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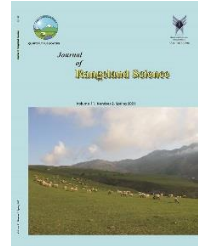

Research and Full Length Article:

Effect of Harvesting Date on Seed Germination and Seed Oil Production of *Salicornia herbacea* L. (Case Study: Gomishan Lagoon, Gorgan, Iran)

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Abstract. *Salicornia herbacea* is a sensitive species to seed shedding. In order to determine the effect of harvesting date on seed shedding, seed germination and seed oil production, an experiment was conducted in Gomishan Lagoon rangelands, Golestan province, Iran. Seed samples were harvested in twenty 1 m^2 plots in 12 times since Nov 6^{th} as the initial date of seed setting until Dec 21^{st} as the final stage of seed maturity in 2019. Sampling was first carried out once a week and increased to every two days at the end of sampling dates. In each plot, the number of shrubs, visual evaluation of plant color changes from green to red, and brown coupled with the weight of the spilled seeds were recorded on each sampling date. Then, the required amount of seeds was randomly taken for the germination test and oil extraction. The obtained data were analyzed using PAST software. The results of analysis of variance showed significant differences between sampling dates on seed germination and oil percent ($p<0.05$). The highest and lowest germination rates with average values of 91% and 98% were obtained in the initial and final stages of harvesting, respectively. Also, the highest and lowest oil yield with average values of 19% and 16% was obtained in the middle and final stages of seed maturity. The results of multivariate analysis showed significant relationships between the seed shedding and plant color (green, red and brown) ($p<0.05$). According to the results, among the visible plant traits, the brown color (dryness) in up to 60% of the plants had the lowest shedding rate and was recommended as a good indicator for determining the suitable date to harvest *Salicornia herbacea* seeds.

Key words: Seed collection, Oil, Seed maturity, Halophyte

Introduction

Among the halophyte plants, *Salicornia herbacea* has been considered for the oil production, forage, and edible vegetables using seawater (Singh *et al*., 2014). It is a wide spread genus in most salty and moist soils at the margin of salt lakes and the salty rivers and the coastal area of the Caspian Sea and Persian Gulf (Ghaffari *et al*., 2006). The *S. herbacea* is an annual plant from the Chenopodiaceae family and the Salicornioideae subfamily (Shepherd *et al*., 2005; Davy *et al*., 2006; Alonso *et al*., 2008). This plant has a spongy stem with small angles similar to leaves, pale flowers and fruits (Patel, 2016). It is halophytic and is known as alkali in Iran (Akhani, 2006). These species are considered food and used in the form of edible vegetables due to their high nutritional values in the minerals and vitamins C and beta-carotene (Lu *et al*., 2001). The *S. herbacea* feeds on salt water and reproduces by self-pollination (Olson *et al.,* 2003). The production potential of *S. herbacea* with seawater has a similar function to the production of *soybeans* with fresh water (Abouheif *et al*., 2000). The *S. herbacea* is rich in dietary fiber and bioactive compounds such as flavonoids and phenolic acids. Many researchers have described glasswort or *Salicornia* as a source of beneficial unsaturated fatty acids. It is comparable to *Carthamus tinctorius* oil in terms of extractable and edible oil production. The use of this oil to produce biofuel in the world is also essential and rather practical (Singh *et al*., 2014). Its seeds (meal) after oil extraction are used to feed livestock and poultry. Its oil is used in traditional medicine to treat such diseases as bronchitis, hepatitis, diarrhea, diabetes, inflammatory and cytotoxic activity. This plant also has antioxidant properties that increase the resistance of the oil to environmental conditions (Isca *et al*., 2014). Moreover, according to the results of Motamedi *et al.,* (2018), halophyte plants can increase the number of nutrients and improve the chemical conditions of the soil around the root system. Nowadays, European and Asian countries are paying attention to this plant so far as the aerial parts of this plant are used in European countries to prepare food and fresh salads, pickles, and drinks in Asian countries (Ahmadi *et al*., 2016). In some societies, the branches of this plant are used in the preparation of a type of nuruk, makgeolli, and vinegar (Song *et al*., 2013; Kim *et al*., 2013). Shin and Lee (2013) made granular salt from *S. herbacea* powder using aquaporin, and they showed that it could be easily used as a substitute for salt in food. The date of seed collection is a pivotal factor in increasing the vigor of seed germination and the quality and quantity of extracted oil from *S. herbacea* seeds. The seed harvest date is usually determined by the amount of moisture or the appearance of the plant (Betty *et al*., 1998). Harvest date is often observed with the change of color in plants from green to red and then brown in *S. herbacea* seed. Relying on the experienced local people, domestic, and foreign sources, the approximate harvest date of *S. herbacea* seed is from mid-November to mid-December in the northern hemisphere (Kadereit *et al.,* 2006; Shahi *et al*., 2017; Farzi *et al*., 2017). Seeds of many plants can germinate shortly after the formation of the embryo, but seed harvest at this stage due to insufficient accumulation of reserve materials during grain filling leads to loss of yield and reduction of seed quality. Grain loss is affected by environmental factors such as relative humidity, drought stress, and strong winds as well as mechanical collision to the plants (Krzymanski, 1998; Minaei *et al.,* 2003). The best seed harvest date is at the end of the growing season and after the stage of physiological maturity. At this stage, the filling and transfer of the material from the mother plant to the seeds have been completed, and the maximum dry weight has been obtained (Elias *et al.*, 2006). On the other hand, the delayed harvest may be associated with various seed wastes such as pest infestation, shedding, and reduced vigor (Madani *et al*., 2008). Harrington (1972) reported that in addition to the maximum dry weight during physiological maturity, the maximum seed quality (i.e., seed vigor and germination percent) also occurs. Although this hypothesis has been confirmed by many researchers (Tekrony and Hunter, 1995), some seed researchers have reported that in some plants, seeds reach their maximum quality before or after physiological maturity (Ghassemi-Golezani and Mazloomi-Oskooyi, 2008). In recent years, different studies have been devoted to the effect of seed collection date on different plant species (e.g.,Ghasemi *et al.*, 2016; Moradi and Bazi, 2016; Gzanchian *et al*., 2007; Madani *et al*., 2008; Fatahi, 1993; Karimi, 1996; Jun and Tao, 2004; Waller *et al*., 1980; Clor *et al*., 1974). The flowering period of *S. herbacea* is relatively long, and the arrival of its seeds is uneven (Ranjbar *et al.*, 2017). It is noteworthy that seed loss is common at harvest date, but the reduction in grain yield is considerable and is a management concern in *S. herbacea* cultivation (Madani *et al.*, 2008). While early harvest reduces seed yield and seed quality, late harvest can lead to an increase in seed shedding (Khajehpour, 2004).

Therefore, this study aimed to determine the most appropriate date or stage of seed harvest to increase seeds germination, reduce loss of seeds, and increase seeds oil extraction.

Materials and Methods

Study area

The *S. herbacea* grows abundantly in the vicinity of Gomishan Lagoon and its neighboring rangelands. This area is located in the western part of Golestan province and lies between the longitude of 2′ 54˚ to 15′ 54˚ E and latitude of 10′ 37˚ to 18′ 37˚ N on the eastern edge of the Caspian Sea, at 20 km from Bandar Torkaman city Golestan province, Iran. The minimum and maximum elevation of the region ranges from -24 to -11 m.a.s.l. The average annual rainfall is 343 mm, and the average annual temperature is 17°C (Kam *et al.*, 2014). Halophyte species in the area are *Halocnemum strobilaceum, Salsola rigida, Halostachys caspica, Tamarixgalica, Salicornia herbacea, Tamarix ramosissima*, which grow on the north and east coasts of Caspian Sea (Karimi, 2010).

Methodology

A homogeneous area of *S. herbacea* was selected in the vicinity of Gomishan Lagoon (natural habitat). Field sampling was taken in mid-November 2019 and morphological traits were recorded before seed ripening to maturity and seed shedding. Sampling was performed 12 times until the last stage of complete seed shedding. Sampling was first carried out once a week and increased to every two days at the end of sampling dates (close to seed shedding). The intervals of sampling were determined as a function of the climatic conditions, yield and the rate of seed shedding. In each sampling, twenty 1 m^2 plots were designed and randomly established across the habitat of *S. herbacea*. In each plot, the number of shrubs was counted. The percentage of shrubs visually estimated using three main colors: 1) green as an indicator of the completeness of the initial stage of seed formation and development; 2) red as an indicator for the middle period of seed maturity; and 3) brown as a sign of the final stage and seed maturity. The weight of the spilled seeds was calculated via shaking the shrubs, and the sampling date was recorded accordingly. Also, in each sampling period, it was ensured to randomly collect an adequate amount of seeds from across the habitat for the germination test and oil extraction.

Germination test

The collected seeds were spread on the newspaper and dried out in the open air for a week. The impure seeds were carefully separated. The pure seeds were placed in zippered plastic bags, labeled, and then placed in a fridge (5°C). At each samples, 150 seeds were used in three replications (i.e., 50 seeds per replication). The Between Paper (BP) method was adopted to perform the germination test (Rezaei, 2001). The petri dishes were washed and then sterilized in an oven at 180˚C temperature for 3 hours. Further, the petri dishes were covered by the standard sterile filter paper. Since the seeds were small, the filter paper method was considered to be the most suitable bed (Rezaei, 2001). Distilled water was added as required for seed germination. The petri dishes were placed in the germinator at 22˚C temperature during the experiment (Young, 1991). The emergence of the roots as the criterion for germination was counted daily for 14 days (Abbasi Khalaki *et al*., 2016).

Oil extraction

The *S. herbacea* seed samples collected during sampling dates (7 samples with three replications) were cleaned and then dried by oven at 70˚C temperature for three hours and ground with mortar. Oil extraction of the samples was performed using Hexane solvent and Soxhlet extractor for eight hours (Institute of Standards and Industrial Research of Iran, no 14880, 2013).

Statistical Analysis

The measured traits were compared at different stages of seed maturity using a one-way ANOVA. A means comparison was made between tratments using the Tukey's test. The normality of data was tested using the Kolmogorov-Smirnov index and Anderson Darling index (Kolmogorov, 1933; Anderson, 1952). The relationship between seed germination and oil production was determined using a linear regression test, while the relationship between seed shedding and plant color percent was assessed using a multivariate regression and Principal Component Analysis (PCA). PAST Ver 3.25 software (Hammer *et al*., 2001) was employed for statistical analysis.

Results

Detail of the estimated field traits at each stage of *S. herbacea* seed maturity after the flowering stage is presented in Table 1.

The results of analysis of variance showed significant differences between seed maturity stage (initial, middle and final stages) on seed germination and oil percent ($p<0.05$) (Table 2).

Means comparisons between three harvesting stages were made using Tukey's test. The highest and lowest germination rates with average values of 91% and 98% were obtained in the initial and final stages of harvesting, respectively. Also, the highest and lowest oil yield with average values of 19% and 16% was obtained in the middle and final stages of seed maturity (Table 3).

Table 1. Different stages of *S. herbacea* seed maturity

Stage		Green color %	Red color %	Brown color %	Shrubs No/plot	Seeds weight g/plots
Initial of seed formation		27	41	32.3	6	0
Full development		12.4	38.1	49	6	0
Full seed maturity	Initial stage	2	14.3	84.0	6	15.4
	Middle stage	1	5.5	94.0	5	16.3
	Final stage	0	1.5	98.5	6	11.0

Table 2. The results of the variance analysis of *S. herbacea* for germination percentage and oil percentage in different stages of seed harvesting

Source of variation	Df	MS	
		Germination percentage	Oil percentage
Harvesting stage	2	136.6**	15.6**
Error	18	2.7	2.7
Total	20		

**= Significant at 0.01 probability level.

Table 3. Comparing the means of germination percentage by Tukey's test in different stages of seed maturity

Seed maturity stages	Germination percentage	Oil percentage
Initial stage	91.3^{b}	17.7^{ab}
Middle stage	92.0^{b}	19.1^{a}
Final stage	97.6^{a}	15.8^{b}

Means of column followed by the same letter was not significantly different (P<0.05)

Results of regression analysis between seed germination percent an independent variable and seed oil percent as a dependent variable showed a negative correlation between them $r=-0.94^{**}$. The regression equation was $Y=-0.5+64.98x$ where: Y= seed germination percent and X= seed oil percent with R^2=0.89 (Table 4). This result indicated that over time, the germination rate increases, whereas the seed oil percent decreases (Table4).

A multiple regression using seed shedding weight as dependent variable and shrub colors (green, red, and brown/dry) as independent variables was developed to determine the best color rate for lower seed shedding (Table 5).

The regression equation was: $Y=-3.86+7.49X_1+1.06X_2+0.53X_3$

Where: Y= weight of seed shedding, X_1= green, X_2= red and X_3= brown/dry color of shrubs. As shown in Table 5, there is a significant correlation between the percentage of seed shedding as a dependent variable and the percentage of green, red, and brown (dry) colors as independent variables (p<0.01). Accordingly, the correlation rate is 79%, and the adjusted coefficient is 62% (Table 5).

As shown in Fig. 1, from mid-December, the color of *S. herbacea* faces more than 50% change was occured; that is the percentage of green and red shrubs had significantly decreased, while the population of dry brownish plants accounts for more than 60% of the entire plants. At this stage, the ripening rate of the seeds is complete; seed shedding initiates and proceeds until the end of December, and the amount of seed shedding reaches its maximum value (nearly 80%) within a week.

Table 4. Results of linear regression analysis between seed germination percentage as an independent variable and seed extracted oil percentage as a dependent variable

Traits	Dec 11 2019	Dec 13 2019	Dec 14 2019	Dec 15 2019	Dec 17 2019	Dec 19 2019	Dec 21 2019
Germination percentage	89.33	89.33	89.33	94.67	95.33	98	97.33
Oil percentage	16.53	20.1	19.77	18.53	16.5	16.17	15.53

Regression equation Y= -0.5+64.98x, R^2=0.89

Table 5. Multivariate regression test between seed shedding as dependent variable and shrub colors (green, red, and brown/dry) as independent variables

Parameters	Coefficient	Standard error	R^2	T test	P value
Constant	a= -3.86	5.69	-	-0.68	0.50^{ns}
Green%	b1= 7.49	0.77	0.39	9.66	0.00**
Red%	b2 =1.06	0.14	0.21	7.79	0.00**
Brown%	b3 =0.53	0.07	0.03	7.50	0.00**

Regression equation Y (seed shedding)= -3.86+7.49 (Green)+ 1.06 (Red)+ 0.53 (Brown) R^2=0.63
**= Significant at 0.01 probability level.

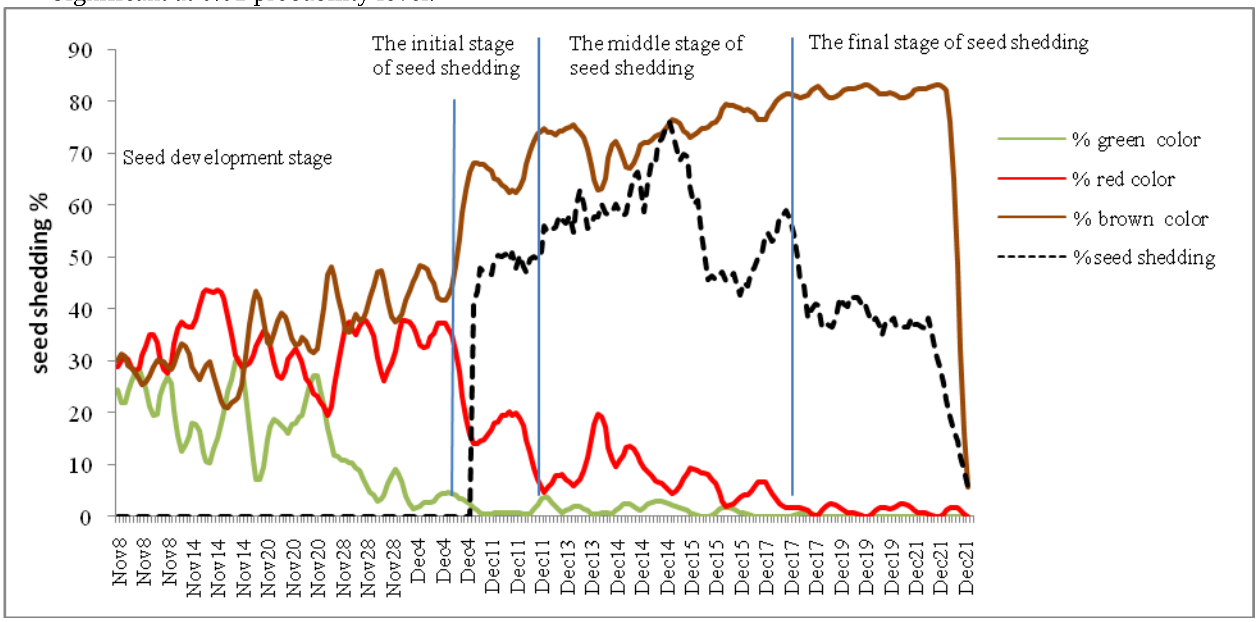

Fig 1. The curve presenting the changes in plant color percentage and seed shedding percentage in different stages of physiological and seed shedding of *S. herbacea*

The results of Principal Component Analysis (PCA) are presented in Table 6. The results of PCA analysis showed that the first two components accounted for 92.5% of the total variation. Seed shedding and brown (dry) color had strong correlation with the first component. Based on the result, the percentage of seed shedding with 96% and the percentage of brown (dry) color with 95% have the most positive correlation with the first principal component axis whereas the green and red colors with the respective percentages of 79% and 89% show a negative correlation with the first axis of the principal component (Table 6).

Table 6. Variance table and Eigen values of Principal Component Analysis (PCA) test and Loading values of each of the variables on the main axes of Principal Component Analysis (PCA)

Traits	Axis1	Axis2	Axis3	Axis4
Seed shedding%	0.96	0.28	0.00	0.00
Green%	-0.79	0.26	-0.42	0.38
Red%	-0.89	0.27	0.35	0.11
Brown%	0.95	-0.27	0.08	0.10
Eigen values	3.33	0.36	0.22	0.07
Variance%	83.49	9.02	5.51	1.96
Cumulative variance%	83.49	92.52	98.03	100

Discussion

The results of this study revealed that the date of seed collection is one of the most important and effective factors in the percentage of seed germination and oil production in *Salicornia* seeds. In the early stages of seed collection, seeds had poor germination due to malnutrition. However, the effective tissues and the accumulation of increased storage materials increase with time. As a result, the absorption of nutrients by the plant concurrently increases the growth and biochemical activity of the plant and leads to increased biological performance of the plant, high quality, and consequently improved seed germination rate (Moradi and Bazi, 2016). On the other hand, as the weather cools and the temperature drops in the fall, seed maturity becomes complete (Gzanchian *et al*., 2007). Hence, the germination percent of seeds collected in late December (final stage of seed harvesting) was higher than that of mid-December (the initial stage of seed harvesting). Clor *et al*., (1974) have conducted series of studies on germination and seed maturity of *Artemisia herba alba*, which expounds that freshly harvested seeds have had low germination during December (15-20%) and the gradual increase in germination capacity has been observed eight months after harvest. In the winter of the following year, the germination rate has reached 80%, which indicates that the seed will arrive after harvest. On the other hand, if the seed collection faces a long delay, the seeds will fall severely and may be eaten by birds and animals or taken else where (Bagheri and Ariapour, 2018). Hence, there should be a trade-off between early and late collections so that maximum quality seeds can be obtained (Baskin and Baskin, 1998). Seed quality often cannot be improved after harvest. Nonetheless, if seeds are collected on time, it would be feasible to maintain seeds quality before consumption (Bagheri and Ariapour, 2018). Determining the efficient date of seed collection from species in rangelands is a pivotal factor to ensure the seed germination of such species (Rabiei, 2001; Ghasemi Firozabadi *et al*., 2012). This is vital, particularly for small seed varieties with a little food stored inside for germination (Azarnivand, 2003; Bagheri, 2006; Ahmadi *et al*., 2004; Gzanchian *et al*., 2007). Ghasemi *et al*., (2016) stated that the delay in seed harvest at the seeding stage increased germination percent in *Salsola rigida* and *Ferula ovina* while it did not affect *Zygophyllum eurypterum.* Waller *et al*., (1980) experimented on *Kochia prostrate* in three regions of the USA where they found out that the last date of seed collection had higher average germination. Moradi and Bazi (2016) stated that with advances in maturity, seed germination of *Prangos ferulacea* has been increased and seeds that were harvested in late May have had a higher germination rate than those of early June. Gzanchian *et al*., (2007) discussed that an increase in the process of seed maturation can congruently increase germination indicators in *Artemisia sieberi*. Zhang *et al*., (2013) showed that a delay in the date of seeds collection (*Brassica napus*) compared to the previous stages can increase growth and germination indicators by far. Karimi (1996) stated that significant characteristic differences are discernible in different seed harvest dates, which is in accordance with the results of this study.

Temperature is a substantial factor in affecting the amount of oil content of seed crops (Alirezalu *et al.,* 2011). It seems that in the case of an early harvest, incomplete storage of materials reduces the oil content of the seeds. As for the delayed harvest, exposure to low ambient temperatures can reduce the percentage of oil (Alirezalu *et al*., 2011). Damian *et al*., (1998) noted that the percentage of *soybean* oil in areas with higher average temperatures is higher than that of areas with lower average temperatures, which is in line with the results of this study.

The best stage to harvest *Salicornia* seeds for oil extraction is at the initial stage of seed harvest because at this stage, the yield of the seed oil is at its maximum level (about 20%). According to Kimber and Gregor (1995), the amount of oil often reaches a constant level during physiological maturity, and there is little fluctuation until the seed is fully ripened. They also stated the observed differences are due to environmental factors, especially temperature, which is in complete agreement with Sutherland and Morey (1982) and Ellis and Copeland (2001). Afterward, due to the relative sensitivity of *S. herbacea* seeds to shedding, the yield of seed oil decreases. Generally, the seed should be morphologically and physiologically mature once harvested. Moreover, seeds should be collected as soon as possible. Early harvesting of *S. herbacea* reduces the oil content of seeds due to the increase in chlorophyll and free fatty acids content of the seeds. The delay in harvest, on the other hand, causes a rise in seed shedding and, therefore, reduces seed yield (Khajehpour, 2004). Since the maturation date between different shrubs and even in different spikes of one shrub differs, harvesting cannot be executed in only one step. Studies show that it is possible to choose a suitable date for seed harvesting based on the conditions of the field that can be attained by continuous monitoring and sampling. The latter should often begin from the end of the flowering stage onwards. Our results suggest that among the visible plant traits, the percentage of brown color (dry) of more than 60% of the plants is a good indicator for determining the suitable time to harvest *S. herbacea*. Since there is not much difference in the germination ability of *S. herbacea* seeds between different seed maturation stages, the prevention of seed shedding during harvest seems to be a key factor. Based on our research structure, the harvest was started when more than 60% of the plants in the field were brown because at this stage, the percentage of seed germination is sufficient; the amount of seed extractable oil is maximized, and the lowest seed shedding during harvest will occur. Monitoring the percentage of plants color changes (from green to red and then brown) can be a biological criterion for managing the selection of the suitable stage to harvest *S. herbacea* seeds in rather similar conditions. It is noteworthy that the seeds collected at the proposed dates will come with specific moisture content. Therefore, they should be dried immediately in the outdoors or with a dryer. Ignoring the technical points in seed collection and accumulation stages may have a significant effect on seed quality and even negate the positive effects of expedited seeding time of this species (Sarmadnia, 1987). Since climatic conditions (e.g., temperature and rainfall) have a great impact on plant phenology including seeding, the architecture of our study is designed in such a way to comply with the conditions of the selected study area. Researchers are advised to repeat this study under different climatic conditions and in different habitats to provide a comprehensive guideline for determining the appropriate date for *S. herbacea* seed collection. Lastly, the relationship between seed harvest date and seed oil combinations in the seed as well as the potentials of increasing the percentage of oil and useful compounds via crop treatments should be further examined.

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بررسی اثر زمان برداشت بر قابلیت جوانه زنی و تولید روغن بذر گونه سالیکورنیا (مطالعه موردی: تالاب گمیشان، گرگان، ایران)

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چکیده. *Salicornia herbacea* یک گونه حساس به ریزش بذر است. به منظور تعیین اثر زمان برداشت بذر بر میزان ریزش بذور، قابلیت جوانه زنی و تولید روغن بذر، آزمایشی در مراتع تالاب گمیشان، استان گلستان انجام شد. نمونه‌های بذر ۱۲ مرتبه، در ۲۰ پلات یک متر مربعی از ۱۵ ام آبان به عنوان تاریخ آغاز رسیدگی بذر تا ۳۰ ام آذر به عنوان مرحله نهایی رسیدگی بذر در سال ۱۳۹۸، جمع آوری شدند. نمونه برداری ابتدا یک بار در هفته و در اواخر نمونه برداری به هر دو روز یکبار افزایش یافت. در هر پلات، تعداد بوته‌ها، ارزیابی چشمی تغییرات رنگ گیاه از سبز به قرمز و قهوه‌ای (خشک) همراه با وزن بذور ریخته شده در هر تاریخ نمونه برداری ثبت شد. سپس، مقدار بذور مورد نیاز به طور تصادفی برای آزمون جوانه زنی و استخراج روغن گرفته شد. داده‌های بدست آمده با استفاده از نرم افزار آماری PAST مورد تجزیه و تحلیل گرفتند. نتایج آزمون تجزیه واریانس نشان داد که بین زمان‌های نمونه برداری و جوانه زنی و درصد میزان روغن، اختلاف معنی‌داری وجود دارد ($p<۰/۰۵$). بیشترین و کمترین میزان نرخ جوانه زنی با مقادیر متوسط ٪۹۱ و ٪۹۸ به ترتیب در مراحل ابتدایی و نهایی برداشت بدست آمد. هم چنین، بیشترین و کمترین میزان عملکرد روغن با مقادیر متوسط ٪۱۹ و ٪۱۶ در مراحل میانی و نهایی رسیدگی بذر به دست آمد. نتایج آزمون آنالیز چند متغیره اختلاف معنی‌داری را بین ریزش بذر و رنگ گیاه (سبز، قرمز و قهوه‌ای) نشان داد ($p<۰/۰۵$). براساس نتایج به دست آمده، از بین صفات قابل مشاهده گیاه، میزان درصد رنگ قهوه‌ای (خشکی) بیش از ۶۰ درصد گیاهان با کمترین میزان ریزش بذور، بعنوان شاخص مناسبی برای تعیین زمان مناسب برداشت بذور سالیکورنیا پیشنهاد شد.

واژه‌های کلیدی: جمع آوری بذر، روغن، رسیدگی بذر، هالوفیت