropism phenomenon under shade, where well channeled light has

tends to attract the plants in their height growth, which is not the case in diffuse light (Djidji et al., 2010). This growth of plants under shade, answers the preference of green vegetable consumers, who when at the market choose green vegetables with long and wide leaves.

Figure 7. *C. crepidioides* plants produced under shade (a) and out of shade (b).

Effect of spacing and amount of water on growth of *C. crepidioides*

The comparison of plants produced during the various seasons indicates that the number of leaves produced during the dry season is higher than the in the rainy season whatever the spacing between plants. However, the leaves grow more in rainy season. This could be a result of insufficient water and high transpiration of plants produced in the dry season when plant evapotranspiration is the highest. The production of this green vegetable during the dry season requires the provision of sufficient quantity of water. It is also observed that there was leaf shrinkage on seedbeds irrigated with 22 liters per day (Figure 8). This shrinkage is even more accentuated on plants with large spacing (30x30 cm and 40x40 cm). Thus evaporation and transpiration are less important when plants are closer.

Figure 8. Morphology of leaves in dry season. (a) seedbeds irrigated with 22 L of water per day; (b) seedbeds irrigated with 44 L of water per day.

Conclusion

It appears clearly from the different experiments that fertilization of soil with cow dung or poultry droppings allows for the best growth of *C. crepidioides* plants to satisfy the preference of green vegetable consumers. Cultivation of this plant under shade induces the best growth of leaves. However, *C. crepidioides* can also be grown out of shade with good growth of leaves. Cultivation of this plant requires a lot of water. The transplanting of seedlings to close spacings limits evaporation and transpiration of the plant in the dry season, which improves its growth. Apart from very low germination rates of the seeds, which forthcoming investigations on production of this green vegetable must improve in terms of its nursery production, there is no difficulty in producing the green vegetable of *C. crepidioides* in regards to its abundant fruiting. It is necessary to valorize this green vegetable because of the nutritional composition of leaves and their importance in terms of health.

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