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Effect of sowing date and foliar application of salicylic acid on forage yields and quality of globe artichoke (*Cynara scolymus* L.)

Wpływ terminu siewu i dolistnego stosowania kwasu salicylowego na plon paszy i jakość karczocha zwyczajnego (*Cynara scolymus* L.)

Summary. In northern latitudes, productivity of forage crops could be affected by weather conditions at sowing as well as during harvesting. Management of sowing date and foliar application of growth regulators can help to avoid severe environmental stresses during these sensitive stages and may improve forage quality. Globe artichoke (*Cynara scolymus* L.) has been recently introduced as a specialty and alternative forage crop in north-western Iran. Morphological characteristics, yield and quality of forage of artichoke leaves were investigated in response to three (April 19, May 5 and 20) sowing dates and three acid salicylic (0, 700 and 1400 μM) rates of foliar spray under field condition. Foliar application of 700 and 1400 μM salicylic acid solution caused to 18% and 26% increase in leaf number, respectively. Results showed that digestible dry matter (DDM), crude protein, crude fat and ash were affected by both sowing date and salicylic acid. The highest digestible dry matter was recorded in third sowing date with 700 μM salicylic acid application. On the other hand the maximum yield and dry matter, crude fat and protein were observed in second sowing date. Based on these results, we estimate that under these environmental conditions, second sowing date (May 5) along with 700 μM salicylic acid foliar application appear sufficient to obtain high yield and acceptable forage quantity of artichoke.

Key words: crude protein, dry matter, forage crop, morphological characteristics

INTRODUCTION

The globe artichoke (*Cynara scolymus* L.) is a perennial thistle of the genus *Cynara* with rosette which is originated from Southern Europe around the Mediterranean. Globe

artichokes have vernalization requirement (low temperatures) and after vernalization fulfillment long days may promote flower formation and stem elongation [Pécaut 1993]. The artichoke leaves may last six or more years, reaching the maximum productivity in the third year [Ceccarelli *et al.* 2010]. This is a useful grazing plant, much browsed by livestock. Artichoke can be nourished fresh or ensilaged, although the forage does not ensilage well because of its high concentration of soluble sugars and high moisture content. The potential advantage of the crop for forage may arise from the fact that it adapts well to a wide range of soils and habitat. Optimal forage quality can be obtained by harvesting tops and leaves during mid September when protein levels will reach to maximum value. Despite, the importance of *C. scolymus* there is no available information on its adaptation with semiarid and moderately cold regions of northern latitudes. Also, there is little information about improvement of the browse production of the species through growth-regulating materials.

In the Mediterranean areas, plants which are sown in spring, complete their juvenile phase before cold seasons, and produce reproductive organs in the next spring. In the most agricultural regions of Iran which are located at arid and semiarid climatic category, where early planting dates increases the risk of the dealing with late spring frosts, another hand late date may increase possibility of coinciding with the hot and dry periods. Water availability and temperature are important factors that determine the rate and extent of germination, since seed germination of globe artichoke may partially be inhibited where temperature is high (30–35°C). However, in most parts the much of globe artichoke should be planted after harvesting of winter plants (wheat and barley), as a second crop. In spite of this fact, few management studies have been conducted on planting dates for globe artichoke forage and this should be investigated particularly in high latitudes.

A promising approach to diminish environmental stress which induces crop losses is the foliar application with chemical desiccant on plants [Khandaker *et al.* 2011]. Salicylic acid naturally occurs in plants in very low amounts and participates in the regulation of physiological processes in plant such as stomatal closure, nutrient uptake, chlorophyll synthesis, protein synthesis, inhibition of ethylene biosynthesis, transpiration and photosynthesis [Khan *et al.* 2003, Piatelli *et al.* 1969, Shakirova *et al.* 2003]. It has been identified that, an important signaling element involved in establishing the local and systemic disease resistance response of plants after pathogen attack [Abdel-Wahed *et al.* 2006]. The effect of salicylic application on production and quality of artichoke was not comprehensively found in the literatures. Therefore, proper information is needed regarding the effect of growth regulators on growth, forage quality and quantity of globe artichoke under different planting dates. This study aimed to evaluate the effect of different rates of salicylic acid at the five-leaf stage, on forage yield and quality of *Cynara scolymus* L. and find the best date.

MATERIAL AND METHODS

The experimental trial was conducted at Agriculture Faculty Farm, Maragheh University, Iran during the spring and summer of 2011. Soil cultivation and seedbed preparation practices were carried out in early April. The field was located at 46°16' East longitude and 37°23' North latitude, at an altitude of 1485 meter from sea level, where the

climate is semi arid and cold temperate. The soil texture of the experimental site is sandy loam, comprising of 53% sand, 31% silt and 16% clay, with pH of 7.45 and EC 0.506 dS m⁻¹. The experiment was conducted in Randomized Complete Block Design (RCBD) using factorial arrangement (3 × 3) with three replicates. The experimental unit area was 20 m² (5 × 4 m). Globe artichoke (*Cynara scolymus* L.) seeds sample of local ecotype were provided from Pakan Bazr Inc, Isfahan, Iran. First factor was consist on three planting dates spaced at about 15-day intervals as 19 April, 5 May and 20 May. Second factor was consist on three salicylic acid concentrations (0, 700 and 1400 μM). Salicylic acid (SA) was dissolved in absolute ethanol then added drop-wise to water (ethanol/water: 1/1000, v/v). Salicylic acid was applied on the foliage of plants with a hand sprayer on 45 days old plants (at the five-leaf stage). A control group of plants was grown sprayed with deionized water. A surfactant teepol (0.5%) was added with the control and SA treatment solutions. The volume of the spray was 50 ml per planter. Seeds were hand-sown onto mounded planting beds in rows 60 cm apart as clump sowing with a distance of 40 cm, so three or four seeds were sown per hill and thinning was carried out as to be one plant per hill after full establishment. N-P-K fertilizers applied with ratio of 200 kg N, 100 kg P₂O₅ and 100 kg K₂O ha⁻¹. All other cultural practices were carried out as recommended for artichoke production during growth seasons.

Chlorophyll content was measured on ten leaves of a plant at each plot, using a portable chlorophyll meter (SPAD) on August 8. For evaluating the plants water status five fresh leaves of same size and same age of five plants from each treatment were collected and weighted (Fw). Leaf segments were kept immersed in distilled water for 24 h at room temperature in the dark. The turgid weight (Tw) of leaves were measured and then oven-dried at 80°C for 72 h until constant weight and reweighting (Dw). The fresh weights, turgidity and dry matter of the leaf segments were used to determine the hydration and relative water content according to Sangakkara *et al.* [1996]. Hydration was determined as $H (\%) = 100 - 100 (Dw / Fw)$. The relative water content (RWC) was determined as $RWC (\%) = [(Fw - Dw) / (Tw - Dw)] \times 100$.

Leaf area (through the indirect method and by comparing the weight and area), leaf number per plant, plant fresh forage yield, dry matter and forage quality were recorded 10 September. At harvest in each plot plants were collected from an area of 2 m² from the middle rows. Dry matter yield was determined through the weighting the dried plants at 70°C for 24 h. this sample subsequently were ground through a 1.0-mm screen for measurement of quality traits.

Chemical composition including crud protein (CP), crud fiber (CF), digestible dry matter, crude fat (CF) and total ash (ASH) were calculated via near infrared spectroscopy (NIRS). Near-infrared spectroscopy is a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum (from about 800 nm to 2500 nm). Near-infrared spectroscopy is not a particularly sensitive technique, but it can be very useful in probing bulk material with little or no sample preparation. Digestible dry matter (DDM) is an estimate of the percentage of the forage that is digestible as determined from Acid Detergent Fiber (ADF) concentration. DDM is used to estimate the energy value of the forage. Crude protein (%) was calculated as $N\% \times 6.25$ [Marten *et al.* 1985]. Nitrogen was estimated using nitrogen exterminator. Total ash were estimated by Ignite a 5 g of sample at 550°C, for 2 h and allowed to cool in desiccators and then weighted. DMD were calculated as method which described by De Boever *et al.* [1994]. Crude fat is an estimate of

the total fat content of feeds. The crude fat was estimated using ether extraction [AOAC 1990]. Crude fat contains actual fat (triglycerides) as well as alcohols, waxes, terpenes, steroids, pigments, ester, aldehydes, and other lipids. Data were statistically analysed by analysis of variance in the General Linear Models Procedures of SAS. Differences among treatments were considered to be significant at $P < 0.05$. Means were compared using least significant difference test (SAS Institute Inc. 2004).

RESULT AND DISCUSSION

Analysis of the variance showed that the main effects and interaction of dates and salicylic acid were statistically significant for all investigated morpho-physiological attributes (table 1). Comparison between sowing dates showed that the highest leaf area was produced through the first sowing date, since leaf area in early sown plants was 8.7% higher than plants sown in second date. Leaf growth and rate of phenological development have critical effect on plant growth and yield and both phenomenon are particularly sensitive to climatic factors. Environmental and climatic factors, including photoperiod, light intensity, temperature, soil moisture, and fertility, are known to affect the number of leaves produced by plants and all of mentioned factors significantly change in growing season. Foliar application of salicylic acid with 700 μM concentration resulted in slight increase in leaf area when compared with control plants. However, the foliar spray with high concentrations of salicylic acid (1400 μM) led to a doubling of leaf area over the control.

Investigation of leaf number per plant showed that the highest number of leaves per plant was recorded in plant sprayed with salicylic acid. Thus, foliar application of 700 and 1400 μM salicylic acid solution caused to 18% and 26% increase in leaf number, respectively (table 1). Mean comparison of water status between sowing dates showed that the second and third sowing date result in significant increase in leaf relative water content (RWC). However the spray of salicylic acid solution could not affect RWC.

In the third planting date chlorophyll content was significantly affected by salicylic acid application, since the highest amount of SA application was accompanied with 6.34% increase in chlorophyll content over the control (table 1). However, Moharekar *et al.* [2003] reported that salicylic acid increases the synthesis of carotenoids and xanthophylls and also enhanced the rate of deepoxidation with a concomitant decrease in chlorophyll pigments in wheat and moong. It seems that salicylic acid treatment induces biosynthesis the some specific carotenoids in plants.

Light interception and photosynthesis are of major importance in yield determination due to their role in dry matter production. In our study plant fresh weight response to time of sowing and salicylic acid which was associated with difference in leaf area development. Mean comparison between sowing dates revealed that second and third sowing dates decreased yield (fresh weight) up to 10% and 16% when compared with first sowing date, respectively. The results of the current study are consistent with those of Diawara [2012] who found a similar decrease in yield of sorghum by delay in sowing date. result showed that foliar application of 700 and 1400 μM salicylic acid solution led to 56% and 127% increase in fresh weight, respectively (table 1). A similar trend was observed for dry matter. It was somewhat surprising that plants sprayed with salicylic acid showed more succulent growth (growth based on water accumulation) in comparison with control.

Table 1. Mean comparison of yield, dry matter and morphological traits of globe artichoke (*Cynara scolymus* L.) as affected by sowing dates and foliar application of salicylic acid
Tabela 1. Porównanie plonu, suchej masy i cech morfologicznych karczocha zwyczajnego (*Cynara scolymus* L.) w zależności od terminu siewu i dolistnego stosowania kwasu salicylowego

Sowing date Data siewu	SA (μM)	LA (m^2)	LN	RWC	CHL	Yield Plon (kg m^{-2})	DM (kg m^{-2})
April 19 19 kwietnia	0	791.19 c	11.66 de	60.55 c	58.10 abc	0.933 cd	0.217 bc
	700	1281.14 bc	14.0 b	64.74 bc	59.72 abc	1.22 b	0.327 b
	1400	2659.87 a	14.66 ab	64.09 bc	60.13 ab	1.81 a	0.417 a
Mean/Średnia	–	1577.4	13.44	63.12	59.31	1.32.	0.320
May 5 5 maja	0	810.28 c	11.33 e	68.0 b	57.46 bc	0.633 d	0.120 d
	700	1151.99 c	13.66 bc	68.61 ab	57.01 bc	1.26 abc	0.243 bc
	1400	2204.37 ab	15.33 a	66.12 bc	59.60 abc	1.71 a	0.407 a
Mean/Średnia	–	1388.55	13.44	67.57	58.02	1.201	0.256
May 20 20 maja	0	872.79 c	12.66 cd	75.59 a	56.06 bc	0.693cd	0.140 d
	700	1241.47 bc	14.33 ab	68.13b	55.06 c	1.087 bcd	0.163 bcd
	1400	2441.05 a	14.66 ab	68.76 ab	62.77 a	1.603 ab	0.360 ab
Mean/Średnia	–	1503.43	13.88	70.82	57.96	1.12	0.221
LSD at 5% NIR przy 5%	–	326.4	1.627	7.02	4.73	0.59	0.094

SA – salicylic acid concentration/stężenie kwasu salicylowego, LA – leaf area/powierzchnia liści, LN – leaf number/liczba liści, RWC – relative water content/względna zawartość wody, CHL – chlorophyll content/zawartość chlorofilu, FW – fresh weight/świeża masa, DM – dry matter/sucha masa

Analysis of forage quality indicated that both dates and salicylic acid influenced significantly portion of digestible dry matter. Foliar application of salicylic acid could significantly increase the digestibility of the forage, as the highest digestible dry matter was recorded in plants planted in May 20 and sprayed 700 μM salicylic acid solution (fig. 1). The major nutritional consideration related to dry matter is that it may affects the density of nutrients (defined as a ratio of nutrient content to the total energy content). Although, early sowing and high level of salicylic acid concentration can result in high dry matter production, but it contains a little nutrients which express as dilution effect hypothesis. Effect of salicylic acid and the interactive effects of sowing date \times spraying salicylic acid were statistically significant for crude protein content. However at same time the effect of sowing date was more prominent than salicylic acid. Maximum amount of protein (14.33%) was observed under third sowing date (May 20th) along with spraying salicylic acid 1400 μM (fig. 2). Forage analysis revealed that ash percentage was affected by the

interaction sowing date \times spraying salicylic acid at 1% probability level. Minimum ash percentage (8.63%) was recorded in the treatment of the third sowing date (May 20th) and application of 1400 μM salicylic acid (fig. 3). However, the difference between second and third planting dates in plants sprayed with salicylic acid statistically was negligible.

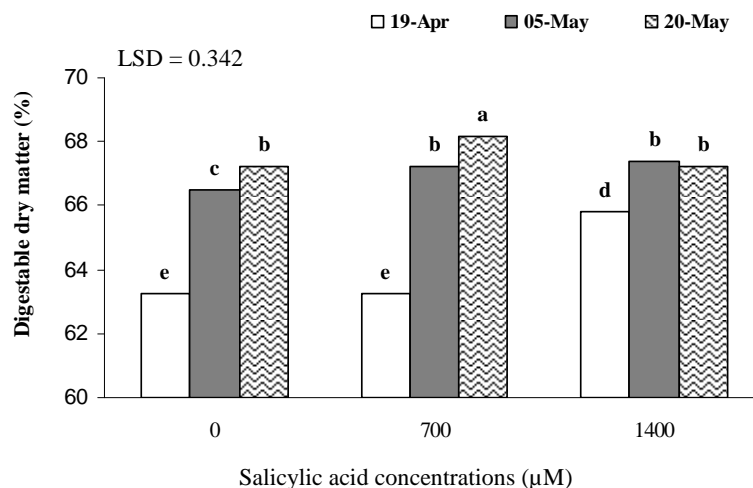


Fig. 1. The effects of sowing date and salicylic acid concentrations on digestible dry matter content in globe artichoke (*Cynara scolymus* L.) leaves (different letters between the columns indicate statistically significant differences)

Rys. 1. Wpływ terminu siewu i stężenia kwasu salicylowego na zawartość strawnej suchej masy w liściach karczocha zwyczajnego (*Cynara scolymus* L.); różne litery między kolumnami oznaczają różnice istotne statystycznie

Although specific macro and micro minerals are required by animals, they are typically only a small fraction of ash. The major effect of the ash content enhancement is reduction of the energy value of forages, and this is why ash concentration should be a routine analysis for forages. Organic matter is the non-ash portion of forages, and it contains all of the major nutrients and the components yielding energy.

Crude fat (CF) percentage significantly affected by planting date and spraying salicylic acid at 0.05 level (fig. 4). Maximum CF (2.68%) was recorded under the combined treatment of the second planting date and 700 μM salicylic acid. On the other hand, the minimum CF (1.52%) was observed in the unsprayed plants under the first sowing date. Fat is a minor element of quality in forages because true fat (triglycerides) or fatty acids are a small fraction (< 4%) of DM. Lipids contain more than triglycerides and fatty acids, and this is a particular problem in most forages, which contain waxes, cutins, and other ether-soluble materials that have limited digestibility and do not provide considerable energy in comparison with carbohydrates. The trend toward fatty acid analysis instead of EE [Palmquist and Jenkins 2003] is beneficial in the measurement of forage quality. However, like using crude protein equivalent, measuring only fatty acids may result in extra matter being included in the non-fiber residue and is not carbohydrate. In forages, about 1 to 2% of DM is ether or hexane extractable but is not fatty acids.

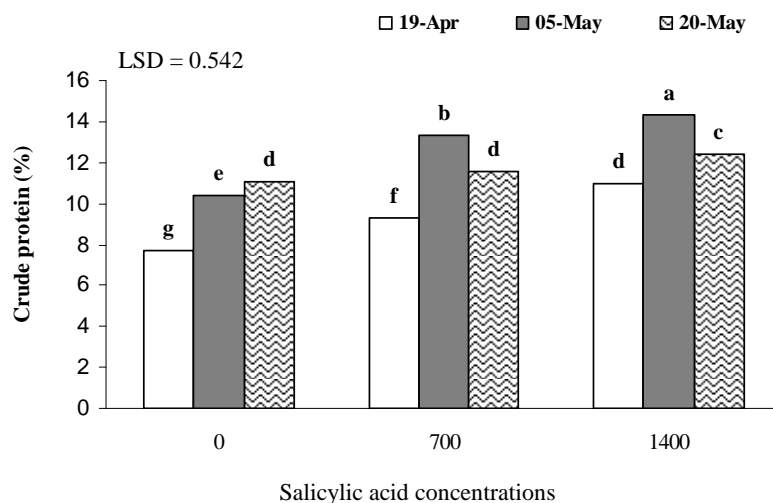


Fig. 2. The effects of sowing date and salicylic acid concentrations on crude protein (%) in globe artichoke (*Cynara scolymus* L.) leaves (different letters between the columns indicate statistically significant differences)

Rys. 2. Wpływ terminu siewu i stężenia kwasu salicylowego na zawartość białka surowego (%) w liściach karczocha zwyczajnego (*Cynara scolymus* L.); różne litery między kolumnami oznaczają różnice istotne statystycznie

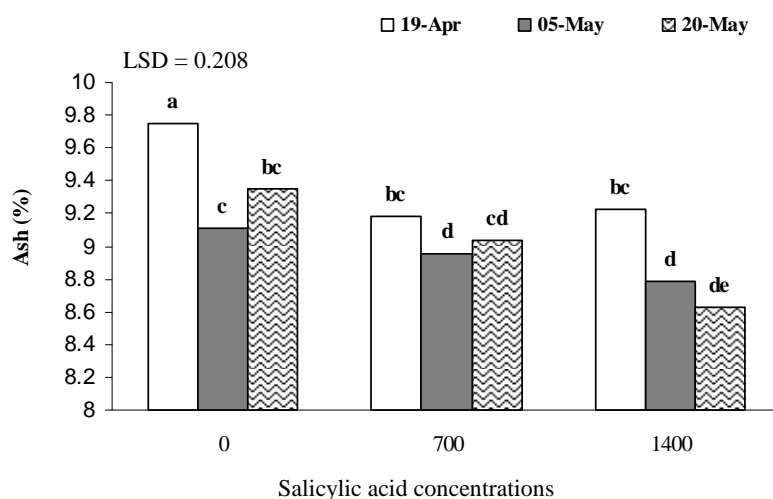


Fig. 3. The effects of sowing date and salicylic acid concentrations on ash (%) in globe artichoke (*Cynara scolymus* L.) leaves (different letters between the columns indicate statistically significant differences)

Rys. 3. Wpływ terminu siewu i stężenia kwasu salicylowego na zawartość popiołu surowego (%) w liściach karczocha zwyczajnego (*Cynara scolymus* L.); różne litery między kolumnami oznaczają różnice istotne statystycznie

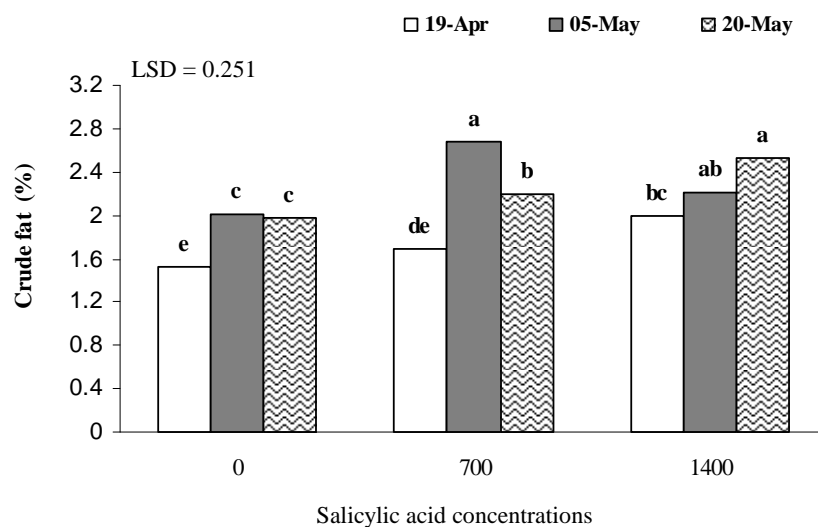


Fig. 4. The effects of sowing date and salicylic acid concentrations on crude fat (%) in globe artichoke (*Cynara scolymus* L.) leaves (different letters between the columns indicate statistically significant differences)

Rys. 4. Wpływ terminu siewu i stężenia kwasu salicylowego na zawartość tłuszczu surowego (%) w liściach karczocha zwyczajnego (*Cynara scolymus* L.); różne litery między kolumnami oznaczają różnice istotne statystycznie

Salicylic acid (SA) or ortho-hydroxy benzoic acid is ubiquitously distributed in whole plant kingdom. SA is a phenolic derivative and is considered to be a potent plant hormone. It is well documented that phenolic compounds can affect different physiological and biochemical processes including, photosynthesis, ion uptake, membrane permeability, enzyme activities, flowering, heat production and growth and development of plants [Abdel-Hakim *et al.* 2012]. Fariduddin *et al.* [2003] observed a substantial increase in dry matter accumulation at a concentration of 5–10 M, supplemented to the leaves of *Brassica juncea* but the concentrations above that proved an inhibitory action. Moreover, they added that wheat seedlings grown from the grains soaked in 5–10 M of SA possessed more number of leaves and higher yield and dry matter in comparison with control. Enzyme activities such as amylase and nitrate reductase were increased by SA application [Chen *et al.* 1993]. On the other hand, SA showed synergetic effect with auxin and gibberellins [Zaghlool *et al.* 2006]. The exogenous application of SA was reported to have a huge effect on a wide range of physiological processes including increased cold germination tolerance in watermelon [Jing-Hua *et al.* 2008] and chilling tolerance in cucumber [Kang and Saltveit 2002]. It was shown that foliar application of acid salicylic on young pepper plants under normal growth conditions provided protection against subsequent low-temperature stress. Besides, the obvious visual symptom, this conclusion was confirmed by chlorophyll fluorescence parameters and electrolyte leakage measurements from the leaves [Abou El-Yazeid 2011].

CONCLUSIONS

These results showed that spraying the globe artichoke (*Cynara scolymus* L.) plants with salicylic acid (SA) in both concentrations enhanced plant growth and improve forage quality. Sowing date affects artichoke growth, development and forage yield depending on salicylic acid concentrations. The effect was shown through reduction in stand establishment with early planting and reduction in forage yield and crude protein with delayed sowing. Although, the significant difference was not observed between second and third sowing dates, reduction in the number of day between different growth stages was observed with delayed sowing and it may increase the risk of encountering with early fall frosts. Although the forage yield of globe artichoke significantly affected by sowing date, foliar application of salicylic acid modified the effects of the sowing dates and in more investigated characteristics their interactions were significant. Our result indicated that under climatic condition of Maragheh second planting date with foliar application of 700 μ M salicylic acid can result in acceptable forage quality and quantity.

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Streszczenie. W strefie północnych szerokości geograficznych na plon roślin paszowych mogą wpłynąć warunki pogodowe podczas siewu oraz zbioru. Zastosowanie odpowiedniego terminu siewu oraz regulatorów wzrostu może pomóc uniknąć stresu środowiskowego podczas tych wrażliwych etapów oraz polepszyć jakość paszy. Karczoch zwyczajny (*Cynara scolymus* L.) został ostatnio wprowadzony do uprawy jako roślina alternatywna w północno-zachodnim Iranie. W doświadczeniu polowym zbadano cechy morfologiczne, plon oraz jakość paszy z liści karczocha w zależności od terminu siewu (19 kwietnia, 5 maja i 20 maja) oraz dolistnego stosowania kwasu salicylowego (o stężeniu 0, 700 i 1400 μM). Dolistne stosowanie 700 i 1400 μM roztworu kwasu salicylowego spowodowało, odpowiednio, 18- i 26-procentowe zwiększenie liczby liści. Stwierdzono, że zarówno termin siewu, jak i zastosowany roztwór kwasu salicylowego istotnie modyfikowały strawność suchej masy, zawartość białka surowego, tłuszczu surowego oraz popiołu. Największą strawność suchej masy zanotowano podczas trzeciego terminu siewu z zastosowaniem 700 μM kwasu salicylowego. Natomiast maksymalny plon i zawartość suchej masy, surowego tłuszczu oraz białka zaobserwowano podczas drugiego terminu siewu. Na podstawie uzyskanych wyników stwierdzono, że w opisywanych warunkach środowiskowych drugi termin siewu (5 maja) oraz dolistne stosowanie 700 μM kwasu salicylowego zapewniają wysokie plony odpowiedniej jakości paszy z karczocha.

Słowa kluczowe: surowe białko, sucha masa, roślina paszowa, cechy morfologiczne