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Research and Full Length Article:

Autecology of Colocynth (*Citrullus colocynthis* L. Schrad) in Gonabad Desert, Iran

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Abstract. Colocynth (*Citrullus colocynthis*) is one of the major medicinal plants, naturally growing in deserts of Middle East and North Africa. Extending cultivation of this species is useful for sand dune fixation and livelihood of the local inhabitants. In spite of numerous studies on seed germination, there are still debates on the best methods of breaking seed dormancy for colocynth. Moreover, seed morphology, phonological stages and habitat conditions of this species is almost unravelled. This study was conducted in 2016-2020 at Gonabad desert, Iran. We established five line transects of 200 m and five plots of 4×4 m, and vegetation parameters, phenology and root morphology, soil charactristics of colocynth were measured. The pH varied between 7.0-7.5 in bare soil and 8.0-8.1 under the canopy of colocynth habitat. The soil of study area was classified as slightly saline. Two weeks pre-chilling at 4°C and night -day temperature range from 25-40°C significantly increased seed germination. In our study area, colocynth was detected as a perennial forb with long and ligneous roots (more than120 cm). Its vegetative growth starts in middle of May, seed ripening and shedding occur in October and November. Average fruit volume was 2.62 cm^3 and seed number counted in each fruit varied between 250 to 420 by the length of 4-7 and 2-4 mm width. Big size fruits contained the highest seed numbers. The best harvesting time in terms of both economic value and seed viability is ripening of fruits.

Key words: Medicinal plants, Plant development, Seed morphology, Seed dormancy, Life duration

Introduction

Citrullus colocynthis L. Schrad is an important medicinal herb (Mardani, 2008; Zargari, 1978) that is adapted to the arid environments of Middle East, West Asia, and North Africa (Schafferman *et al.*, 1998; Ghahreman, 1978; Jayaraman *et al.*, 2009; Amamou *et al.*, 2011; Taxiera da silva and Hussain., 2017). Essential oil of *Citrullus colocynthis* is useful for biological pests control such as improved whitefly resistance in watermelon cultivars (Ramzi *et al.*, 2013; Coffey *et al.*, 2015). There are also numerous studies on medicinal properties of colocynth which according to them this plant is important for its seed oil, fruit, pulp materials, and some diseases specially diabetes (Althawadi *et al.*, 1986; Amamou *et al.*, 2011; Hussain, 2014; Taxiera da silva and Hussain., 2017; Sargezi, 2014; Soltani and Mohammad-zadeh, 2015).

Poor seed germination is one of the main problems in propagation of colocynth. Therefore, several studies have been designed and performed to solve this problem. The effects of applying KNO_3, gibberellin acid, pre-chilling, scarification by sulphuric acid, and sandpaper with day and night temperatures have been tested on seed germination of colocynth (Chomicki and Renner, 2014; Abdollahi *et al.*, 2010, 2011; Ebadi and Miri, 2013; Miri *et al.*, 2012; Tavassoli *et al.*, 2015; Soltani and Mohammad-zadeh, 2015; Ahmadi *et al.*, 2015; Ghasemi and Masoumi-asl, 2014; El-Keblawy *et al.*, 2017). Based on the studies of El-Keblawy *et al.* (2017), germination of *C. colocynthis* is very sensitive to light and incubation temperature as well as to the environmental conditions associated with the time of seed maturation. The collected seeds of March almost did not germinate in both light and dark at the three temperature ranges of 15-25, 20-25, and 25-35 ºC. At the lowest temperatures, seeds of all collections did not germinate in light, but those of June, October, and December collections were germinated in dark (El-Keblawy *et al.*, 2017). Chomicki and Renner (2014) reported under natural habitat, seeds shed in late November, and the area experiences 3 to 5 months cold temperature during late autumn and winter. Miri *et al.* (2013) found alternate temperature of day and night at 35 and 15ºC and 12-week stratification as the best treatments. Abdollahi *et al.* (2010) mensioned that scarification by sulfuric acid and 6 weeks pre-chilling were the best seed dormancy breaking treatments. Therefore, in previous studies, both physiological (immature seeds) and morphological (hard seed coat) aspects were the main reasons for low germination of colocynth. But, there are still controversies on the best seed germination treatment. Sen and Bhandari (1974) studied recruitment in the natural habitats of colocynth and concluded poor regeneration rate of this species. They found the main regeneration method by production of adventitious roots at older nodes, so a number of new plants are produced in the next season and help to improve plant propagation. Once the Colocynth was established in the desert, the plants will perennate year after year by vegetative propagations as well as by seeds. Results of Sabri *et al.* (2017) have been shown that the root depth and branch length of this species in Zabol habitat was 2 meters. Mainwhile, 48 hours of hot water soaking was identified as the best treatment for breaking seed dormancy and improving germination of *Citrullus colocynthis*. The result of Guzzon (2018) studies provided novel insights about drought avoidance adaptive traits, and detected *Citrullus*, linked with the plants growth environments. Levi1 *et al.* (2017) proved that *C. colocynthis* can be a viable source to introduce biotic and abiotic stress resistance genes into cultivated watermelon.

In spite of rich literature on medicinal properties and germination requirements of colocynth, there are still debates on the best seed dormancy breaking treatment.

Furthermore, seed morphology, phonological stages, and habitat conditions of this species are almost unravelled. Therefor, this research aimed to study these ecological aspects of colocynth at desert of Gonabad in Iran.

Materials and Methods

Botany of colocynth

Citrullus colocynthis L. Schrad (common syn. names: colocynth, Aboujahl watermelon, bitter apple, and wild gourd) belonged to the Cucurbitaceae family and its genus, species, and varieties are shown in Table 1.

Table 1. Family, species, and varieties of colocynth (Taxiera da silva, 2017; Chomicki and Renner, 2014; Zargari *et al.*,1978)

Family	Genus and Species	Varieties
Cucurbitaceae	*Citrullus amarus* Schrad	
	Citrullus mucosospermus (Fursa)	
	Citrullus colocynthis L. Schrad	*lanatus* (Thunb.) Matsum. & Nakai
	Citrullus colocynthis L. Schrad	capensis (Alef.) (Alef.)
	Citrullus colocynthis L. Schrad	*insipidus* (Pangalo.) Fursa
	Citrullus colocynthis L. Schrad	*stenotomus* (Pangalo.) Fursa

This perennial forb has rough angular stem with fleshy and rough leaves. The blooms are solitary pale yellow. They are monoecious, so the male (stamens) and the female reproductive parts (pistils and ovary) are born in different flowers on the same plant. In the male, flowers' calyx is shorter than the corolla. They have five stamens, four of which are coupled and one is single with monodelphous anther. The female flowers have three staminodes and a three-carpel ovary. The two sexes are distinguishable by observing the globular and hairy inferior ovary of the female flowers. Each plant produces about 30 round fruits with almost 5-6 cm extremely bitter taste. The calyx encloses 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. Each plant produces 15 to 30 fruits. Seeds are brown and 5 mm long by 3 mm wide.They are edible but similarly bitter, nutty-flavoured, and rich in fat and protein. They are eaten whole or used as an oilseed (Saberi *et al.*, 2011; Ghahreman, 1978; Rechinger *et al.*, 1977; Zargari *et al.*, 1978).

Geographic distribution

C. colocynthis is originally from tropical Asia and Africa. But it is now widely distributed in the Sahara-Arabian, Africa, and the Mediterranean regions included the countries of Cyprus, Syria, Lebanon, Jordan, Iran, Turkey, Afghanistan, Pakistan, India, Israel, and Egypt (Schafferman *et al.*, 1998). *C. colocynthis* occupies sand dunes and wadi habitat in hot deserts of Middle East.

In Iran, this species occurs in many deserts such as Gonabad, Bajestan, Sarakhs, Zabol, Zahedan, and Kerman (Fig.1). Based on studies of Schafferman *et al.* (1998) in Negeve desert, the growth of colocynth starts in late March and April, blooming between May and August and its fruit ripens in November and December.

Study area

The study area is located in Gonabad at longitude of 58°33'24" E and at latitude of 34°35"8' N with the elevation of 1100 m. Colocynth usually grows in ephemeral streams of flood plains at the slops less than 5 precents with no specific aspect. Using data of Climatological Research Institute of Khorasan Razavi (2017) for the city of Gonabad, average rainfall in the study area is 155 mm. Average annual temperature is 14.2 ºC. The minimum and

maximum temperature are -1.6 and 44.6, respectively. According to Domarten method, the climate of Gonabad is classified as arid.

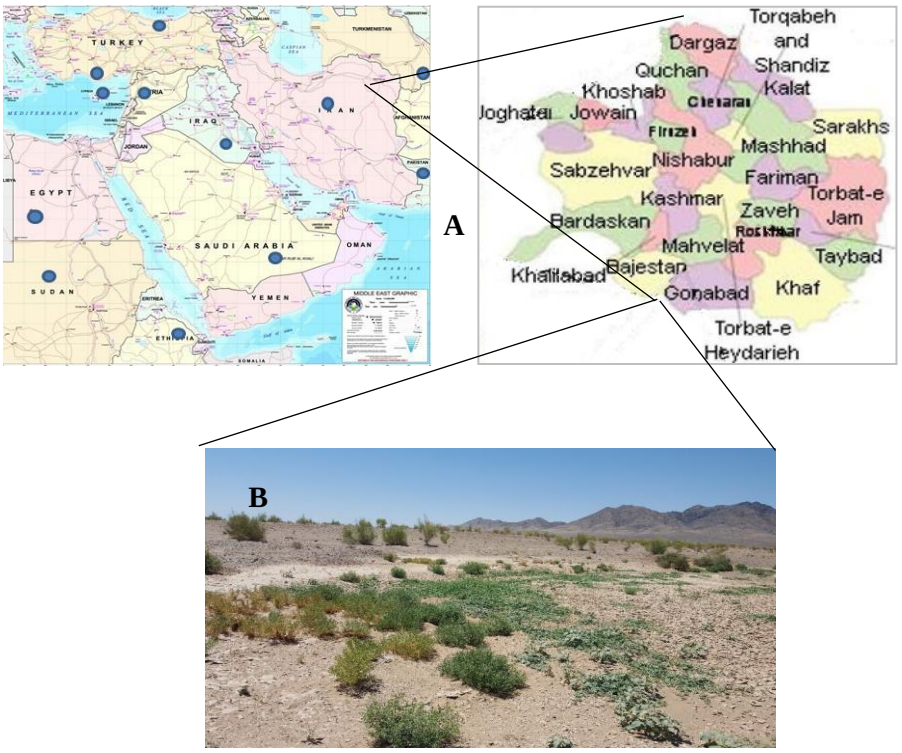

Fig. 1. Distribution of *Citrullus. colocynthis* in Middle East and in Iran. **A.** The map is from Asia Atlas based on Robinson and Decker-Walters (1997); **B**. Natural habitat of *C. colocynthis* in the flooded terrain of Gonabad Desert, Khorasan Province, Iran. The left of study species is *Peganum harmala* and in background *Haloxylone persicum* is shown.

Sampling method

To assess vegetation of the study area, a representative stand of colocynth habitat was first selected. Based on the dimension of habitat which was occurred in a flooding catchment, five line transects of 200 m were established vertical to the slope down to the catchment. Based on the average size of the colocynth stands (about 10 m^2), five quadrates of 3×4 m were established along each transect which are intercepted with the Colocynth stands. And vegetation parameters such as plant cover, litter, and bare soils were measured in full flower stage in July-August, 2016 and 2017.

The phenological stages of colocynth were recorded every two weeks during the middle of May to early November as described for forbs (Lieth, 1974; Sargezi, 2014, and Haratirad *et al.*, 2016). Seeds of colocynth were collected at the stage of seed dissemination in early November of 2017.

To estimate the number of seeds in each fruit of colocynth, 10 fruits were randomly selected and their diameter and volume were measured. To count the seed numbers, the fruit were dissected.

Three soil samples were collected at each depths of 0-30, 31-60, 61-90, and 91-120 cm from understory and adjacent (1-meter distance) of colocynth. Soil samples were selected along the line transects from understory of colocynth and in the nearby open areas. And soil texture, moisture, pH, Electrical Conductivity (EC), Organic Matter (OM), Sodium Adsorption Ratio (SAR), and Exchangeable Sodium Percentages (ESP=1.95+1.03×SAR) were measured (Head, 2009; Tan, 2005;

Koveyligilaneh and Wahabi, 2011; Ranjbar *et al.*, 2015).

Seed viability and germination tests

Seeds were washed by distilled water and tested by tetrazolium chloride for 24 hours for their viability. Based on the pervious experiments (e.g. Miri *et al.*, 2012; Ebadi and Miri, 2013), to simulate seed germination of colocynth in its natural habitat condition, our experiments were conducted as separate tests and under temperature ranges of 14-27, 15-32, and 25-40 °C (low for night and high temperature for days), pre-chilling, and control treatments with 3 replicates of 20 seeds.

Pre-chilling includes placing on double layered Whatman No.1 filter paper moistened with 5 ml of distilled water in sterilized Petri dishes with 15 cm diameter in the fridge at 4 °C.

Mean germination time (MGT) and seed germination rate (SGR) were also measured by using the folowing Equation:

$$MGT = \sum(d_1 \times n_1 + d_2 \times n_2 + ... + d_k \times n_k / \sum(n_1 + n_2 + ... + n_k)$$

Where: *n* is number of new germinated seed, and *d* is the number of germination day from the beginning of experiment (Copeland and McDonald, 2001).

SGR is calculated by dividing the number of normal seedling to the days to final count (Pire and Simon, 2019).

Statistical analyses

Effects of two temperature treatments of 15-32 and 25-40 °C on seed germination were separately analysed by using paired t-test.

The relationships between cumulative values of germination rate (*Y*) and germination days (*X*) were analysed for both control and pre-chilling treatments by using linear regression equation of $Y = \beta_\circ + \beta_1 X + \varepsilon$ Where: X and Y are regression coeffecients and ε is sampling errors.

The relationships between number of seeds (*Y*) and the volume of fruits (*X*) were analysed by using the same linear regression model which was mentioned above.

Results and Discussion

Habitat conditions

The soil characteristics of the study location in different soil depths are summarized in Table 2. The study area is classified as slightly saline soil, where pH varied between 7.0-7.5 in open area and between 8.0-8.1 under the canopy of colocynth. ECs were more than 4 and ESPs were less than 15% (Table 2). Soil moisture was lowest at the upper most layers (0.73%) and was highest at the depth layer of 120 cm (4.56%). The texture of soils was sandy loam. The organic matter was 0.23% in bare soil and 1.40 % under the canopy of colocynth.

Table 2. Some of soil characteristics in understories and bare grounds of colocynth habitat at Gonabad Desert.

Sample point	Depth (cm)	Soil moisture (%)	OM (%)	pH	EC	ESP
Understory	0-30	0.73	1.91	8.08	8.5	8.47
	30-60	2.04	0.90	8.11		
	60-90	2.90	-	-	-	
	90-120	3.22	-	-	-	
Mean		2.22	1.40	8.10	8.5	8.47
Bare soil	0-30	0.48	0.23	7.08	7.6	7.56
	30-60	0.52	0.22	7.54		
	60-90	1.47	-	-		
	90-120	2.86	-	-		
Mean		1.33	0.23	7.31	7.6	7.56

The cover percentages of Colocynth and accompanied species are shown in Fig 2. *Peganuma harmala* and *Tribulus terrestris* were the major plant species that accompanied colocynth in its habitats. The *Tribulus terrestris* canopy cover was so low to show, so it was eliminated from the pie bar of cover percentage.

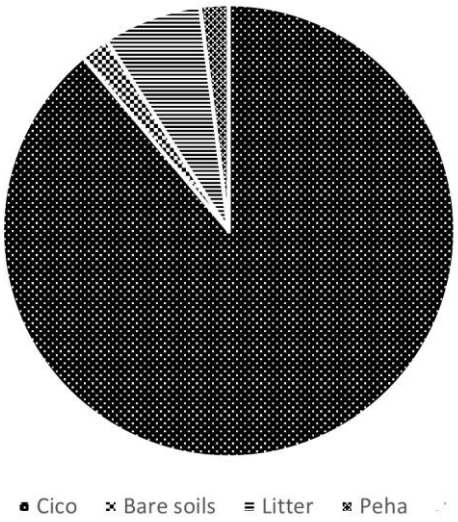

Fig. 2. The percentages of different measured characters: Cico=*Citrullus colothynthis*, Peha= *Peganum harmala*

Phenology and life duration

The phenological stages of colocynth are shown in Fig 3. It starts vegetative growth at middle May. Floral buds appear from middle July but full flowering stage happens at late August. Fruits ripe at October. Finally, seed ripening and shedding occur in October and November.

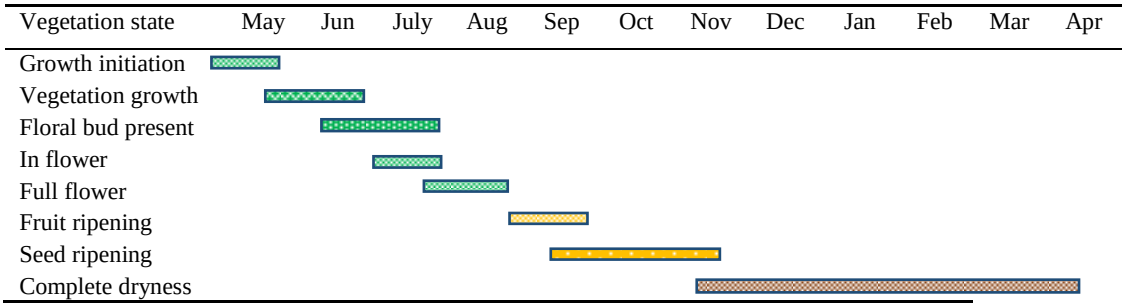

Fig. 3. The phenological stages of Colocynth in Gonabad study area, Iran

We did not find comprehensive studies on phenology of colocynth under field conditions. Ghahreman (1978) indicated flowering of Colocynth at March, without referring to a particular geographic zone. Also, Taxiera da silva and Hussain (2017) reported the flowering season to be in September and fruit maturity in October. Both references show similar phenological periods with colocynth in our study area, and a small difference can be related to the differences in climate condition between Iran and Negev deserts. We could not find any studies about accompanied species of Colocynth, but in our study area, *Peganum maururum* and *Tribulus terrestris* were accompanied species.

There are contradictions on the life duration of Colocynth in literatures. Ghahraman *et al.* (1978) reported it as an annual and Althawadi and Grace (1986) reported it as a perennial forb with extensive root system. In a manual published by CJP (Centre for Jatropha Promotion & biodiesel), it was emphasized

that both annual and perennial species of Colocynth occurs in natural habitats. But based on extended roots, our result confirmed only perenniality of colocynth with long and ligneous roots which was also approved by extensive root system in excavated soil profiles. (Sen and Bhandari,1974; and Althawadi and Grace,1986

Seed morphology and germination

The seed characteristic of Colocynth was shown in Fig. 4. Seed numbers were counted and ranged between 250 to 420 per fruit. The seed sizes were 4 to 7 mm length and 2 to 4 mm width. There was a linear relationship between volume of fruits and seed numbers in each fruit (Fig. 5). The average of fruit volume was 2.62 cm^3.

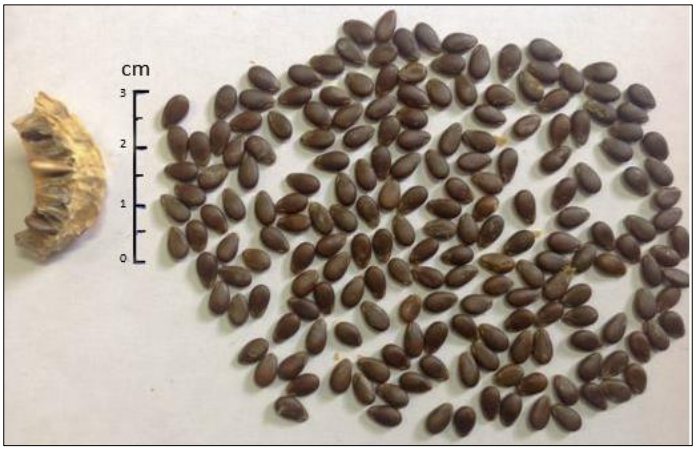

Fig. 4. Sample of colocynth seeds showing a part of mesocarp (left) and the brownish ripped seeds (right)

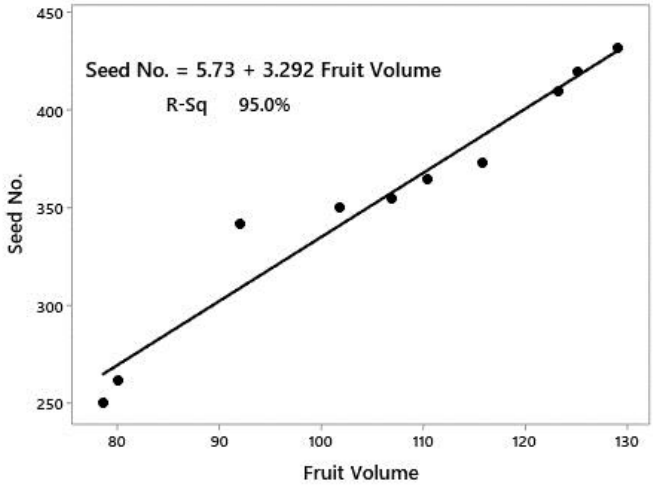

Fig. 5. Relationship between volume of fruits (cm^3) and seed numbers

The results of testing Tetra Zolium on seeds showed 98% viability. There were not considerable seed germination at the temperature levels of 15-32 °C. Many zeros were entered in data causing the skewed distribution so, the analyses were restricted to two levels of temperature and using t-test instead of ANOVA. Both SGR and MGT were significant at 2 levels of temperature ($p<0.05$) (Fig. 6).

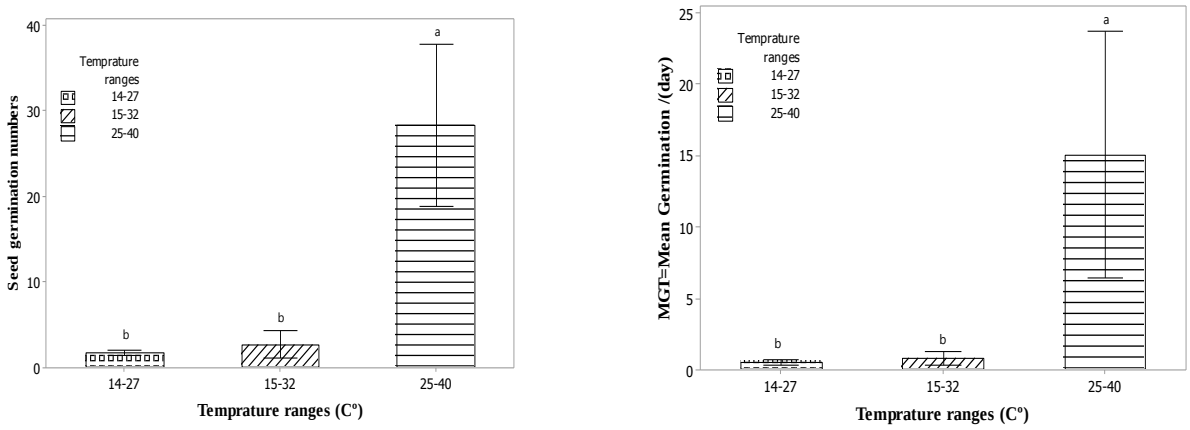

Fig. 6. (left) The seed germination numbers (SGN) and (right) the mean germination time (MGT) were significant at two levels of temperature ($p<0.05$)

The regression relationship was tested between times (days after seed sowing in petri dishes) and germination rate (Figs.7a) and germination number of colocynth (Fig.7b). For germination rate, there was no significant difference between control and pre-chilling treatments (A). Hence, the two regression relationships were combined and a quadratic relationship was presented. However, pre-chilling treatment had increased the seed germination of colocynth, which led to significant difference in the regression relationship between days after sowing and germination time under the control versus pre-chilling treatments.

Based on El-Keblawy *et al.* (2017) in Dubai, testing germination of *C. colocynthis* at the three temperatures showed that in the lowest temperature treatment, seeds of all collections did not germinate in light which was different with our results as we had germinated seed in the same low temperature. The best temperature range for germination of colocynth 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 starts (Ebadi and Miri, 2013; Miri *et al.*, 2012). That could prove the main important role in germination of the species in the habitat that is temperature with high range between day and night. Furthermore, hot water soaking treatment was identified as the best treatment in Saberi *et al.* (2017), for breaking seed dormancy and improving seed germination and seedling growth of *Citrullus colocynthis*. Also, a positive and significant effect of prechilling on seed germination number of colocynths that was also found by Abdollahi *et al.* (2011) who indicate the physiological seed dormancy. As Chomicki and Renner (2014) studies, under their natural habitat, seeds shed in late November, and experience 3-5 month cold temperature during autumn to winter months, which enforce the natural prechilling treatment. However, the seeds we had collected in summer and manually stored in the room temperature needed 3 month prechilling Treatments (placed on double layered Whatman No.1 filter paper moistened with 5ml of distilled water in sterilized Petri dishes with 15 cm diameter in the fridge at 4°C).

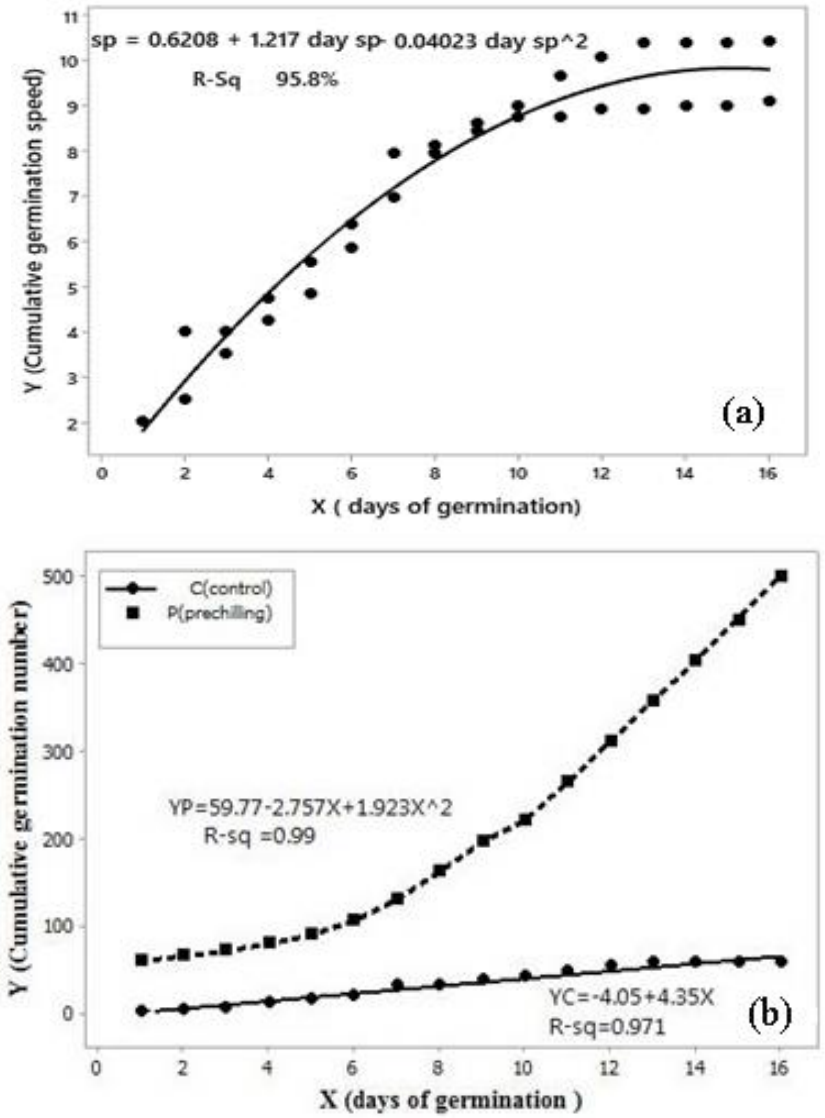

Fig. 7. (a) The relationships between cumulative germination rate and time and (b) between cumulative germination number and time

Conclusions

Colocynth is a highly adapted to the desert conditions in slightly saline soils. In our review of literature, we noticed the potential source of edible oil and high medicinal value of this species. Hence, it can be a source of income for the local inhabitants. Colocynth is a perennial herb forb with extensive root system. The seasonal growth stages start in mid-May and continues till mid-autumn. Its fruits mature and seeds were shed in early November. The best temperature range for germination of colocynth in our study area was 25 to 40°C from night to day, which was close to daily temperature regimes of its natural habitats. A linear relationship between fruit size and seed number indicates that the best harvesting time is at ripening stage in middle of November.

Acknowledgements

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بررسی اکولوژی فردی هندوانه ابوجهل (*Citrullus colocynthis* L. Schrad) در منطقه بیابانی گناباد، ایران

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چکیده. هندوانهٔ ابوجهل(*Citrullus colocynthis*) از جمله گیاهان دارویی مهمی است که به طور طبیعی در بیابان‌های خاورمیانه و شمال آفریقا رشد می‌کند. گسترش سطح زیر کشت این گونه برای تثبیت شن‌های روان و همچنین ایجاد درآمد برای ساکنان محلی مفید است. علیرغم مطالعات متعدد در مورد جوانه زنی بذر، هنوز هم ابهاماتی در مورد بهترین روش شکستن خواب بذر این گیاه وجود دارد. علاوه‌براین، مرفولوژی بذر، مراحل فنولوژیکی رشد و شرایط زیستگاهی این گونه ناشناخته مانده است. این گونه بین سال‌های ۱۳۹۵ تا ۱۳۹۹ در بیابان گناباد ایران مطالعه شد. با استقرار پنج ترانسکت ۲۰۰ متری و پنج پلات ۴×۴ متر، پارامترهای پوشش گیاهی، فنولوژی، مرفولوژی ریشه و خصوصیات خاک این گونه اندازه‌گیری شد.pH خاک لخت بین هندوانه‌ها بین ۷٫۵-۷٫۰ و در زیر تاج آن بین ۸٫۱-۸٫۰ متغیر بود. خاک منطقه در طبقهٔ کم‌شور قرار گرفت. دو هفته پیش‌سرمادهی مرطوب در ۴ درجهٔ سانتی‌گراد و دمای متغیر روز و شب از ۲۵ تا۴۰ درجهٔ سانتی‌گراد به طور معنی‌داری جوانه‌زنی بذر را افزایش داد. در منطقهٔ مورد مطالعه، هندوانهٔ ابوجهل به عنوان یک گیاه چند ساله با ریشه‌های بلند و چوبی (بیش از۱۲۰ سانتی متر) تشخیص داده شد. رشد رویشی این گونه از اواخر اردیبهشت ماه آغاز می شود و رسیدن و ریزش بذر به ترتیب در ماه‌های مهر و آبان رخ می‌دهد. میانگین حجم میوه ۲٫۶۲ سانتی‌متر مکعب، تعداد بذر در هر میوه بین ۲۵۰ تا ۴۲۰ متفاوت بود. طول بذر بین ۴ تا ۷ میلی‌متر و عرض آن بین ۲ -۴ میلی‌متر تغییر می‌کرد. میوه‌های بزرگ‌تر بیش‌ترین تعداد بذر را داشتند و بهترین زمان برداشت هندوانه از نظر ارزش اقتصادی و قابلیت زنده‌مانی بذر، زمان رسیدن میوه‌ها بود.

کلمات کلیدی: گیاهان دارویی، توسعهٔ گیاه، مرفولوژی بذر، خواب بذر، طول عمر