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EFFECTS OF ENVIRONMENTAL FACTORS AND SOWING DEPTH ON SEED GERMINATION IN *CLEOME GYNANDRA* L. (CAPPARACEAE)

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Abstract

Cleome gynandra is a wild vegetable that is rich in nutrients especially vitamins, mineral elements and protein. It is consumed in most parts of South Africa as a vegetable. The leaves and seeds of this plant are used in folkloric medicine for the treatment of head and stomach aches. Despite its high dietary-medicinal value, the plant is still regarded as a weed in many Provinces of South Africa while the conditions necessary for its optimum growth in the wild are still obscure. Therefore, this study was designed to investigate the effect of various environmental factors and sowing depth on the germination of two types of seeds of *C. gynandra* in the Eastern Cape Province of South Africa.

The result shows that the average seed weight was 1.2 ± 0.003 mg and the viability of Lot A and B were 22.6 ± 2.3% and 67.3 ± 5.0% respectively. The optimum germination was achieved at 30°C for both Lots A and B when watered bi-weekly at a sowing depth of 0.5 cm. The result also showed that germination was best in the dark (28.7%) for both Lot A and B. In overall, the germination rate under all the conditions was highest in Lot B. This study indicates that *Cleome gynandra* has the potential of thriving successfully under varied environmental conditions despite the great fluctuations of temperatures in South Africa during summer and winter respectively.

Key words: Wild vegetable, Temperature regimes, Seed germination, Sowing depth.

Introduction

Traditionally, the use of wild leafy vegetables is an important component of the diet of the people of the Eastern Cape in South Africa. These vegetables are a rich source of micronutrients and vitamins (Nesamvuni *et al.,* 2001; Steyn *et al.,* 2001). In addition, they play a significant role in nutrition as well as food security and serve as supplements for the management of malnutrition (van Wyk & Gericke, 2000; Steyn *et al.,* 2001; Odhav *et al.,* 2007; van Rensbury *et al.,* 2007). Some of the most common wild vegetables found in this Province include *Physalis viscosa, Amaranthus paniculatus, Solanum nigrum, Rumex obtusifolius, Physalis peruviana, Sonchus asper*, *Corchorus olitorius* and *Cleome gynandra* (van Rensburg *et al.,* 2007).

Cleome gynandra L. is an erect herbaceous annual plant belonging to the Capparaceace family. It grows up to 1.5 m in height. The leaves are alternate, palmately compound and its petals are white, pink or lilac. This plant is commonly known as African spider. It grows as a weed in most tropical countries but it is sometimes a semi-cultivated popular tropical leafy vegetable in many parts of sub-Saharan Africa especially in countries such as Botswana, Kenya, Tanzania, Uganda and Zimbabwe (van Den Heever & Venter, 2007). In South Africa, the plant is commonly found in Limpopo, North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free-State and the Northern Provinces (Smith & Stearn, 1972).

C. gynandra is a wild vegetable that is rich in nutrients especially vitamins A and C, calcium, iron, magnesium and protein (Chweya & Mnzava, 1997). The succulent shoots are boiled and eaten as a pot herb, stew or side dish in South Africa. In Kenya, several nutritional uses of this plant have been proven (Opole *et al.,* 1995). These leaves are used, in Thailand, for making a fermented product known as Paksian-dong, (Anon., 1990). Indians use the leaves as a flavoring agent in sauces.

In folkloric medicine, *C. gynandra* leaves and seeds are used, especially, in the treatment of head and stomach aches. Bruised leaves, which are rubefacent and vesicant, are used to treat neuralgia, rheumatism and other localized pains (Chweya & Mnzava, 1997). In India, the sap from the leaves is used as an analgesic for headaches, epileptic fits, ear aches and for the treatment of inflammation (Narendhirakannan *et al.,* 2005; Mule *et al.,* 2008). Regular consumption of this vegetable has been reported to relieve childbirth complications, to treat scurvy, marasmus and malaria (Onyanyo & Kunyanga, 2013). Despite its ethnopharmacological importance, this plant is still regarded as a wild vegetable that grows naturally in this part of the world though wild vegetables have been proved to be of potential significance to rural life and development (Flyman & Afolayan, 2006).

Although the natural regeneration of *C. gynandra* is mainly by seed, the seeds of this plant do not germinate readily (Chweya & Mnzava, 1997). To improve its seed germination, pretreatment methods and alteration of environmental factors have been reported (Ochuodho & Modi, 2005) while no other methods have been devised to promote its proper propagation in South Africa. This study was, therefore, designed to investigate the effect of various environmental factors and sowing depth on germination of the seeds of *C. gynandra* in the Eastern Cape Province of South Africa.

Materials and Methods

Seed collection: Mature seeds of *Cleome gynandra* were harvested from the University of Fort Hare research farm in Alice (Latitudes 32° 47' 0" South and Longitudes 26° 50' 0" East). The freshly harvested seeds were removed from their capsules, air-dried and used for the germination studies whereas another batch of the seeds were stored in an air tight container at room temperature (15-25°C) for

eight months and later used for various germination trials. For the purpose of this report they are referred to Lot A seeds and Lot B seeds respectively.

Seed weight determination: Seed weight was determined by weighing 200 seeds using an analytical balance and the mean weight of one seed was calculated.

Viability test: To ensure that the seeds used for experiments were viable, viability test was carried out using the tetrazolium technique (Anon., 2003). Four replicates of 50 seeds each were used for each seed Lot. Adopting the procedure of Peters (2000), the seeds were imbibed for 24 h in water, pierced along the margin without damaging the embryo and soaked in colorless 0.1% solution of 2,3,5 triphenyl tetrazolium chloride (TTC) for 16 h at 25°C in the dark. The seeds were then removed from TTC solution, washed with distilled water and soaked in 10 ml of 95% ethyl alcohol to permit direct observation of the embryo. They were then viewed under a light microscope to observe the stained embryos. Embryos of viable seeds appeared bright red in color.

Germination trial: Seeds were surface sterilized in 0.1mercuric chloride solution for 60 s to prevent fungal attack before being rinsed in several changes of sterile water. They were then placed in a 9 cm sterile petri dishes lined with two Whatman No.1 filter papers moistened with 3 ml of distilled water. Treatments were arranged in a completely randomized design with three replicates of 50 seeds each. The experiment was repeated thrice and the pooled mean values were separated on the basis of least significant Differences (LSD) at a probability level of 0.05.

Effect of temperature: The effect of temperature on germination was investigated by placing the petri dishes in incubators set at 10, 15, 20, 25, 30 and 35°C. This was observed daily for 14 days. Distilled water was added when necessary.

Effect of light: Seeds were exposed to illuminations produced by sun rays whereas Petri dishes were covered with three layers of aluminum foil in the dark treatment (Baskin & Baskin, 1998) and daily observations were assessed in a dark room illuminated with a green light.

Effect of water regimes: The following water treatments were investigated. These include daily, once a week and bi weekly watering. The seeds were examined every 24 h and considered viable when the radicle was observed (Thanos & Rundel, 1995). Germination was recorded daily over a period of 14 days and germinated seeds were removed promptly. At the end of the period, non - germinated seeds were dissected to check for viability (if the embryo and endosperm are intact and not discoloured) (Anon., 2003). Germination percentage for each treatment was calculated using the formula cited by Czabator's index (1962).

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds per replicate}} \times 100$$

Effect of sowing depth: Plastic pots were filled with 2 kg of garden soil and five seeds of *C. gynandra* were sown at 11 different depth of 0 (soil surface), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 cm. The pots were laid out in a completely randomized design with three replicates. Each treatment was irrigated daily with water. Germination counts was measured daily for 14 days. The germination rate was determined using a modification of Timson's index (Khan & Rizvi, 1994) of germination velocity.

$$\text{Germination velocity} = \sum G/t$$

where G is the percentage of 2-day- interval germinated seeds and t is the total germination period. The greater the index value, the higher the germination rate.

Statistical analysis: Data were presented as means ± standard deviation of triplicate determination. One way analysis of variance (ANOVA) was used to determine the effect of various factors on germination percentage. Significant level was set at 5% probability while Duncan's multiple range test were used to segregate the treatment means. All analyses were done using MINITAB student version 12 for windows statistical soft ware.

Results and Discussion

Seed weight and viability: In this study, the average seed weight was 1.2 ± 0.003 mg and the viability of Lots A and B as determined were 22.6 ± 2.3% and 67.3 ± 5.0% respectively. The low seed viability observed in Lot A could be due to immature embryo of the seeds at harvest, thus, such seeds will require to be stored for ripening processes to occur. This leads to promoting or inhibiting hormones needed for the germination processes. For example, high concentration of Abscisic Acid (ABA) has been shown to delay germination of freshly harvested seeds. The concentration of ABA tends to decrease with time of storage (Rehman & Park, 2000).

The viability of seeds in Lot B was found to be much higher than Lot A. This could be ascribed to the presence of gibberellic acid (GA_3) in the seed. The long term storage promotes biosynthesis of this GA_3 in seeds thereby enhancing germination (Finkeltein *et al.,* 2008). This finding concur with that of Kamotho (2004) who found out that dormancy in freshly harvested seeds of this species was broken by storing the seeds from six months to one year. In another study, a much higher germination was also reported in seeds stored for one year (Kwack & Kang, 1985). It is, therefore, essential to keep freshly harvested ripe seeds of *C. gynandra* in storage for about six months and up to two years. This will improve the shelf life of the seeds and allow the immature embryos to reach their maturity (Ochuodho, 2005).

The results revealed that the highest germination was recorded at 30°C, followed by that of 25°C in Lot B while there was no germination recorded at 10°C in Lot A (Fig. 1). The result showed that germination increased with temperature up to 30°C before it began to decrease in both Lots. The result also showed that the germination was significantly affected by varying temperature regimes. Generally, germinated seeds in Lot B were significantly higher than those in Lot A in all temperature treatments. The rate of germination increases linearly with temperature

within a well defined range (Hegarty, 1977). Hence, the corresponding increase in germination of *C. gynandra* seeds with increasing temperature of 30°C was expected. Enzymes are known to be affected by temperature. For instance, an increase in temperature of 10°C has been shown to double the rate of enzymatic activity (Mader, 1993). This partially accounts for the increase in germination of *C. gynandra* with increasing temperature up to a threshold of 30°C. However, the germination of seeds at 25°C was still worthwhile and the fact that this seed germination occurred at low temperature of 10°C in seed Lot B suggested that the plant can thrive under varied temperature conditions.

Watering regime results indicate that the highest germination was recorded at the bi- weekly watering regime in Lot B, followed by that of once a week and the least germination was observed in Lot A. This investigation has shown that the various watering regimes produced varied effects on the germination of *C. gynandra* seeds. Watering once a week gave intermediate results as shown in Fig. 2. Watering of seeds bi-weekly significantly increased the number of seeds with radicle protrusion compared to daily watering. Seeds watered daily showed low germination. This was due to the seed coat imbibing excess water which was detrimental to the emergence of the radicle (Sesay, 2009).

The effect of light and dark conditions on the germination of *C. gynandra* seeds indicated a high number of seeds germinated in the dark when compared to the light condition (Fig. 3). This is due to the fact that *C. gynandra* seeds are negatively photosensitive when exposed to light for more than 12 h in a day. This response to light exposure could be explained as a survival variation of this species because it is a short day plant requiring at least 12 h of darkness for germination to take place (Ochuodho, 2005).This phenomenon showed that this species has preference for darkness and this should be taken into consideration during field establishment. Gutterman *et al.* (1992) reported similar results on *Amaranthus* species stating that inhibition of seed germination by light seems to be a common phenomenon in wild plants. Seed germination in many plant species is inhibited by continuous white light and such seed germinate well in darkness (Bewley & Black, 1994).

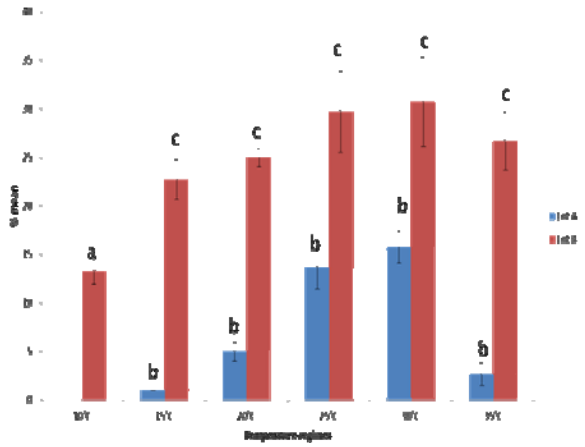

Fig. 1. Effects of varied temperature on germination of *Cleome gynandra* seeds. Different superscript letters showed seed germinations are significantly different ($p<0.05$).

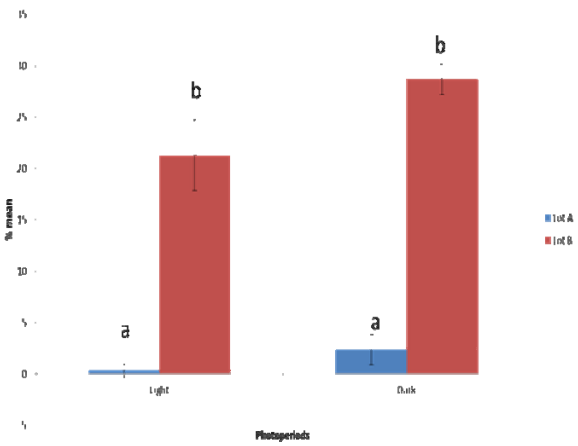

Fig. 3. The effect of light and dark conditions on germination of *C. gynandra* seed. Different superscript letters showed effects of light and dark conditions are significantly different ($p<0.05$).

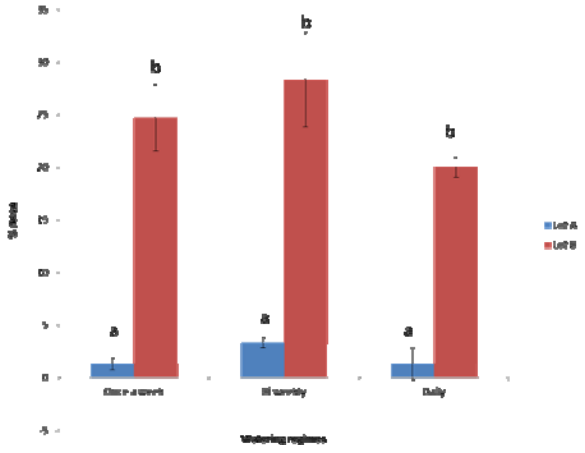

Fig. 2. The effect of various watering regimes on germination of *C. gynandra* seed. Different superscript letters showed effects of watering are significantly different ($p<0.05$).

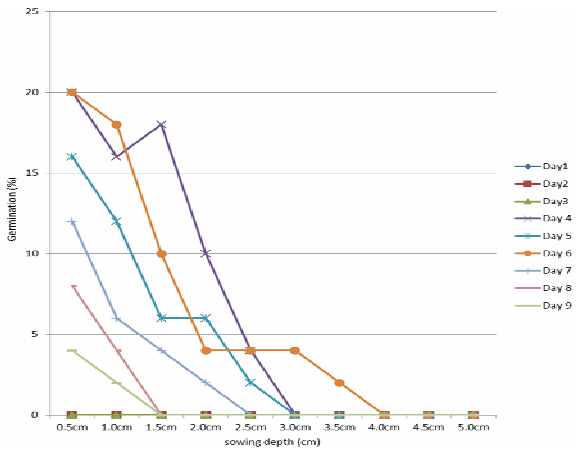

Fig. 4: Relationship between germination and depth at different days after sowing.

At the end of the experiment, the highest seed germination percentage was observed at a depth of 0.5 cm followed by 1 cm and there was no germination at the soil surface (Fig. 4). This occurrence was also observed in 4 cm, 4.5 cm and 5 cm depths respectively. Similar trend has also been observed in the rate of germination of *Echinochloa colonoa* due to flooding (Smith & Fox, 1973). Generally, the rate of germination observed in this study decreased with increasing depth. While Liu Guojun *et al*. (2015) obtained a similar result in *Haloxylon* species having the highest germination percentage at sowing depth of 0.5 cm to 1.0 cm, Chauhan *et al.* (2006) reported that *Galium tricornutum* seeds showed no germination when placed on the soil surface. This could be ascribed to some environmental factors such as soil-to-seed contact, light conditions on the soil surface and water availability (Ghorbani *et al.,* 1999).

Conclusion

This study has shown that the optimal temperature for germination of this species is between 20^{o}C-30^{o}C. The species also germinates better in dark conditions with bi-weekly watering regime at a sowing depth of 0.5 cm. This study also suggests that *C. gynandra* has the potential of thriving successfully, despite the great fluctuations of high and low temperatures in South Africa, characteristic of summer and winter, respectively.

Declaration of interest: The authors declare that they have no competing interest.

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