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The Interaction Effect of Irrigation Regimes and Sodium Selenate Foliar Application on Oil and Chemical Compositions of Milk Thistle (*Silybum marianum*)

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The aim of this study was to determine the effects irrigation interval and sodium selenate foliar application on phytochemical traits of milk thistle. Irrigation regimes consisted of 30, 60 and 90 mm evaporation from class A Pan and the second factor is sprayed with sodium selenate 0, 25 and 30 mg/L. The chemical composition of the essential oil was examined by GC and GC-MS and they were significantly affected by Sodium Selenate Foliar and Irrigation Intervals. The results proved analysis of variance indicated that the effects of drought and the interaction between sodium selenate spraying and irrigation had significant effect on leaf proline, oil content, silymarin and silybin percent. The total oil content, silybin and silymarin decreased by 2% due to increasing the water scarcity but the amount of the proline increased under the drought stress. The highest amount of the silybin (19.7%) and silymarin (3.617%) and oil content (19.53%) were measured in treatment 30 mm evaporation from pan and spray 25 mg sodium selenate whereas the highest amount of the proline (13.79%) in treatment of 60 mm evaporation from pan and 30 mg spray sodium selenate. It has proved that the drought stress increased the quality of milk thistle seed oil.

Key words: *Silybum marianum*, seed oil constituents, irrigation regimes, foliar application.

Milk thistle, *Silybum marianum* from *Asteraceae* plant family, is a well-known medicinal plant, native to the Mediterranean region of Europe and widely dispersed to many countries throughout the world. Its medicinal effects are documented among the alternative medicines referred to as liver and bile-related diseases remedy[1]. Hence milk thistle widely cultivated due to its striking medicinal values. It is important when known that for obtaining silymarin (the main important active compound); special difficulty is isolation of the considerable quantity of fatty oil that may reach to approximately 20 to 35% which is similar to many vegetable oil seeds[2]. Drought is the most important abiotic factor limiting crop productivity but in some cases the change in plant metabolites could be an advantage of the production. Although selenium (Se) is not an essential element for plants[3], several studies Demonstrate that selenium supply may exert diverse beneficial effects, Including growth-promoting activities[4-5] Moreover; some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses, e.g. drought[6-7-8] salinity[9-5] and heavy metals[10] stresses. Selenium exerts beneficial effects on growth and stress tolerance of plants by enhancing their antioxidative capacity[9-11]. In fact, coping with drought responses needs to be continued through the primary metabolic processes and the plant is ready to deal with it. Usually in drought conditions produced active ingredients to prevent intracellular oxidation increases[8]. The drought stress increased the quality of milk thistle seed oil[2].

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Recent researches have demonstrated that Se is not only able to promote growth and development of plants, but also increases resistance and antioxidant capacity of plants subjected to various stress[12-5]. Stress factors such as drought trigger common reactions in plants and lead to cellular damages mediated by reactive oxygen species (ROS).The present study aims to determine the irrigation regimes and sodium selenate foliar effects on oil content and of milk thistle seed oil grown in Urmia-Iran. In this paper for the first time we investigated some biochemical responses of this plant when submitted to water deficit.

MATERIALS AND METHODS

This study was conducted under field conditions at the Urmia University west Azerbaijan-Iran in the 2010 and 2011. Soil physical and chemical properties of the experimental area are well drained sandy loam soil. In 2010 the mean annual precipitations was 464.3 mm and mean annual temperature was 28.8°C, while the corresponding values for 2011 were 495.7 mm and 28.9°C. The experiments were established in a split–split plot design in RCBD with three replications. The main plots had Duration of test, the sub plots were three irrigation regimes (30, 60 and 90 mm evaporation from class A Pan). The sub-sub plots were three different dozes of Sodium selenite, sprayed with zero, 25 and 30 mg per liter was administered in the flowering bud stage on Urmia ecotype. Irrigation interval applied in the treatment of non-irrigation water shortages, crop water needs to be done. Plant water needed calculation using the pan and the pan evaporation measured daily, and on the pan and crop coefficient ratio, the amount of irrigation water needed at each stage was determined. Irrigation by polyethylene pipes and the volume of water entering the water meter plot with cancer. Spraying Sodium selenate doses determined according to supply and jointing stage were sprayed with a hand sprayer. The most important characteristics of the Milk thistle that measured are: Proline content, oil yield, oil percentage, silybin and Silymarin percentage. Silymarin extracting was done according to Duan et al, 2004 method[13].

Plants of 2 m^2 in each plot were harvested, dried and winnowed. The dry milk thistle seed powdered sample (50 g), and distilled water (500 ml) were placed in a round bottom flask and connected to the Clevenger distillation unit and distilled for 3 h.

The gas chromatography (GC) characteristic was: a FID Hewlett- Packard 5890 was used using a fused silica capillary SE54 (30 m × 0.25 mm id.) column. Temperature program was: 2 min at 60°C, 60 to 100°C (2°C/min) and 100 to 250 °C (5°C/min), then isothermal for 20 min; carrier gas was helium at a flow rate of 1.0 ml/min.

The gas chromatography–mass spectrometry (GC–MS) characteristic was: a Hewlett Packard 5989A GC-MS system, equipped with library software, Wiley 138 and NBS75, was used. Capillary GC conditions, as above were employed for the fused silica capillary column SE54. Injection volume was 1.0 ul at 1:50 split. Significant MS operating parameters: ionization voltage 70 eV, and scan mass range from 40 to 350 u. Compounds were identified by matching of their mass spectra with those recorded in the MS library and further confirmed by injecting the authentic samples of different compounds with the volatile oil and by comparison of the mass spectra with those of reference compounds or with published data[13].

Sterilization and oil extraction

In preparation for extraction, seeds were sterilized using 70% ethanol followed by 5% bleach / 1% sodium dodecyl sulfate (SDS) solution[14]. 100 g of each seed lots were dried and then, grounded to fine powder in a grinder. Then, 15 g of the powder were extracted with organic solvent -n-hexane-using a 250 ml capacity traditional Sox let apparatus for 8 h (60°C) in 3 replications[15]. The extract containing the oil were then separated and rotary-evaporated under reduced pressure at 35°C.

Physicochemical properties of oil

The oil content of seed lots was measured by weighting of oil per 100 g of seed. Moisture content of oil has been measured by AOCS methods [15]. The total chlorophyll content (mg of pheophytin A /kg oil) of oil was measured by spectrophotometer according to the method of Pokorny et al., (1995) was used[16]. To measure the infiltration of Bates et al., (1973) was used[14]. Proline concentrations were determined spectrophoto metric ally using a using the net on the molecular weight of 0.011,513 grams of pure proline in 10 ml of distilled water and this solution a solution of

100 ppm as the standard curve of purified proline as a witness at a wavelength of 520 nm was measured in micrograms per mol. Proline to prepare standard solutions Kalinova methods[16]. The refractive index was measured by refracto meter apparatus at 25°C. Acid value of the samples were measured and expressed by mg NaOH / g oil and the pH value of the oil samples also was measured[15].

Proline extraction

To measure the infiltration of[14] was used. Proline concentrations were determined spectrophotometric ally using a standard curve of purified proline as a witness at a wavelength of 520 nm was measured in micrograms per mol. Proline to prepare standard solutions using the net on the molecular weight of 0.011,513 grams of pure proline in 10 ml of distilled water and this solution a solution of 100 ppm as the mother solution was prepared. The solution was prepared and purified proline concentrations of 2 ml each were collected and monitored.

Statistical analysis

The data were statistically analyzed by (ANOVA) with SPSS software. Results are expressed as the mean of three separate replicates. Means were compared by the Duncan's multiple range test (1955) at $P < 0.01$ significance level.

RESULTS

Combined analysis of two-year results from effect of irrigation regimes and selenat sodium foliar application on the essential oil, and oil content of milk thistle are presented in Table 1. Therefore, to investigate these differences, the average genotypes of the individual characteristics were compared using the Duncan test.

The effect of treatments on percentage of proline

The results of the combined analysis of the data shows that the interaction of stress and interactive effects of sodium selenate spraying and irrigation had no significant effect on leaf proline Thus, the reduction of stress, proline accumulation in leaves increased. The average value of proline in water stress, severe, moderate, mild, respectively, decreased So that the irrigation of 30 mm evaporation from class A pan Least proline levels in about 9.98 and a maximum amount of irrigation of 90 mm evaporation from class A was about 13.34. The interaction of sodium selenate spraying and irrigation highest percentage of proline to the 13.79 of 60 mm evaporation from pan And spraying least 25 mg Sodium selenate and proline accumulation in leaves treated with 30 mm evaporation from pan and spray Sodium selenate was obtained Indicate that increased efficiency in the use of sodium selenate in proline synthesis, control and drought stress effects is inferior. Proline increased stress in plants, it is a defense mechanism. Proline by several mechanisms such as osmotic adjustment, preventing degradation of the enzyme, maintains and enhances protein synthesis plant resistance against stress. Sodium selenate application of the tension can be increased proline[7]. Research has shown that proline plant (*Ocimum basilicum*) under salt stress significantly increased, indicating a failure of the system to produce Symylat plant resistance against damage caused by salt stress in plants One reason for the increase in proline, which induces the production of endogenous ABA, proline is likely to increase. Sodium selenate probably by inducing the synthesis of intermediate compounds such as ABA, the reaction created to protect and reduce damage from drought stress in plants[17].

The effect of treatments on oil content

The irrigation effect on oil and selenate spraying interaction was significant at the one percent of the oil content. The results of the comparison table The impact of irrigation treatments was show The highest percentage of oil in the treatment of irrigation of 30 mm evaporation from class A to the 19.233 percent, respectively, and the lowest value of 90 mm evaporation from class A to the 18.174 percent. In the foliar sodium selenate data results showed significant differences between treatments at the 5% level. The highest and lowest percentage of oil respectively 19% and 18.611% for 25 mg and 30 mg treatments Experimental data indicated that sodium selenate as compared to control foliar increase the amount of oil is due to increased photosynthesis, root growth and facilitate impact floret inoculation[10]. Due to the significant interaction between foliar and irrigation Treatment. The results of testing Show, The highest oil content in the treatment solution 30 mm evaporation and 25 mg sodium selenate and a minimum of 90 mm evaporation and lack of treatment was spraying

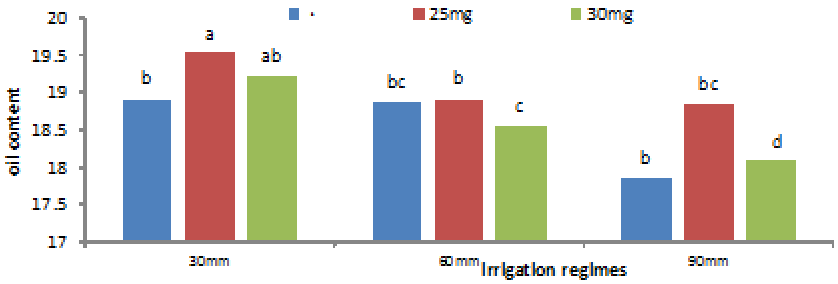

Fig. 1. Comparison of the interactions between irrigation regimes and foliar sodium selenate on seed oil content

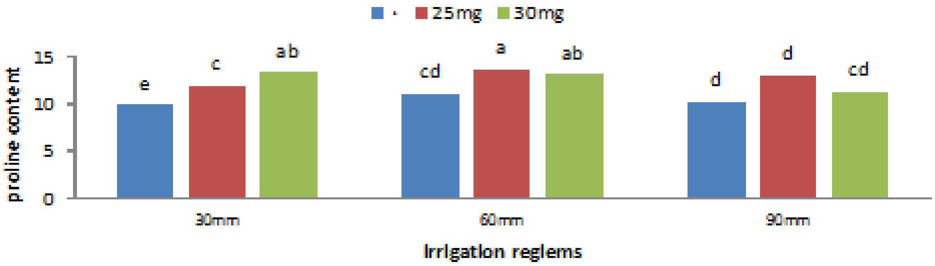

Fig. 2. Comparison of the interactions between irrigation regimes and foliar sodium selenate on proline

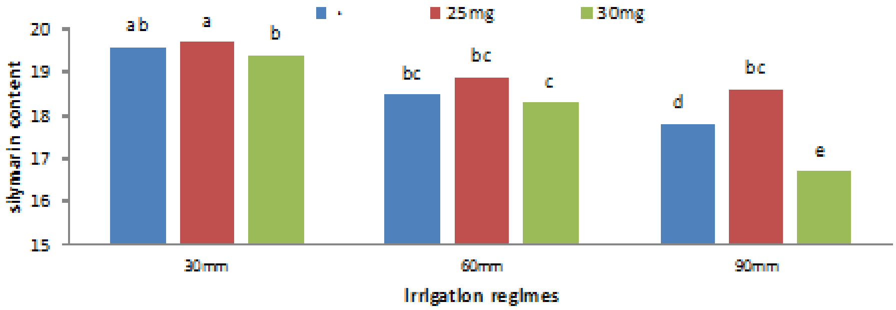

Fig. 3. Comparison of the interactions between irrigation regimes and foliar sodium selenate on silymarin content

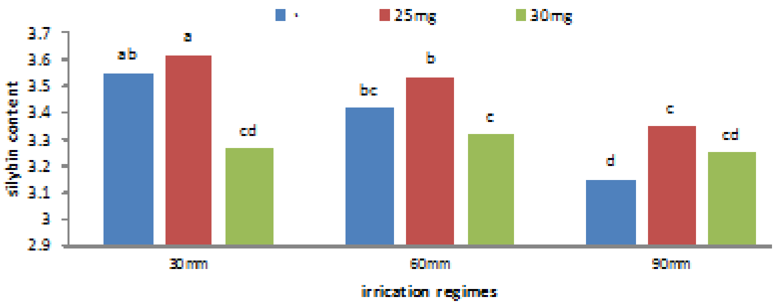

Fig. 4. Comparison of the interactions between irrigation regimes and foliar sodium selenate on sliybin content

sodium selenate The results of testing on turakanian et al.,2004corresponded to the low water stress is increased catalase activity[4].

Corresponded to the stress on potato increased catalase activity is low water consumption of 21 grams per hectare of selenium levels and the activity of this enzyme[17]. Given that this is one of the most essential components of the system and the level of activity of the enzyme glutathione peroxidase activity in the presence of certain amounts of sodium selenate glutathione peroxidase is an indicator of the strength of the immune system, also tested on the activity of antioxidant enzymes and also the maximum amount of soybean peroxidase activity in stress and selenium intake of 20 grams per hectare were reported. Lehigh formation of free radicals that cause cell damage and destruction is evident[7].

The effect of treatments on percentage Slymarin

According to the analysis of variance were significant differences between treatments in the percentage of oil in the $P < 0.01$ level interaction effects were found at the $P < 0.05$ level. The results of the comparison (Table 4) with Duncan, the effect of irrigation treatments on percentage Slymarin showed The highest amount of treated 30 mm evaporation from pan and lowest of treated 90 mm evaporation from pan to order about 3.478 and

Table 1. The total mean square (MS) test every two years

S.O.V	d.f	Proline content	Silybin content	Silymarin content	Oil content
Repeat	2	641338.58	**0.15	** 618.135	** 189339.776
year	1	^{ns} 54.642	^{ns} 0.110	0.031^{ns}	^{ns} 0.081
error	2	1.585	0.117	0.006	8.63
Irrigation regimes	2	305.530**	2.25**	0.254**	** 5.083
Year*irrigation regimes	2	37.203^{ns}	^{ns} 0.024	0.012 ^{ns}	^{ns} 0.021
error	6	3.92	0.011	0.2	0.032
Spraying Sodium selenate	2	2.489**	5.80**	0.272**	1.005**
year*Spraying Sodium selenate	2	0.231 ^{ns}	^{ns} 0.009	^{ns} 0.017	^{ns} 0.021
Irrigation regimes* Spraying Sodium selenate	4	** 0.039	** 5.17	** 0.033	** 0.248
year*irrigation* Spraying Sodium selenate	4	^{ns} 0.196	^{ns} 0.028	^{ns} 0.035	^{ns} 0.008
error	24	0.455	0.12	0.027	0.06
c.v	-	3.848	4.276	2.867	15.529

Table 2. Effect of irrigation regimes on prolin and phytochemical traits of milk thistle

Irrigation regimes	Prolin content	Silybin content	Silymarin content	Oil content
Evaporation 30mm	9.98c	c 18.0	c 3.250	a 19.233
Evaporation 60mm	12.58b	b 18.4	b 3.422	18.777 b
Evaporation 90mm	13.34a	19.8 a	3.478 a	18.174 c

Table 3. Effect of sodium selenate foliar application on proline and phytochemical traits of milk thistle

Sodium selenate	Prolin content	Silybin content	Silymarin content	Oil content
0 mg	10.24c	18.1 c	3.244 c	18.573b
25 mg	13.52a	18.8 a	3.428 b	19.00 a
30 mg	11.12b	18.5 b	3.478 a	18.611 b

Table 4. Effect of sodium selenate foliar application and irrigation regimes on proline and phytochemical traits of milk thistle

Irrigation interval	Sodium selenate	Prolin content	Silybin content	Silymarin content	Oil content
	0	9.87e	a 19.6	3.550ab	18.983b
Evaporation 30mm	25 mg	11.95c	a 19.7	3.617a	19.530a
	30 mg	13.41ab	b 19.4	cd 3.267	ab 19.217
	0	11.18cd	bc 18.5	3.417bc	bc 18.880
Evaporation 60mm	25 mg	13.79a	bc 18.9	3.553b	b 18.917
	30 mg	13.23ab	c 18.3	c 3.317	18.553c
	0	10.24d	d 17.8	3.150d	17.857e
Evaporation 90mm	25 mg	13.11a b	bc 18.6	3.350c	bc 18.853
	30 mg	11.29cd	e 16.7	cd 3.250	d 18.082

3.250 was. Sodium selenate on the effect of foliar application of silymarin showed the greatest amount of silymarin treated with 30 mg Sodium selenate the lowest average 3.478 and 3.244 was treated with an average consumption of sodium selenate(Table 2). The results of the comparison interaction and spraying with irrigation, showed the greatest amount of silymarin in the treatment of 30 mm evaporation from pan and spray 25 mg Sodium selenate with the lowest rate of 3.617 and 90 mm evaporation from pan and irrigation sodium selenate was observed with the use of 3.150(table 2). Catalase activity increased with increasing stress and tension at all levels of sodium selenite concentrations of 20 and 25 milligrams per liter increase in enzyme activity was, But it was only 30 mg per liter decrease in the level of enzyme activity was increased. Based on the results, an increase in water stress will increase as other antioxidant enzymes catalase activity was and Sodium selenate increased the enzyme activity to protect plants under drought stress was In other words, the decomposition of hydrogen peroxide plant and plant tolerance against active oxygen radicals, to the enzyme concentration was increased under stress conditions. The enzyme catalase as a protective antioxidant defense system will cause decomposition of hydrogen peroxide[2].

The effect of treatments on percentage Slybin

The results obtained showed the influence of irrigation on the amount of active ingredient Slybin at $P < 0.05$ level of significance was tested in the first year, the highest level Slybin related to the treatment of 30 mm evaporation and the lowest rate of 90 mm evaporation from pan was evaporated.

The overall success rate was increased relative to the control of tuberculosis in the second year of the experiment (repeated) The highest level of treatment, 30 mm evaporation Slybin 19.8 and the lowest was 90 mm evaporation from The impact of spraying on of Slybin seed at $P < 0.05$ level of significance (Table 2) The comparison showed that the highest percentage of Slybin of foliar Sodium selenate to 30 mm evaporation and 25 mg spray selenate made and minimum of 90 mm evaporation and lack of spraying is In general foliar spray Sodium selenate at all levels were elevated relative to control citizens (Table 1), Considering the composition of substances and materials synthesis of secondary metabolites Come sodium selenate prospective effect that results in increased grain ingredients[17].

CONCLUSION

Given the importance of water in the production of medicinal plants, and given that the stress of some active ingredients in herbs increases, Specific management actions to lower the amount of water necessary to produce acceptable Performance looks with increasing irrigation intervals and drought conditions caused by the use of materials such as sodium selenat and prevent damage caused by oxidation of intracellular reduced stress and increased final yield and the percentage of active ingredient plant.

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