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ORIGINAL ARTICLE

The Effects of Exogenous Salicylic acid on some Quantitative and qualitative attributes of Medicinal pumpkin (*Cucurbita pepo* L. var. Styriaca) under drought stress

Hossein Rabbi Angourani [1]*, Jaber Panahande Yangajeh [2], SahebAli BolandNazar [3], Jalal Saba [4], Fariborz Zare Nahandi [5]
1-PhD student of Horticulture, Faculty of Agriculture, Departement of Horticulture, University of Tabriz, Tabriz, Iran
3 and 5- Associate Professor of Horticulture, Faculty of Agriculture, Departement of Horticulture, University of Tabriz, Iran
4- Associate Professor of Agronomy and plant breeding, Faculty of Agriculture, Department of Agronomy and Plant breeding, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
Corresponding author- Hossein Rabbi Angourani Email: Rabbihosein@Gmail.com

ABSTRACT
One of the most important results of drought stress is reducing economic function and increasing the quantity of active constituents in the dry weight of medicinal plants. Salicylic acid is a phenolic compound used as a growth regulator in the induction of resistance to drought. In this study, the effects of salicylic acid on yield components and Active constituents of medicinal pumpkin under drought stress were investigated. This experiment was conducted in a completely randomized design by factorial method with three replications investigating the effects of two factors of drought stress treatments in four levels of control, (moderate stress), (medium stress) and (severe stress) respectively adjusted in the potential uptakes of 0.1, -1, -0.5, and -1.5 MPa and salicylic acid included foliar application in 4 levels of zero = foliar application with distilled water of 0.5, 1, and 1.5 mgL^{-1}. The results showed that increasing drought stress levels reduced the oil yield, but in contrast, increased the Beta-sitosterol. In addition, functional attributes such as leaf area and 100-seed weight and seed yield were decreased, while salicylic acid at 0.5 and 1mgL^{-1} increased the balanced yield of oil and Beta_sitostrol by raising oil percent and seed yield under mild and medium stress conditions, presenting increase in plant resistance to drought as a result of salicylic acid application.
Keywords: *Active constituents, Beta-sitosterol, Drought stress, Medicinal pumpkin, Oil yield, Salicylic acid*

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INTRODUCTION
Medicinal pumpkin (*Cucurbita pepo* L.var. Styriaca) is an annual plant which belongs to the Cucurbitaceae that their oil and active constituents (Beta-sitosterol) are widely used in the prevention of prostate cancer and treatment of urinary tract irritation [42,20,16] .Drought stress is considered as the most important abiotic factors to reduce growth and agricultural plants yield in arid and semi-arid areas of the world [41], the effects of this stress in medicinal plants include the decisive reduction of economic function, which is accompanied with increased quantity and quality of active constituents in most cases [52,41], drought stress causes bad effects on metabolic processes of plants including water relations, nutrient uptake, photosynthesis and assimilate distribution [24 ,9]. The effects of water deficiencies in plants are usually appeared as decrease in photosynthesis and plant growth [52,13]. At the molecular level, the negative effects of stress associated with harms of oxidative to plant cells are due to the imbalance between the production of Reactive oxygen species (ROS) and mechanisms of antioxidant

defense [46,32] that cause accumulation of ROS, damage to proteins, membrane lipids and eventually cell death [12]. In medicinal plants, one of the effective applications of drought induction is to increase the quality of plants or the active ingredient [46]. Of course, although the content of secondary metabolites in some medicinal plants may show increase under drought stress, but the yield of the produced active constituents decreases under stress condition[4]. Nowadays, the use of hormones and controller substances of potential growth are created to improve stress tolerance [58,57].Salicylic acid (SA) or ortho hydroxy benzoic acid is a growth regulator that has a phenolic nature and has an important role in the uptake and transmission of ions and increase in the photosynthesis amount [17], salicylic acid is an endogenous hormone [21], and regulates the synthesis of antioxidant compounds during biotic and abiotic stresses [48, 45 ,20] which are effective to create tolerance against drought [23], usage of salicylic acid in the leaves of the corn and soybean increased leaf area and its dry yield but plant height and root length remained constant [49,48,10]. Foliar application of salicylic acid in terms of drought stress of basil increased the essential oil yield and reduced oxidative damage [2]. As a result, it is likely that the exogenous use of it may have impact on the reduction of biotic and abiotic stresses [53,52,16]. Thus, the aim of this study is to investigate the effect of foliar application of salicylic acid on yield components, oil yield and active constituents of medicinal pumpkin under drought stress conditions in which appropriate quantity of the active constituents can be obtained by creating a balance to eliminate the effects of economic function reduction caused by drought stress with positive effects of salicylic acid.

MATERIALS AND METHODS

The present research was conducted in the research field located at the Faculty of Agriculture of Zanjan University, Zanjan province, Iran (latitude: 36°:41'N, longitude: 48°:24'E and altitude of 1620 m above sea level).plant materials was applied seed of medicinal pumpkin(*Cucurbita pepo* L.var. Styriaca) from Zardband company with the origin of Hungary, The study was arranged as factorial experiment based on a randomized complete block design with three replications with investigation of two factors, each in 4 levels (a total of 16 treatments),. Salicylic acid treatments include foliar application in 4 levels (foliar spray with distilled water), 0.5 , 1 and 1.5 mgL^{-1} and also drought stress in four levels of control = (100% of field capacity complete irrigation), mild stress = (85% of field capacity), medium stress = (70% of field capacity) and severe stress = (55% of field capacity) which were respectively adjusted in the potential matrixes of -0.1, -0.5, -1 and -1.5 MPa. The actions of drought stress treatments were done by the emergence of the flowers and in order to reach various levels of drought stress in the soil, irrigation avoidance method was applied [25], In this case, experimental terraces were not irrigated again after irrigation and reaching the soil moisture value to the field capacity level. To measure the soil moisture and determine irrigation time, TDR device was used [36]. The amount of available water in the soil determined based on soil moisture curves, and the amount of available water in the soil was calculated for different treatments according to soil moisture, in order to ensure the accuracy of calculation of soil moisture values, weighting method was used in some cases [36,25]. Foliar application of salicylic acid was done at full bloom of flower in the early hours of the morning and its amount was to the extent that reached to the limit drops of solutions after the leaves completely soaking [19,11]. During the experiment, all agricultural factors were applied uniformly and drip irrigation system was used to control and manage irrigation better [31].

Parameters of yield components:

Seed yield, 100-seed weight, bush height and leaf areas per bush were measured with 5 randomly selected bush from each treatment. Seed yield, after fruit ripening and removing the margin of the terrace level and splitting the fruits, the seeds were harvested and were weighted after drying in an oven at 40 ° C for 72 hours with a precise scale with 0.01 precision and the seed yield was reported in terms of kg in hectare. Then, to calculate the 100-seed weight, 10 samples of 100 numbers of seeds in each treatment were randomly prepared and weighed and averages were calculated. The heights of the bushes in five randomly selected bushes were measured and the averages were calculated. For measuring the leaf area concurrent with the end of stress phase, leaf area per bush independently read by Leaf area meter of model VM-900E / K after the random selection of three bushes and immediately separating the related leaves, and finally the average was calculated for each treatment.

Extracting oil content of the seed:

In order to extract the oil, the seeds were put in the oven at 40 degrees Celsius for 24 hours so that moisture of the seeds became reduced and equalized for all treatments. From each treatment, 5 samples of 1 gram were prepared after grinding to extract oil. The samples were packed with a string in filter papers with certain weighs and then the n-hexane in Soxhlet was applied for 8 hours to extract oil, after this time the samples were came out from the device and were placed for 24 hours in the oven at a temperature of 50 ° C to be desiccated. Oil percent of samples was obtained from the difference of the primary and secondary weight (before and after Soxhlet) then Oil yields were obtained from multiplying seed yield to the oil percent in each terrace, respectively and it was reported in terms of kilograms per hectare.

Measuring the active constituents (beta-sitosterol):

It was read from the samples of the extracted oil to measure the beta-sitosterol of absorption spectra by beta-sitosterol standard and pure dichloromethane in the wavelength of 231.5 nm by Spectrophotometr device with model (perkinelmer, lambada) 25 usa uv/vis [43,7,6].

Statistical calculations:

Data obtained from the experiment were analyzed by analysis of variance using SAS statistical software (ANOVA ; SAS ,Version 9.1) and mean scores were compared using Duncan's multiple range test at *p* = 0.05 level.

RESULTS AND DISCUSSION

Components of seed yield: (seed yield in hectare and 100-seed weight):

Seed yield:

According to the results of variance analysis table (Table 1), drought stress at 5% level and foliar application of salicylic acid, each in (P≤0.01)level and the interaction of these two factors at (p≤0.05) level had a significant effect on this attribute. Increase in the intensity of drought stress reduced the seed yield that its lowest value was observed in 55% level of the field capacity (Table 2). Water deficiency is a limiting factor in the production of many agricultural plants in field conditions [34], the lack of water causes a series of physiological, morphological and biochemical changes in plants, including a reduction in leaf area and cell division [56], stomata blockage and transpiration reduction [13], restrictions of photosynthesis [52] and disorder in absorption and distribution of water and food [14].

Table 1. Analysis of variance of data for effect of different levels of salicylic acid on some qualitative and quantitative characteristics of medicinal pumpkin

		Mean square						
Source	**df**	**Dry seed yield**	**100-seed weight**	**Bush length**	**Leaf area**	**Oil content%**	**Oil yield**	**Beta_sitosterol**
Replication	2	48256	5.35	0.55	0.021	0.018	10089.8	0.0141
Drought stress (D)	3	51483*	20.83**	7.12**	0.56**	0.596**	10637.9**	0.2634**
Salicylic acid (SA)	3	91337**	14.57*	0.17ns	0.132**	0.733**	38130.8**	0.7378**
D*SA	9	33138*	2.033*	0.531*	0.058*	0.033*	6117.2*	0.0265**
Error	30	12426	5.029	0.246	0.021	0.018	2306.1	0.0098
% cv	–	13.85	10.77	15.35	22.51	10.77	15.09	10.9

*,** significant at p≤0.05 and at p≤0.01respectively.ns: non_significant

As a result, photosynthesis ingredients transferred to the seeds decreased, causing reduction in the ultimate yield of the field conditions [52,13]. On the other hand, foliar application of the non-stressed plants with salicylic acid increased the seed yield at levels of 0.5 and 1 mgL^{-1} but at the level of 1.5mgL^{-1}, this attribute was reduced (Table 2), in interaction of plants foliar applications under the stress with salicylic acid, the seed yield in comparison to the plants under stress increased in most treatments and foliar application of salicylic acid in levels of 0.5 and 1 mg, showed greatest increase of 100-seed weight respectively in the levels of 85% and 70% of field capacity, but in the density of 1.5mgL^{-1}, seed yield had declining trend (Table 2), in the beans, occurrence of moisture stress during blossoming and seed filling caused to decrease seed yield [31,30]. Salicylic acid and acetyl salicylic-acid effectively protected tomato and bean plants under drought stress in the density of 0.1 and 0.5mmol which ultimately increased the yield and growth of fruit and plant seeds in these conditions. However, for densities above and below this range, positive results were not reported [45] justifying the decline in the application of salicylic acid with the density of 1.5 mg per mgL-1.

100-seed weight

According to the results of variance analysis table (Table 1), drought stress at (P≤0.01) level and foliar application of salicylic acid and the interaction of these two factors at (p≤0.05) level had a significant effect on this attribute. By increasing drought stress levels, 100- seed weight decreased and the minimum value of it was observed on 55% FC (Table 2), that had similar results with drought stress effects on Milk thistle under water stress which reduced the number and percent and yield of seed oil by increasing stress [33].

Table 2.Means of the effect of different levels of salicylic acid on some qualitative and quantitative characteristics of medicinal pumpkin

Treatment								
Salicylic acid	**Drought stress**	**Dry seed yield (kg/ha)**	**100 -seed weight(gr)**	**Bush length (m)**	**Leaf area (m^2)**	**Oil content %**	**Oil yield (Kg/ha)**	**Beta-_sitosterol (gr/100gr Oil)**
0 mg L^{-1}	Control	1580 bc	10.787 fg	6.312 a	2.6093 cd	39.411 bcd	622.69 def	0.43954 g
	85% FC	1478 de	10.78 fg	6.2825 a	2.2745 e	36.604 bcd	541 def	0.47408 g
	70% FC	1374 ef	8.318 hi	5.7924 ab	1.7415 h	36.213 bcd	497.6 ef	0.74757 ef
	55% FC	1278 f	6.838 i	4.1585 ghi	1.1942 i	34.781 d	444.5 f	0.81818 e
0.5 mg L^{-1}	Control	1957 a	18.542 a	5.6027 bc	3.7268 a	42.705 ab	835.7 a	1.05302 cd
	85% FC	1731 b	15.451 b	5.053 cde	3.3547 b	45.336 a	787.7 ab	1.32046 a
	70% FC	1762 b	11.461 efg	4.4329 fgh	2.2887 e	42.351 abc	746.2 abc	1.20473 ab
	55% FC	1664 bc	12.332 def	4.2454 fgh	1.9384 gh	40.871 abc	680.1 cde	1.09556 bcd
1 mg L^{-1}	Control	1914 a	17.635 a	4.8158 def	2.7303 c	40.241 abc	770.21 bcd	1.04616 d
	85% FC	1761 b	14.737 bc	4.4847 efg	2.4115 de	38.594 bcd	679.6 cde	1.1739 bc
	70% FC	1755 b	11.89 efg	3.7761 i	2.0135 fg	37.671 bcd	661.1bcd	1.04754 d
	55% FC	1673 bc	14.28 bcd	3.2159 j	1.7384 h	35.556 cd	594.8 cde	1.02847 d
1.5 mg L^{-1}	Control	1431 e	13.711cde	5.3809 bcd	2.5704 cd	39.028 bcd	558.5 def	0.54045 g
	85% FC	1470 de	13.084 cde	4.6984 efg	2.5494 ef	41.571 abc	558.4 def	0.64658 f
	70% FC	1471 de	12.152 def	4.2383 ghi	1.8514 gh	39.328 bcd	578.5 def	0.65678 f
	55% FC	1373 ef	10.362 gh	3.9318 hi	1.4141 i	39.492 bcd	542.22 f	0.9743 d

†. Means sharing the same letters in a column do not differ significantly at $p \leq 0.05$ according to Duncan's multiple range tests. FC: Field capacity

Also, the number of seeds and oil amount reduced in caraway under drought stress [28]. 1000 seed weight decreased by reducing soil moisture because assimilate transmission to the seed and seed size reduced [53]. Water stress which occurs especially in the early stages of seed filling period usually reduces weight and thereby leads to reduced seed yield [22] that under drought stress, seed yield has significant positive relation with leaf area, plant height, 1000 seed weight. 1000 seed weight increases with low water stress and the number of seeds decreases in the plant [27,23,26]. Any type of water stress in plant breeding stage can have a significant impact on the weight of seeds. In Rapeseed, stress during blossoming affects final seed weight and reduces the seed 100-seed weight [26,21]. On the other hand, the foliar application of the non-stressed plants with salicylic acid increased 100-seed weight at levels of 0.5 and 1 mgL^{-1}, but decreased this attribute at a level of 1.5mgL^{-1} (Table 2), similar results was found in basil [2]. The other interaction of foliar application of stressed plants with salicylic acid increased 100-seed weight in comparison to stressed plants in most treatments and foliar application at levels of 0.5 and 1mgL^{-1} of salicylic acid at 85% FC (Table 2), that had similar results with drought stress effects stress level showed the most increase in 100-seed weight but had a downward trend in the density of 1.5 mgL^{-1} of salicylic acid (Table 2), but the values were higher than the plants with stress [31]. In peas, the number of seeds is also affected by drought and this situation is based on the genotypes, especially in the subsequent stages of blossoming [35,26]. Overall, emerged water deficiency stress at blossoming stage which is the most sensitive stages of growth inhibited the growth of it [47]. In the condition of water stress, salicylic acid treatment protected the activity of nitrate reductase and retained protein content and nitrogen of leaves compared to the plants in enough water conditions [49,43], the results indicate the role of salicylic acid in setting plants drought response that can be used as a growth regulator for improving the growth of plants under stress. The salicylic acid also reduced the effects of drought stress and promoted the yield in tomatoes under salt by affecting the activity of antioxidant enzymes and metabolites such as ascorbic acid, glutathione [45,38].

Bush length

According to the results of variance analysis table (Table 1), drought stress at ($P \leq 0.01$)level didn't have a significant effect on this attribute but foliar application of salicylic acid didn't have a significant effect on this attribute and the interaction between these two factors at ($p \leq 0.05$) level had a significant effect on this attribute. By increasing drought stress levels, bush length decreased (Table 1), and the minimum value of it was obtained in stress operation on 55% FC (Table 2), that had similar results with drought stress effects (Table 2). Bush length in most plants has a significant correlation with the yield [2]. Plant height in drought stress conditions in comparison to the seed weight of the main stem has a more important role in terms of participation in seed yield in the plant [50]. Plants which showed a greater reduction in the height under drought stress are more placed under drought stress and plant height and length can be used as a type of response to the drought, as a criterion for diagnosis of critical tolerant genotypes [63], thus, the bush height reduction can be considered as plant's efforts to counter water deficiency. On the other hand, foliar application of stressed plants with salicylic acid didn't have a positive impact on the length and a decreasing trend was seen in comparison to the stressed plants without the use of salicylic acid, so that by increasing the levels of salicylic acid, plant height was lowered and the lowest value was observed in 1 mgL^{-1} treatment and 55 percent of field capacity (Table 2), which according to its effect in nitrogen metabolism and its impact on the production of compatible metabolites such as proline [59,51] represent the efforts of the plant for transferring the assimilates into the seed and production of osmolytes and antioxidant enzymes to deal with water deficiencies and sustain plant better, which seems logical in producing more foliage to more assimilates production and seed filling, In general, plant stress continues its growth, so with the use of salicylic acid, the direction of assimilate movement to the seeds is changed and causes reduction in the bush length [59].

Leaf area

According to the results of variance analysis table (Table 1), drought stress of foliar application and salicylic acid, each at ($P\leq0.01$)level and the interaction between these two factors on the level of ($p\leq0.05$)had a significant effect on this attribute. This value decreased with increased levels of drought, when plants are faced with any kind of stress, reductions in leaf area and biomass are response to drought stress [40]. Water stress in the early vegetative growth moderately decreases leaf area, plant height, crop growth rate and nitrogen absorption and greatly reduces these growth parameters in the late of growth and in the reproductive stage [37]. On the other hand, foliar application of non-stressed plants with salicylic acid could have a positive impact on leaf area and the highest values were observed at levels of 0.5 mgL^{-1}. In foliar application of stressed plants, the maximum value of leaf area was obtained at the density of 0.5 mg and 85% FC (Table 2), that had similar results with drought stress effects, these values had a declined trend at the level of 1.5 (Table 2). Water deficiency has little effect on the rate of leaf appearance, but decreases the total leaf area by decreasing development and increasing the amount of leaf senility [8]. Reducing leaf area under drought stress is a compatible mechanism that can be achieved to avoid plant dehydration. By reducing the level, leaf transpiration decreases and reaches to its lowest amount. On the other hand, preservation of leaf area is very important during periods of stress because more carbon for photosynthesis is absorbed [39] .Drought tolerant species reduce water waste through reducing leaf area [29,1] and limiting opening of stomata or both of them [37]. On the other hand, salicylic acid with nitrogen metabolism activation tries to develop assimilate construction of centers [51] and expands leaf area that ultimately affect the total seed yield and 1000 seed weight [58].

Oil content and yield:

According to the results of variance analysis table (Table 1), drought stress in ($P\leq0.01$) level, and foliar application of salicylic acid and the interaction between these two factors at ($p\leq0.05$) level, had significant effect on this oil percent, By increasing levels of drought stress, oil percent decreased (Table 2), By application of salicylic acid in non-stressed plants, this oil percent had its maximum value at level of 0.5 mg per mgL-1, then it tended to decline. In foliar application of plants under stress, the highest increase was observed in the levels of 0.5 and 1 mgL^{-1} but at the level of 1.5 mgL^{-1} it had declining trend, this oil percent decreased with increased periods of drought (Table 2). According to the results of variance analysis table (Table 1), drought stress and foliar application of salicylic acid in 1 percent level and the interaction between these two factors at $p\leq0.05$ level, had significant effect on oil yield, By increasing the intensity of drought stress, oil yield decreased and its minimum value was observed in the 55% FC (Table 2), that had similar results with drought stress effects on Milk thistle under water deficiency stress, which reduced seed oil percent and yield by increasing drought stress [33]. It was also seen in the study of drought stress on caraway that oil amount decreased due to drought stress [28], in Rapeseed yield and production also decreased due to drought stress [5]. Foliar application of stressed plants with salicylic acid increased the oil yield at the levels of 0.5 and 1 mg/mgL-1, but at a level of 1.5 mgL^{-1} this attribute reduced. On the other hand, foliar application of stressed plants with salicylic acid increased the oil yield in comparison to stressed plants in many treatments. And foliar application at the level of 0.5 mgL^{-1} of salicylic acid showed the greatest increase in oil yield in 85% FC (Table 2), that had similar results with drought stress effects stress levels, but at densities of 1 and 1.5 mg per mgL-1, the trend was almost stable and declining (Table 2). In contrast, salicylic acid especially at the level of 0.5

mgL^{-1} increased the oil production. Higher levels increased the oil yield, but decreased compared to prior treatments at the level of 1.5 mgL^{-1} (Table 2) that was compatible with the results of salicylic acid foliar application in corn, which resulted in a significant increase in the oil of corn seeds, while higher densities of salicylic acid reduced the amounts of these compounds [55], oil yield is the function of oil percent and seed yield and by applying drought, all three parameters decreased, so due to the positive effect of salicylic acid in increasing seed yield and oil percent under stress conditions, oil yield also rose along with it.

Active constituents (Beta_sitostrol):

Phytosterols, especially beta-sitosterol are of secondary herbaceous metabolites, which are constructed like other sterols after breathing on the path of mevalonic acid with the origin of acetyl-CoA enzyme. Steroidogenesis are derivatives of triterpenes and considered as the essential component of plasma membrane that are settled in this place through interaction with phospholipids, the production of this path is related to growth regulator ingredients. According to the results of variance analysis table (table 1), drought stress and foliar application of salicylic acid and interactions between these two factors were significant at ($P \leq 0.01$) level. By increasing drought stress the amount of beta-sitosterol (active ingredient) increased compared to the control (Table2) demonstrating certain applications of moisture regimes increase secondary metabolites in some medicinal plants [44]. This issue was consistent with the results of the rice that the amount of phytosterols, especially beta-sitosterol increases in the plant facing drought stress [27], The greatest amount was achieved in the stress action at levels 55% FC (Table 2), that had similar results with drought stress effects, on the other hand, plants foliar application with the density of 0.5 mgL^{-1} of salicylic acid had positive impact on increasing the amount of beta-sitosterol than the plants that were just under drought stress, But foliar application of salicylic acid at the level of 1.5 mgL^{-1} was associated with a decrease in beta-sitosterol in prior treatments (Table 2) that had a consistency with the results reported in basil in drought conditions, foliar application of salicylic acid increases antioxidant enzymes and aroma yield and reduces oxidative damage in addition to increasing active ingredients [8]. On the other hand, foliar application of plants under drought stress with salicylic acid could have a positive impact on the amount of betasitosterol, so that the external application of salicylic acid of 0.5 mgL^{-1} at the levels of 70 and 85 percent of field capacity, increased the betasitosterol amount more than the plants that were only under drought stress. But the densities of 1 and 1.5 mgL^{-1} of salicylic acid had declining trend in comparison with the treatment of 0.5 mgL^{-1}, but had higher values than under stress plants without salicylic acid treatment (Table 2), In some medicinal plants, drought stress increases the percentage of secondary production, because due to the growth reduction under stress, carbon fixation during photosynthesis is used to produce secondary metabolites, and increasing these ingredients will prevent oxidation within the cells [2,3]. But the yield of secondary metabolites reduces under drought stress due to reduced biomass production [54]. On the other hand, in more severe stresses, the plant mostly uses photosynthesis ingredients to produce osmotic regulator ingredients and processes that are associated with energy consumption, which ultimately affects the quantitative and qualitative attributes of the plant and may not increase the amount of active ingredients [18]. It appears the use of salicylic acid has exacerbated assimilate remobilization from leaves to the seeds under the impact of drought conditions at the beginning of seed filling. Due to the improved yield of the seed in stress conditions, betasitosterol yield which is a function of seed yield and seed beta-sitosterol percent, have showed increase under stress by application of salicylic acid, that was in accordance with the results of foliar application of Milk thistle under drought stress with growth regulators which increased the total silymarin with seed yield increase per hectare [15].

CONCLUSIONS

Usage of drought stress and foliar application of salicylic acid led to a balanced development of attributes such as oil percent and seed and oil yield, decreasing effect of oil yield affected by drought stress at levels of salicylic acid was recovered, thus the beta_sitosterol yield which is the function of seed yield and seed's oil yield showed growth. The rise in the balance of this valuable material is under the positive impact of drought stress and application of salicylic acid, had best results in treatments of salicylic acid with levels of 0.5 and 1 mgL^{-1} and moderate stress=(85% of field Capacity) set in matrix potential of -0.5 MPa and medium stress = (70% of field capacity) set in matrix potential of -1.0 MPa.

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