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RESEARCH ARTICLE

IN VITRO PROPAGATION OF THE RARE *CARALLUMA RETROSPECIENS* PLANT IN EGYPT

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Abstract

The study is an efficient protocol to describe *in vitro* the propagation of an endangered medicinal cactus plant belongs to Apocynaceae, namely: *Caralluma retrospeciens* (Ehrenb.) N.E.Br. naturally grows in some wadis of Elba Mountain at the southeastern part of Egypt. The current study is the first one in Egypt to propagate this type of cacti *in vitro* using seeds. Germination trials through seeds and propagation by cuttings were done. Also photosynthetic pigments; Chlorophyll (a & b), Carotenoid and Anthocyanin were estimated. Result of this study revealed that the germination rate ranging between 30-70% at different rates depending on temperature and storage period. From the results, it is clear that *C. retrospeciens* is vulnerable to extinction and does not spread naturally like any other species, despite its very high yield of seeds, Due to the spread of seeds and their transmission to far distances from the mother plant, due to their light weight or attachment of these seeds to other branches and fall in environments inappropriate for germination. In addition, the pigment in *C. retrospeciens* was differ during winter and summer, most of them are higher in summer and lower in winter. In summer season, Chlorophyll a and Carotenoid had a highest value, while Chlorophyll b and Anthocyanin had a lowest value, vice versa in winter season Chlorophyll b and Anthocyanin had a highest value, while Chlorophyll a and Carotenoid had a lowest value.

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Introduction:-

Caralluma is a xerophytic cactus related to family Apocynaceae that comprise (240 genera and 3400 plant species, including shrubs, woody or herbaceous climbers and herbal parts falling overhead, stem succulents that have a water or milky sap. The members of this family spread throughout the world (Worldwide) especially in the Tropical or Sub Tropical regions where the environment is suitable for the growth and prosperity of such plant species. The juicy species in this family represent (61) plant species only (Albers and Meve, 2002).

C. retrospeciens (Ehrenb.) N.E.Br. is a cactus plant with quartet stem, reaching one meter height, growing in stout dense bushes, thick stem with very compacted corners, s Broad deltoid re-curved, succulent, serrated leaves (Täckholm, 1974) it is green at the beginning of their formation and then tend to brown later, at the beginning the seed germinates and the embryonic peduncle emerges in the form of an inverted triangle with its base up and its head down. Also the lower part called hypocotyl with a length of approximately 5-8 mm, appears on both sides of this part, one leaf at each end, then perpendicular crosses appear on both sides of the base, each carrying one leaf where the four leaves appear from a vertical crossed perspective. The area of a single leaf reaches from 1.5-2 mm.

The size of these leaves gradually decreases with the development of the flowering process and the increase in the size of the inflorescences until they become a remnant (Boulos, 2000). It is found in some valleys of Elba Mountain, where it grows between rocks and sometimes rare grows in the course of the valley where the gravel soil arising from the decomposition of rocks (Abdel Ghani and Abdel Khalik, 2006)..

The etymology of '*Caralluma*' is derived from the Arabian word, 'qarh al-luhum', meaning wound in the flesh or abscess. *Caralluma* is also regarded as the synonym of genus *Boucerosia*; however, the only difference between the two genera is the arrangement of floral parts (Stewart, 1972). Plant species belonging to the genus *Caralluma* have normally small caducous leaves. Mostly, they are succulent perennial herbs, some of which are reported as edible species (Naik and Krishnamurthy, 2012). Various medicinal uses of *Caralluma* species have been documented in the Arabic and Indian traditional medicine including treatment of diabetes, cancer, tuberculosis, snake and scorpion bites, skin rashes, scabies, fever, and inflammation (Aruna *et al.*, 2009). Most common uses of this genus have been recorded as a famine food without any reported adverse effects till date.

Species that belong to subfamily Asclepiadoideae have poor seed germination and viability. *Caralluma* species are adapted to sunny conditions with good aeration in controlled conditions (Albers and Meve, 2002). Humidity in excess is not needed and thus can be grown outdoors in mild regions with the temperature not less than 10°C; otherwise, they can be grown in a greenhouse or window garden. An ongoing experiment on *C. tuberculata* has shown some good results so far under the shade in controlled conditions at Kohat, Pakistan. *C. tuberculata* is also used for ornamental purpose in various areas of Pakistan (Ali, 1983). Most suitable methods of propagation are through seeds and cuttings, while division is considered as the preferable one. Usually, there are few roots at the bottom of shoots, which can be detached and immediately planted in small pots. The cuttings are inserted in sandy soil to reproduce roots in controlled conditions. Irrigation is only recommended when the soil become dry and would be continued until roots are formed (Samyudurai and Thangapandian, 2012). In case of plants being raised from the seeds, pots are filled with pieces of gravels and sandy compost. Later, the seeds are sowed with a slight cover of soil. The pots are then placed in a controlled environment with 55°C temperature and are covered with a transparent material until sprouting. As the seedlings attain a certain height, they can be transplanted in a pot of sandy soil keeping plant to plant distance of 1 inch (Mustafa, 2014).

In despite of its very high yielded of seeds, *Caralluma* species vulnerable to extinction and does not naturally spread due to (1) very light weight of seeds that enable them to transmit a distance far off from the mother plants; (2) presence of long fluff (pubescence), seeds can be attached to tree branches and fall on an inappropriate habitats for germination. For the above mentioned reasons, the present study was done to verify the ecological bearing of photosynthetic pigments, especially anthocyanin, in accommodation *Caralluma* plant to such very drastic habitats. Also, it is the first attempt to propagate a cactus plant through germination of seeds in Egypt. Further, this research will highlight the *in vitro* propagation of *C. retrospeciens*

Materials and Methods:-

Seeds and plant collection

Mature Seeds and cuttings of *C. retrospeciens* were collected from wadi Izab, Elba Mountain in Egypt. It extends between the longitude of 35 west to 36 east, and the latitude of 23 north to 22 south, running along a length of about more than 100 km in the western region of the Elba mountain ranges and runs towards the northeast until it reaches the coast of the Red Sea, consisting of a large fan (Zahran and Willis, 2009).

Phenological observation

During the period of field work in 2015-2016, phenological observation of *C. retrospeciens* made by monthly visits. Six stages of phenology were recorded as follow: vegetative, flower buds, flowering, fruiting (unripe), fruiting (ripe) and dry. Growth Percentages were recorded in a quadrat of (5×5) m² in each selected stand (Abdelraouf *et al.*, 1996).

Germination and propagation

It is difficult at all to obtain seeds of *Caralluma* plants, so some cuttings were brought from their natural habitats and were planted in nursery at the Desert Research Center to be under control and seeds can be easily collected. The collected seeds were planted in pottery pots (40×30 cm) size, filled with sand and clay (1:1v). Germinated seeds were followed until seedling phase (Schopmeyer, 1974.).

Physiological studies and propagation

Seasonally, during summer and winter, fresh plant materials were collected in glass packages containing 80% acetone solution. Then crushed and subjected to measure chlorophyll (a & b), carotenoid compounds and anthocyanin pigments according to (Sims and Garmon, 2002).

Results and Discussion:-

Phenological aspects

C. retrospeciens is widely distributed in Tropical or subtropical regions such as Elba Mountains, southern Eastern part of Egypt. It dominates open wadis with sandy and gravelly soil surface as Wadi, Izab. During the first (main) period, flowering is in March to May and fruiting in May and July. The second flowering period in July and August and fruiting in September. Seeds can be collected during July and August (Fig. 1). Amateur shrub produces about 55-75 pods each pod with 25-28 seeds. These results agreed with (Albers and Meve, 2002; Kishor *et al.*, 2010; Mahmoud *et al.*, 2010).

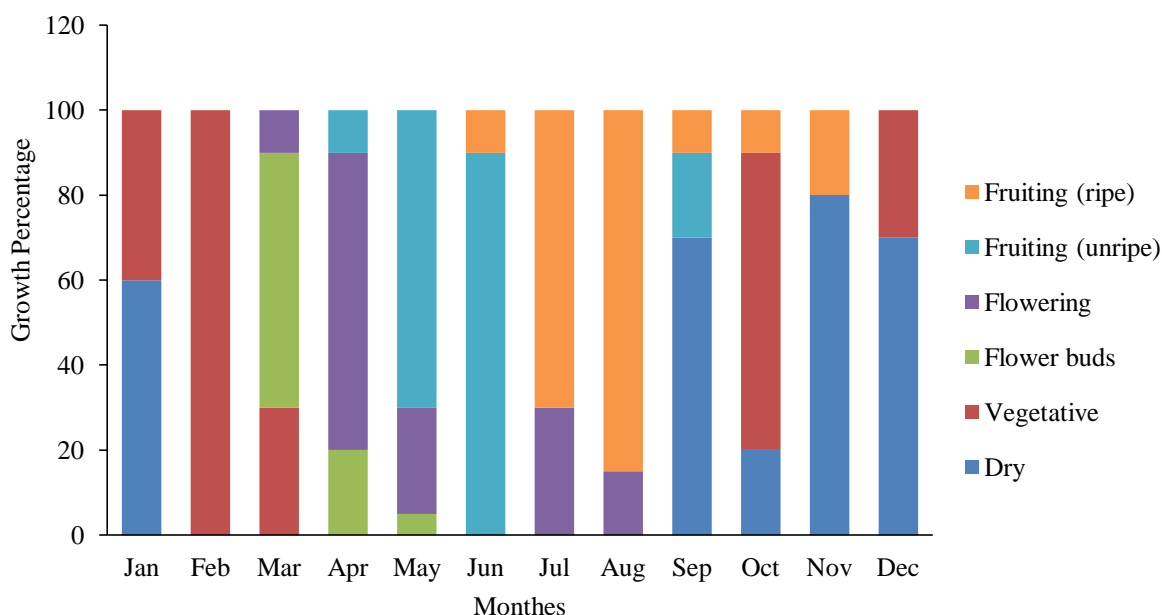


Figure 1:- Phenology aspects of *C. retrospeciens*.

Germination and Propagation

The germination rate ranging between 30-70% at different rates depending on temperature and storage period, when the seeds were germinated in Petri-dishes. The germination percentage of recently harvested seeds reached to 80% at temperatures ranging between 25-35⁰ and they decreased to 40% at temperatures between 18-25⁰. While, after two months of storage, the germination rate increased (70-80%) at temperature decreased between 25-35°. After a period of approximately one year, the germination rate increased (40-60%) (Fig. 2). From the previous results, it is clear that the *Caralluma* is vulnerable to extinction and does not spread naturally like any other plant species, despite its very high yield of seeds. Due to the spread of seeds and their transmission to far distances from the mother plant, due to their light weight or attachment of these seeds to other branches and fall in environments inappropriate for germination as (Kishor *et al.*, 2010; Mahmoud, *et al.*, 2010). Extreme high temperature, such as in a fire can increase germination and emergence of species.

Flowering and fruiting

Caralluma plants are flowering four times per year, according to the emergence of modern vegetative growths of the plant. Every modern vegetable growth is given one inflorescence in the form of a tent that contains many flowers. These growths appear in four stages throughout the year starting from March to October. The flowering stage, through fruiting and seed propagation, takes approximately three months at high temperatures (spring and summer) and extends to nine months in the remainder of the year, meaning that there is a clear overlap in the flowering and fruiting processes. This timing difference may be due to the difference or variation in temperature throughout.

Brown flowers tend to blush, grouped in terminal tents, and one tent contains approximately 55-75 flowers. The number of flowers increases according to the size of the tent. Few of these flowers continue to complete the life cycle (fruiting and seed formation) by a rate ranging from 4-10% of the total number of flowers, with a decrease in temperature. This percentage decreases noticeably until they are absent at the beginning of the winter. The flower when it opens is about 15 mm wide and the height from the beginning of the pink rhombus from 18-20 mm (Fig. 3). Usually the flower is five-petal, but sometimes very rarely the flower appears hexagonal or seven-petal. These mutations may be mutations unknown. Fruits paired follicles, cylindrical, green with alternative and interfered-stratified green dark spots as the findings of (Albers and Meve, 2002; Kishor *et al.*, 2010; Mahmoud *et al.*, 2010).

The pod is hooked at the top, reaching 8-10 cm in length. Duplication of the fruit means that the single pink rasp carries two numbers of fruits (the fruit is divided into two) and very rarely is the single (Kishor *et al.*, 2010) the fruit contains (pod) of 25-28 brown seeds pressed at the ends connected to it White fluff with a cottony texture at the point of the hilum pit. The length of this fluff reaches about 2.5 cm, as it helps in the speed of transmission, the spread of light seeds to long distances by wind. The seed is very light, with a weight of 100 seeds reaching approximately 1g (Fig. 4). At the end of the growth stage, receptacles remain as traces on the old branches of the *C. retrospeciens* (Fig. 5). These results agreed with (Albers and Meve, 2002; Kishor *et al.*, 2010; Mahmoud *et al.*, 2010).



Figure 2:- Seed germination and flower shape of *C. retrospeciens*.



Figure 3:- Flowering stage of *C. retrospeciens*.



Figure 4:- Fruiting and pod shape of *C. retrospeciens*.



Figure 5:- Receptacles as remnants on the branches of *C. retrospeciens*.

Physiological studies and propagation

The results obtained in table (1) showing the determination of pigments during summer and winter. The pigment in *C. retrospeciens* was differed during winter and summer; most of them were higher in summer and low in winter. Chlorophyll a and Carotenoid have a highest value (3.12 mg/ml and 1.09 mg/ml, respectively) in summer season and lowest value (1.53 mg/ml and 0.63 mg/ml, respectively), while Chlorophyll b and Anthocyanin had a highest value (0.7 mg/ml and 0.31 mg/ml, respectively) in winter season and had a lowest value (0.55 mg/ml and 0.089 mg/ml, respectively) in summer season. Result of the present study clearly showed that the photosynthetic pigments differed in between them and between the two seasons of the study. The most permanent point of this investigation was the evident progressive increasing of the red anthocyanin pigment during winter where very low temperatures dominate. The study suggests that anthocyanin was responsible for warming the plant tissue. This phenomenon accommodate the plant to survive during very low temperature seasons. This result is the same in the finding of (Sims and Garmon, 2002 and Roobha *et al.*, 2011), who determined that the Anthocyanin pigment destructed in high temperature.

Table 1:- Effect of seasonal temperature of *C. retrospeciens*.

Items	Chlorophyll a mg/ml	Chlorophyll b mg/ml	Carotenoid mg/ml	Anthocyanin mg/ml
Seasons				
Winter	1.533	0.70	0.631	0.316
Summer	3.122	0.554	1.09	0.089

Conclusion:-

Caralluma is an endangered medicinal plant has a therapeutic importance for many diseases, so that *Caralluma* plants need more studies for propagation. This study is the first one in Egypt to propagate this type of *Caralluma* *in vitro* using seeds. In addition, it gives an evidence on effects temperature and storage period on the seeds germination, also, the photosynthetic pigments differed in between them and between the two seasons of the study.

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