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Proceedings edited by the National Organizing Committee of 2021 IGC/IRC Congress

Published by the Kenya Agricultural and Livestock Research Organization

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Habitat, phenology, and seed studies of *Citrullus colocynthis* in Lut Desert, Iran

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Keywords: colocynth, medicinal plants, desert restoration, seed morphology, plant development

Abstract

Colocynth is one of the major medicinal plants that naturally grows in deserts of Middle East and North Africa. Extending cultivation of this species is useful for sand dune fixation and for making incomes for the needy inhabitants. Despite of numerous studies on seed germination, there are still debates on best method of seed dormancy breaking for colocynth. Moreover, seed morphology, phenological stages, and habitat conditions of this species are not fully studied. In a flat plain of Gonabad, located at the edge Lut desert, we established line transects of 200 m and plots of 4×4 m, where vegetation parameters, phenology, and soil characteristics of Colocynth habitats were studied. Soil of study area was classified as slight alkaline, where pH varied between 7-7.5 in open and 8-8.11 under the canopy of colocynth. Two weeks pre-chilling at 5°C and day/night temperature range from 25-40°C significantly increased seed germination. In the study area colocynth was detected as a perennial herb with long and ligneous roots (>120 cm depth). Its vegetative growth starts at mid-May, seed ripening and shedding occur in October and November respectively. Average fruit volume was 2.62 m³ and seed number counted in each fruit varied between 250 to 420 by the length of 4 to 7 mm and 2-4 mm width. Bigger size fruits contained highest seed number, hence the full maturity stage is best harvesting time in terms of both economic value and seed viability.

Introduction

Citrullus colocynthis is an important medicinal herb (Adam, et. al, 2001) that is adapted to the arid environments of Middle East and North Africa (Shafferman, et al., 1998; FAO, 2017). According to studied sources-Colocynth is important for its seeds oil, fruit, and pulp materials. Poor seed germination is one of the main problems in propagation of colocynth, hence several studies have been designed and performed to solve this problem. The effects of applying KNO₃, gibberellic acid, pre-chilling, scarification (by using sulfuric acid and sandpaper), and day/night temperatures have been tested on seed germination of colocynth (Abdollahi et al., 2010; Ebadi and Miri, 2013; Ghasemi and Masoumi, 2014). Miri et al. (2013) found that 12-week stratification and alternates temperatures of day and night (35 and 15 degrees) were the best treatments. Whereas, Abdollahi et al. (2010) reported scarification by sulfuric acid; and 6 weeks prechilling as the best seed dormancy breaking treatments. Therefore, previous researches indicate both physiological (immature seeds) and morphological (hard seed coat) aspects as the main reasons for low germination of colocynth, but there are still controversies on the best seed germination treatments. Sen and Bhandri (1974) studied regeneration of colocynth in natural habitats and concluded poor regeneration rates of this species. They also found that by producing adventitious roots at older nodes of colocynth, the new plants would be reproduced in the next season and improve propagations. Once the new plants are established in the desert, the propagations persist by seeds years after years. Despite of a rich literature on medicinal properties and seed germination requirements of colocynth, there are still debates on the best seed dormancy breaking treatment. Furthermore, geographic distribution, seed morphology, and phenology of this species are not intensively studied. Therefore, our research was aimed on studying the autecology of colocynth in Lut desert of Iran.

Material and Methods

Botany: *Citrullus colocynthis* (L.) Schrad (Colocynth) as a perennial forb has rough angular stem with fleshy and rough leaves. The blooms are solitary pale yellow. Each plant produces about 15 to 30 round fruits (almost 5-6 cm) with extremely bitter taste. The calyx englobes the yellow-green fruit which becomes marble (yellow stripes) at maturity. The mesocarp is filled with a soft, dry, and spongy white pulp, in which the seeds are embedded. Each of the three carpels bears six seeds. Seeds are brown and 5 mm long by 3

mm wide. They are edible but similarly bitter, nutty-flavored, and rich in fat and protein. They are eaten whole or used as an oilseed (Rechinger et al., 1997). Both male and female flowers are on same plant.

Distribution: *Colocynthis* is widely distributed in the Sahara-Arabian, Africa, and the Mediterranean regions including the countries of Cyprus, Syria, Lebanon, Jordan, Iran, Turkey, Afghanistan, Pakistan, India, Israel, and Egypt (Shafferman et al. 1998). This species occurs in many places in Middle East, including the hot deserts, in sandy soils and in wadies. In Iran, this species occurs in Gonabad, Bajestan, Sarakhs, Zaboul, Zahadan, and Kerman. (Fig. 1).

Study area: Our study area was located at longitudes of 58° 33' 24" E and at latitudes of 34° 35' 8" N with the elevation of 1105 m in Gonabad, Iran.

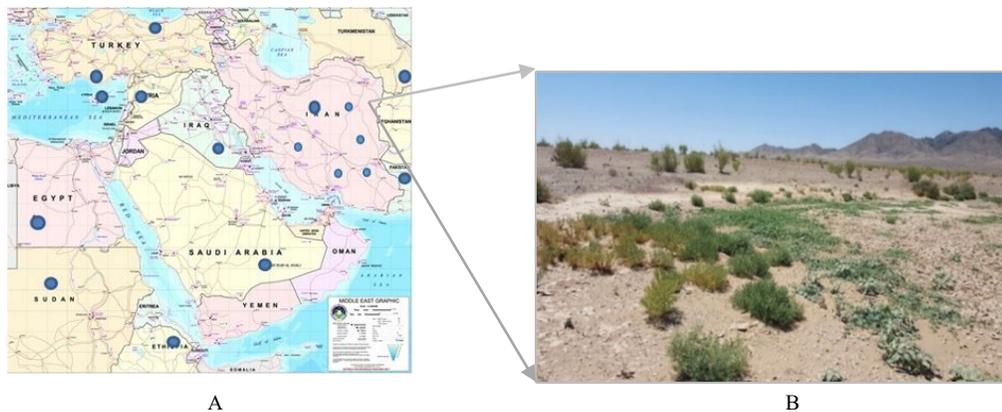


Fig.1. A. Distribution of *Colocynthis* (blue circle) in Middle East (Robinson and Decker-Walters, 1997) and B. Natural habitat of *C. colocynthis* in the flooded terrain of Gonabad Desert, Iran.

Sampling: To measure the vegetation of study area, a representative stand of *colocynthis* habitat was selected. Based on the dimension of habitat which occurred in a flooded catchment, 5 line transects of 200 m length were established vertical to the slope down to the catchment. Plots of 4×4 m were established at intercepted plants of *colocynthis*. Vegetation parameters of plant cover and litter were measured at early stage of flowering (July-August, 2016 and 2017). The phenological stages of *colocynthis* were recorded at every two weeks during growing season (May-November) of 2016 and 2017. Seeds of *colocynthis* were collected in early November of 2017. Soil samples were collected at depths of 0-30, 31-60, 61-90, and 91-120 cm in understories of *colocynthis* and in adjacent open area (1-meter distance from plants). The soil characteristics of texture, moisture, pH, EC, sodium adsorption ratio (SAR), exchangeable sodium percentages (ESP), and organic matter were measured (Kim 2005).

Seed viability and germination tests: Seeds were washed by distilled water and tested by tetrazolium chloride in 24 hours for their viability. To simulate seed germination of *colocynthis* in its natural habitat condition and based on the previous experiments (e.g. Ebadi and Miri 2013.), our experiments were conducted as separate tests under treatment of temperature ranges of 14-27, 15-32, and 25-40°C. Lower and higher temperatures were for nights and days, respectively. Pre-chilling (keeping seeds for 10 days in 5 °C.) and control treatments were applied with 20 seeds in each of 3 replicates. Mean germination time (MGT) was measured (Copeland and McDonald 2001).

Statistical analyses: Germination percentages and mean germination time (MGT) of three temperature levels under control and pre-chilling treatments were separately analyzed by using one-way ANOVA. The relationships between cumulative values of germination speed and germination days were compared for control and pre-chilling treatments using group regression linear model. To estimate the number of seeds in each fruit of *colocynthis*, 10 fruits were randomly selected and their diameter and volume were measured. The fruits were dissected to count the number of seeds. The relationship between volume and number of seeds were analyzed by using simple linear model.

Results

Habitat conditions: The study area was classified as sandy loam with slight saline, where pH varied from 7 to 7.5 in open areas and from 8 to 8.11 in closed canopy of colocynth. The organic matter varied from 0.22 % in bare soils to 1.41% in closed canopy. Soil moisture varied from 0.73% at the upper layers to 4.56% at the lower layers of 120 cm.

Phenology: This species starts vegetative growth at mid-May. Floral buds appear from mid-July but full flowering stage happens at late August. Fruits appear at mid-July and ripe in September. Finally seed ripening and shedding occur in October and November respectively.

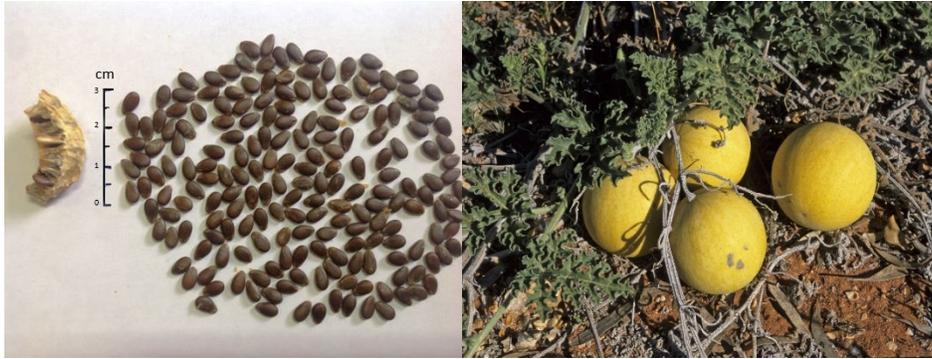


Fig 2. Sample of colocynths seeds showing a part of mesocarp and the brownish ripped seeds.

Seed Studies: The seeds characteristics of Colocynth are shown in (Fig. 2). The number of seeds per plant were 250 to 420 and the length and width of seeds ranged 4-7 and 2-4 mm, respectively. There was a linear relationship between volume of fruits and seed number in each fruit with correlation of 0.95, that is, number of seeds positively and linearly increased with fruit volume.

Germination: The mean germination speed and number of germinated seeds were compared for colocynth at three temperature ranges. Accordingly, the means of germination time and number of germinations were significantly ($P < 0.05$) higher under 25-40°C than other treatments (i.e. 14-27 and 15-32°C). The relationship between time (days after seed sowing in Petri dishes) and germination speed and also, time and germination number of colocynth was investigated by using regression groups for control and prechilling treatments (Fig.3a and b). For germination speed, there was no significant difference between control and prechilling treatments (Fig.3a), hence the two regressions were combined and a quadratic relationship was presented. However, prechilling treatment had increased the seed germination of colocynth, which led to significant difference in the relationship between days after sowing and germination time under the control and prechilling treatments.

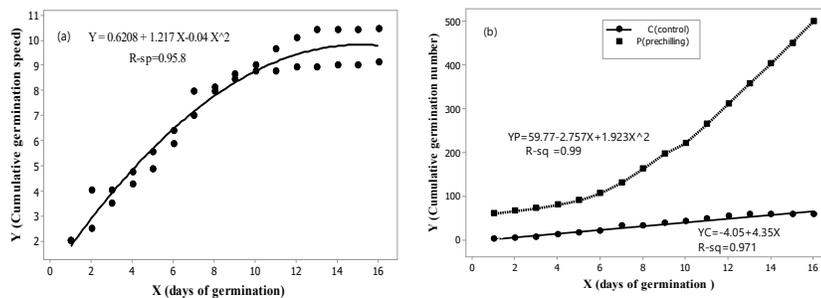


Fig 3. (a) The relationships between cumulative germination speed and time and (b) between cumulative germination number and time.

Conclusions

Colocynth is a highly adapted plant to the desert conditions with alkaline soil. It has a potential source of edible oil and high medicinal value hence can be a source of income for the resource poor local inhabitant. It is a perennial herb with extensive root system. The seasonal growth stage starts at mid-May and the growth continues till mid-autumn, when its fruits matured and seeds shed at early November. The best temperature ranges for germination of colocynth in the study area was 25 to 40 °C from night to day, which was close to daily temperature regime of its habitat at the time of seasonal growth. A linear relationship between fruit size and seed number indicate the best harvesting time (for economic value being at the latest growth stage in Mid-November).

Acknowledgements Financial support for this research was obtained by grant no 41810, by Ferdowsi University of Mashhad, Iran.

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