Advances in Bioresearch
Adv. Biores., Vol 8 (5) September 2017: 87-95
©2017 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.5.8795

Advances in Bioresearch

ORIGINAL ARTICLE

Nitric oxide exogenous improves quantitative and qualitative grain yield in milk thistle (*silybum marianum* L.) under drought stress

[1]Esmaeil Zangani, [2]Saeid Zehtab-Salmasi, [3]Babak Andalibi, [4]Abbas-Ali Zamani
[1]Dep. of Plant Ecophysiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran, Email: [2]Dep. of Plant Ecophysiology,Faculty of Agriculture ,University of Tabriz, Tabriz, Iran
[3]Dep. of Agronomy and Plant Breeding, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
[4]Dep. of Environmental Science, Faculty of Sciences, University of Zanjan, Zanjan, Iran
Corresponding author – Esmaeil Zangani
Email: zangani@znu.ac.ir

ABSTRACT

Drought stress is one of significant constraining factors in the growth and diversion of some metabolic processes in medical plants. Nitric oxide (NO) plays a salient role in the reactions of plants towards environmental stress such as drought. A split split plot design was used in a field experiment to evaluate the role of sodium nitroprusside (SNP) as a NO donor in improvement of yield in two genotypes of milk thistle. Research findings showed that drought stress reduces grain and oil yield in both genotypes. However, reduction in silymarin yield was only significant in the stem elongation stress stage. The exogenous of 100 µM SNP improved grain and oil yield in both stages of stem elongation and anthesis stress, particularly in Sari ecotype, whereas application of the same level of SNP only caused a significant increase in silymarin yield during anthesis stress in both of these genotypes. Spraying with 100 µM SNP compensated the reduction of grain oil content, but this increase in silymarin grain content was significant just in the Hungarian cultivar. Withholding irrigation reduced the parameters of grain yield components, except for1000-seed weight, whereas application of 100 µM SNP alleviated theses negative effects. Parameters of yield components indicated the significant positive correlation with the quantitative and qualitative yield of the plant, while the silymarin grain content was only affected by the grain weight. Hence NO can play an important role in tolerance of milk thistle under drought stress, also NO signaling by exogenous of 100 µM SNP was more efficient than of 200 µM SNP for the protection of milk thistle with the stress severity.

Keywords : *Milk thistle, Sodium nitroprusside, Silymarin, Active constituents, Drought stress*

Received 18/02/2017 Revised 09/06/2017 Accepted 01/08/2017

How to cite this article:
E Zangani, S Zehtab-Salmasi, B Andalibi, A-Ali Zamani. Nitric oxide exogenous improves quantitative and qualitative grain yield in milk thistle (*silybum marianum* L.) under drought stress Adv. Biores., Vol 8 [5] September 2017: 87-95.

INTRODUCTION

Milk thistle (*silybum marianum*), native to Mediterranean area, is an annual or biennial from Asteraceae family which also grows in many parts of the world, particularly in warm and dry regions [30]. Milk thistle's active constituents is known as silymarin which is composed of valuable flavonolignans such as silibin, silychristin and silydianin that accumulate in the fruits of this plant [21]. These flavonolignans are efficient in treating liver disorders and some other diseases [12]. Environmental stress such as drought influences secondary metabolites produced in medicinal plants. Although secondary metabolites are not directly affected by primary metabolic processes such as photosynthesis, respiration and transpiration[6], the produced active constituents' yield is influenced by the economic plant yield and is consequently subject to environmental conditions.Studies have shown that various farming conditions such as available water can affect the levels of active biological components (silymarin) in milk thistle [17]. Although secondary metabolite contents in some medical plants may increase under drought stress, active components production yield decreases under stress. Therefore one strategy to improve medicinal plants yield is to produce plants which are relatively tolerant towards drought and have greater production under drought stress. Drought stress has deleterious effects on metabolic processes of plants such as water relations, nutrients uptake, net exchange and assimilate partitioning [9,20]. The effects of water deficit usually manifest in the form of reduction in photosynthesis and plant growth in the whole-plant [14,38]. At the molecular level, however, the negative effects of stress link with oxidative damage to plant cells through imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms [26,34]. Plants employ a series of defensive mechanisms such as closing stomata, transpiration reduction [14], antioxidant enzymes accumulation [13,26] and rapid accumulation of osmolytes such as sugar alcohols, proline and glycine betain to enhance tolerance capacity during water shortage [31]. Hence one of the effective application of drought induction in medicinal plants is quality enhancement or increasing secondary metabolites [35]. Today signaling molecules application such as Nitric Oxide have created a potential for the enhancement of stress tolerance in plants [40,44]. Nitric oxide (NO) is a small, fairly stable gas molecule that is soluble in water and lipid, and is considered as an important biological messenger molecule in plants and that diffuses through membrane [29,44]. Nitric oxide participates in adjusting many physiological processes of plants such as germination stimulation, leaf expansion, metabolism and pathogenic factors [22,25], programmed cell death [10], stomatal movement [14,42], photosynthesis and flowering [36], and iron homeostasis [29]. Recently NO has been recognized as a key signaling molecule in plant response to biotic and abiotic stress, and acts as a mediator and messenger and takes part in the functions of plant growth adapters [8]. NO creates certain protective effects for plants under drought stress that are related to anti oxidant defense [5]. NO exogenous application in *Dendrobium huoshanense* raised relative water content (RWC) levels and antioxidant enzymes, but higher NO concentrations aggravated the effects of drought stress[10]. In an experiment which studied the effects of NO in rice concluded that 100 μM SNP concentration had a greater influence on photosynthesis increase and seedling growth in stress condition[14]. As Regards, most studies have been about seedlings and no such study has been conducted about the physiological role of NO in drought stress alleviation on the final yield of milk thistle under farming conditions , this study investigated the role of NO in tolerance improvement towards drought in Milk thistle based on quantitative and qualitative grain yield and its relationship with parameters of yield components in the field.

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). The soil where the experiment was conducted possessed clay loam texture with a pH of 7.85. The research was conducted in split split plot restriction in a randomized complete block design with three replications. Foliar spray with sodium nitroprusside (SNP) as an NO donor at three levels of 0 (without spraying), 100 and 200 μmol l^{-1} was considered as the main factor, while drought stress at three levels of control (without withholding irrigation to the end of growth period), stress at mid stem elongation (withholding irrigation to the end of growth period) and stress at anthesis stage (withholding irrigation at the stage of grain filling to the end of the growth period) were regarded as secondary factors. Finally two genotypes of Milk thistle comprised the sub-sub factors. Milk thistle (*Silybum marianum* L.) seeds comprised an indigenous landrace (Sari ecotype) and an imported cultivar (Hungarian cultivar) from Pakan Seed Company located in Isfahan.The seeds were sterilized by 0.2% carboxin thiram fungicide prior to sowing. The seeds were sown on March 28, 2014. Sub-sub plots comprised six 4-m rows spaced 50 apart with a density of 8 bushes per square meter. Fertilizer N was applied in irrigation water, based upon soil test totaling almost 60 kg N ha^{-1} in split application before cultivation and again before stem elongation. Spraying with SNP at the stage of stem elongation was conducted on the main plots and this was repeated a week later. Nitroprusside sodium was supplied from Merk-Germany Company.

Grain yield

After the physiological maturation of seeds, grain yield was reported following the elimination of margins from the area of 4 m^2 harvest and expressed in kg/ha.

Determination of oil content

For the purpose of oil extraction, grains were first put in the oven for 24 hours at the temperature of 45^{oC}, so as to reduce the moisture of the grains and equalize them for all treatments. From every treatment, 6 samples each weighting 3.5 g were prepared for oil extraction after grinding. Samples defatted in a soxhlet apparatus with 180 Ml of petrolueum ether solution at $60^{\circ C}$ for 8 h, so as to extract oil. After that period the samples were placed in the oven for 24 h under the temperature of $50^{\circ C}$ to get dried. The oil samples rate was gained through the weight difference in primary and secondary stages (before and after soxhletion).

Silymarin extraction procedure

To extract silymarin, the residue (after measuring the oil content) were completely dried and weighted. Next they were soxhleted with methanol solution for 10 h. After this period, the obtained methanol extracts were evaporated to dryness at $50^{\circ C}$. Once the methanol vaporized, yellow powder was remained which contained silymarin.

Spectrophotometric determination of silymarin

The powder gained from the extraction stage was solved in methanol and reached to volume in a 50 mL volumetric flask [37]. 1 mL of the sample solution was transferred to a 10 mL volumetric flask and then 2 mL of 2,4-dinitrophenylhydrazine- sulphric acid solution was added to that. It was then placed in bath water under the temperature of $50^{\circ C}$ for 50 minutes. After cooling the flasks, its volume was reached to 10 mL by adding the methanolic solution of potassium hydroxide (C= 10%, m/v). After two minutes, 100 µL of the experimental solution was transferred to a test tube and 2 mL of methanol was added to that. The solution was centrifuged at 3000 rpm min^{-1}. The supernatant solution obtained from the centrifuge was transferred to a measuring vessel and next 2 mL methanol of the remaining sample solution was added to the tube and centrifuged. The supernatant solution gained from the second centrifuge was added to the measuring vessel as well. Finally contents of the measuring vessel reached the volume of 5 mL by methanol and the optical absorption of methanol solution was measured at 490 nm wavelength by UV/VIS Spectrophotometry Device (Perkin – Elmer lambada 25) using the standard solution as blank. The silymarin rate (calculated as silibinin) was calculated by the following formula [37].

$$x = \left(\frac{A}{585} \right) \times \left(\frac{25 \times 10^{3}}{G \times d} \right)$$

Where *X*: The rate of silymarin calculated as silibinin(%), *d*: The cuvete thickness of Spectrophotometry [= 1 cm], *A*: The absorption of examined solution at 490nm, 585: Specific coefficient of absorption [A1 1% 1cm=585] , and *G*: The weight of sample.

Silymarin and oil yield

The oil and silymarin yield were determinated by multiplication of grain yield in oil and silymarin content respectively and were reported in kg/ha.

Parameters of yield components

The number of capitula per bush, plant height and the number of branches per plant were measured through random selection of 20 bushes in each treatment. Furthermore, the number of grain per capitula, 1000-seed weight and capitula diameter were obtained through random selection of 50 capitula from each treatment. Finally capitula diameter was measured through digital caliper.

Statistical analysis

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. Correlation coefficients between traits were determined through treatments means (n=18) and using SAS software. Finally the figures were drawn using Excel software.

RESULTS

Grain yield

Although maximum grain yield was obtained under well irrigation condition, drought stress exertion at the stem elongation and anthesis stages significantly decreased grain yield in both genotypes. In fact, this decline at the stem elongation stage was greater compared with anthesis phase (Table 1). NO exogenous application through spraying with 100 µM SNP under stress increased grain yield in both genotypes compared with its non-application and this improvement was more effective in Sari ecotype yield compared with the Hungarin cultivar. Application of 100 µM SNP in Sari ecotype at the time of bolting stress significantly increased grain yield by 41% and during stress at the stage of anthesis by 37% compared with non-spraying during stress. However in the Hungarian cultivar, the application of the same level of SNP (100 µM) only led to a significant increase of 23% in grain yield during grain filling. Moreover the application of 200 µM SNP in both genotypes during withholding irrigation did not have a significant effect on grain yield.

Silymarin yield and content

Drought stress reduced silymarin yield compared with the control (Table 1). Moreover, spraying with 100 µM SNP enhanced silymarin yield in both genotypes under stress condition, although it significantly increased silymarin yield under anthesis stress compared with its non-application (approximately 50 percent). The maximum silymarin yield was obtained by 100 µM application of SNP under well irrigation conditions (125kg/ha), and the minimum silymarin yield was gained by 200 µM SNP at anthesis stage in Sari ecotype (38kg/ha), (Table 1). Drought caused a relative increase in the active constituents of milk thistle in both genotypes. However the increase was insignificant (Table 2). Silymarin content in Sari ecotype in both stages of irrigation withholding was greater compared with the Hungarian cultivar, and this difference was significant. NO application through spraying with 100 µM SNP caused a 30 percent increase in silymarin in the Hungarian cultivar during stem elongation compared with its non-application. Moreover the application of 200 µM SNP had a negative effect on silymarin of Sari ecotype during anthesis (Table 2).

Oil yield and content

Oil yield indicated a significant decline in both genotypes as drought intensity was aggravated (Table 1), whereas the application of 100 µM SNP in both stages of stem elongation and anthesis improved oil yield compared with its non-application (Table 1). Moreover, spraying with 100 µM SNP exerted more influence on oil yield enhancement in Sari ecotype in both stages of stress exertion compared with the Hungarian cultivar. Drought stress significantly decreased grain oil content in both genotypes and this decline in the period of stem elongation was greater compared with the anthesis stress (Table 2). On the other hand, spraying with 100 µM SNP compensated the negative effects of oil content reduction at the stem elongation stage and significantly increased it.

Grain yield components

Table 1: Effects of NO application on silymarin, oil and grain yield of Milk thistle under drought stress.

Treatment	Genotype	Hungarian	Sarri	Hungarian	Sarri	Hungarian	Sarri
SNP	Drought stress	Grain yield (kg/ha)	Grain yield (kg/ha)	Silymarin yield (kg/ha)	Silymarin yield (kg/ha)	Oil yield (kg/ha)	Oil yield (kg/ha)
0 µM	Control	2341.5 a-c	2516.5 ab	80.57 c-e	96.85 bc	568.4 ab	634.9 a
	DS1	1423.5 g	1452.6 g	49.09 fg	67.15 d-f	317.36 f	336.72 ef
	DS2	1852.9 d-g	1706.3 d-g	62.98 d-f	75.05 c-e	438.01 c-e	397.62 d-f
100 µM	Control	2042.9 c-f	2678.2 a	87.84 b-d	125.35 a	508.52 b-c	656.63 a
	DS1	1617.0 fg	2049.0 c-f	72.48 c-f	78.57 c-e	391.21 d-f	510.38 bc
	DS2	2285.3 a-c	2333.1 a-c	94.20 bc	108.72 ab	559.24 ab	574.8 ab
200 µM	Control	2123.9 b-d	2107.4 b-e	82.15 c-e	83.69 c-e	514.40 bc	515.76 bc
	DS1	1680.3 e-g	1506.7 g	71.50 c-f	59.77 e-g	392.79 d-f	350.84 ef
	DS2	2042.8 c-f	1733.8 d-g	78.79 c-e	37.99 g	490.46 b-d	418.4 c-f

Means sharing the same letters in a column or row in per trait do not differ significantly at $p \le 0.05$ according to Duncan's multiple range tests.

Control: Well watered ; DS1:Drought stress in the middle of the bolting ; DS2: Drought stress in the anthesis ; SNP: Sodium nitroprusside.

Drought stress significantly decreased number of capitula mean in the bush (Table 3). No exogenous application compensated this decline under stress condition, such that at stem elongation stress stage, the application of 100 µM SNP increased the number of capitula in Sari ecotype by 44 percent and the level of 200 µM SNP caused a 20 and 35 percent increase in both Sari ecotype and Hungarian cultivar respectively, compared with its non-application. As drought stress intensity increased,the number of grain per capitula indicated a downward trend, but this decline was significant only at the Hungarian cultivar (Table 3). The number of grain per capitula at the Hungarian cultivar was more than Sari ecotype and this increase was significant. At the stage of stem elongation stress, the application of both levels of SNP significantly increased the number of grain per capitula in Hungarian cultivar compared with its non-application, while this improvement in Sari ecotype was merely gained through 100 µM SNP application. Drought stress did not significantly decrease the 1000-seed weight in both stages of irrigation withholding, but spraying with 100 µM SNP at the stage of stem elongation significantly increased 1000-seed weight compared with its non-application under stress condition (Fig. 1). Plant height in both stages of withholding irrigation manifested a significant reduction in irrigated plants (Table 2). However the number of branches of the plant (Table 3) and also the diameter of capitula (Fig. 2) showed the greatest significant decrease in stem elongation stress. The exogenous application of 100 μM SNP prevented height and capitula diameter decline of the plant, particularly at the stage of stem elongation, whereas this level of SNP at this stage improved the number of branches in plant only in Sari ecotype. Similarly, the level of 200 μM was effective in increasing the number of branches in Hungarian cultivar. The factors influencing plant yield shows that there is a positive and significant correlation between yield constituents, particularly capitula number in plant and grain,oil and silymarin yield (Table 4). As a result, increase in the quantitative yield will also enhance qualitative yield under stress condition. Among the constituent components of grain, the grain oil content had a positive and meaningful correlation with all the parameters of yield components, whereas grain silymarin content was only influenced by grain weight, such that increase in grain weight improved the active constituents and finally silymarin yield (Table 4).

Table 2 : Effects of NO application on silymarin and oil seed content and average of plant height of Milk thistle under drought stress.

Treatment	Genotype	Hungarian	Sarri	Hungarian	Sarri	Hungarian	Sarri
SNP	Drought stress	Silymarin content (%)	Silymarin content (%)	Oil content (%)	Oil content (%)	Plant height (cm)	Plant height (cm)
0 μM	Control	3.41 d	3.85 b-d	24.13 a-d	25.21 a	138.99 a-d	146.17 ab
	DS1	3.46 cd	4.63 ab	22.34 e	23.19 de	112.5 h	112.27 h
	DS2	3.38 d	4.39 ab	23.67 b-e	23.33 b-e	123.22 f-h	124.83 e-g
100 μM	Control	4.31 ab	4.68 ab	24.83 ab	24.52 a-d	148.83 a	140.83 a-c
	DS1	4.49 ab	3.84 b-d	24.12 a-d	24.78 a-c	125.4 e-g	127.79 d-g
	DS2	4.12 a-d	4.70 a	24.50 a-d	24.66 a-d	138.61 a-d	132.05 c-f
200 μM	Control	3.88 a-d	3.96 a-d	24.29 a-d	24.49 a-d	130.67 c-f	135.67 b-e
	DS1	4.27 a-c	3.98 a-d	23.41 b-e	23.3 c-e	118.61 gh	116.55 gh
	DS2	3.88 a-d	2.12 e	24.03 a-d	24.04 a-d	127.98 d-g	126.51 e-g

Means sharing the same letters in a column or row in per trait do not differ significantly at $p \leq 0.05$ according to Duncan's multiple range tests.

Control: Well watered ; DS1:Drought stress in the middle of the bolting ; DS2: Drought stress in the anthesis ; SNP: Sodium nitroprusside.

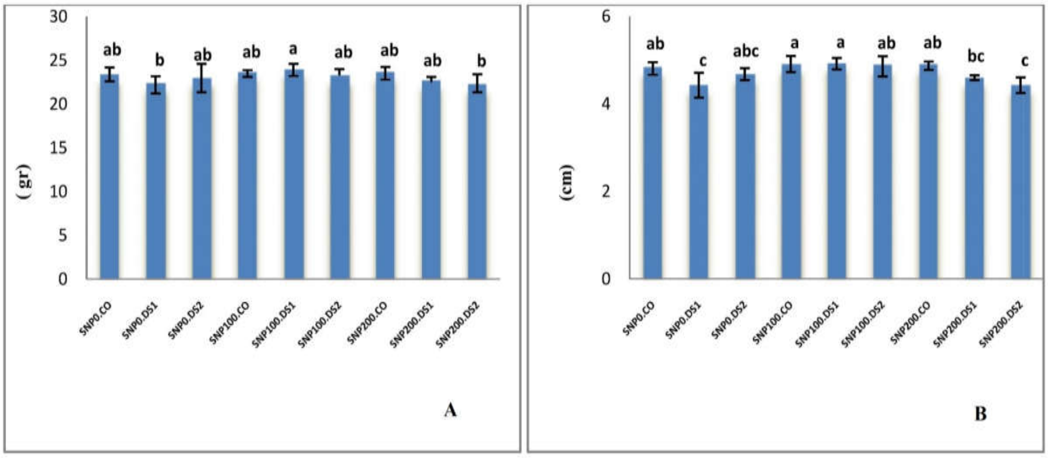

Figure 1. Effects of NO application on 1000 seed weight (A) and bloom diameter (B) of Milk thistle under drought stress. Co : control, DS1:Drought stress of Stem elongation stage, DS2: Drought stress of anthesis stage .SNP : Sodium nitroprusside , bars = s.e

DISCUSSION

In general, water shortage is a constraining factor in producing many crops under farming conditions [31]. Water deficit causes a series of physiological, morphological and biochemical changes in plants including reduction in leaf area and cell division [2,41], closure of stomata and transpiration decline [14], photosynthesis limitations [38] and disruption of absorption and distribution of water and nutrition [15]. Thus photosynthesis assimilates transferred to grain will reduce [32] and this decreases the ultimate plant yield under farming conditions. With water deficit, the milk thistle grain yield manifests a decline[18]. NO application induces stomata closure and protects cells against oxidative Stress[33]. Hence NO capacity relates to stomata opening adjustment by affecting the Ca^{2+} transduction signal pathway [14]. Moreover NO exogenous application can improve fluidness of the membrane especially phospholipid bilayer and lead to enhance plant growth [24]. As a result NO exogenous application enhances cell membrane stability, photosynthesis, and water status of the leaf by increasing synthesis combinations [11] and finally improves the growth of the plant. In an investigation of water stress in Milk thistle concluded that stress significantly decreased stem height and diagonal of inflorescence, but had no influence on 1000-grain weight[6]. Furthermore, decline in the number of bloom and plant height in milk thistle, under irrigation treatments has been reported [18]. Plant height decline in response to drought may be due to xylem and phloem vessels blockage and hence prevention of any transfer in this manner [27]. The exogenous application of 100 μM SNP reduced the negative effects of stress and enhanced it. Reported that Nitric oxide is effective in regulating processes such as leaf expansion and vegetative growth of branch[7].

Table 4: Pearson's correlation coefficients between yield and yield component of parameters on Milk thistle under drought stress and NO application. (n=18).

Trait	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Seed per capitula (1)	1										
1000-seed weight (2)	n.s.	1									
Plant height (3)	0.692**	0.528*	1								
Branch per plant (4)	0.677**	0.543*	0.746**	1							
capitula diameter (5)	0.522*	0.806***	0.563*	0.513*	1						
capitula per plant (6)	0.726**	0.622**	0.853***	0.906***	0.649**	1					
Oil content (7)	0.671**	0.628**	0.866***	0.734**	0.623**	0.842***	1				
Grain yield(8)	0.539*	0.591*	0.854***	0.621**	0.619**	0.823***	0.809***	1			
Oil yield(9)	0.563*	0.607**	0.875***	0.644**	0.634**	0.842***	0.851***	0.996***	1		
Silymarin content (10)	n.s.	0.524*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1	
Silymarin yield (11)	n.s.	0.706**	0.683**	n.s.	0.586**	0.647**	0.662**	0.839***	0.833***	0.657**	1

n.s. , * ,**, and *** indicate non-significant or significant differences at $p < 0.05$, 0.01 or 0.001, respectively.

Increasing irrigation intervals in milk thistle, grain oil content declined up to 5 percent compared with the control[38]. In caraway (*carum carvi*) [23] alo, observed that drought stress reduced the oil content. The exogenous application of 100 μM SNP increased the water use efficiency and reduced transpiration (Data have not been reported), It consequently increased grain filling period and improved grain oil content and oil yield under stress. As a result, regarding the positive effect of NO in grain yield and oil content enhancement under stress, oil yield increased as well. In examining water stress in milk thistle under greenhouse conditions, concluded that there is no significant difference between treatments in total silymarin content[6], Nevertheless, among the constituent components of silymarin, the highest amount of taxifolin was related to low level of irrigation. Also reported that the maximum content of silymarin was obtained in plants grown in field capacity of 60%, and treatments of 75% and 40% of field capacity possessed lower silymarin and completely similar content[17]. Conversely, in other studies argued that as irrigation intervals increased, the total silymarin and its constituent components increased [18]. A similar finding is reported [1] in *Hypericum brasiliense*. Flavonoids are secondary metabolites whose biosynthetic is influenced by environmental conditions [21]. In some medical plants, drought stress increases secondary productions rate because due to stress, decreases plant growth and carbon fixation during photosynthesis is consumed to produce secondary metabolites. Enhancement of these metabolites avoids oxidation inside these cells [39]. However secondary metabolites' yield declines under drought stress, because of produced biomass decline [18]. Nevertheless under more intensive stress, the plant uses most of its photosynthesis assimilates to produce osmotic adjustment materials and processes which come along with energy consumption and finally affect the quantitative and qualitative features of the plant, that Hereon the content of active components may not increase [3]. In addition, reported the effects of sufficient irrigation in boosting growth and the amount of peppermint (*Mentha piperita* L.) essential oil, and argued that irrigation treatments have had no effect on the constituent components of the essential oil [4]. NO application at 100 µM SNP causes relative increase in silymarin content and also significant silymarin yield in area unit, particularly under anthesis stress (Tables 1 and 2). It seems that NO application has aggravated the retranslocation of active constituents (silymarin) from leaves to grain due to the influence of drought stress at the beginning of grain filling stage. Considering the improvement of grain yield by NO application under stress, silymarin yield that is subject to grain yield and silymarin content of grain indicated an increase under stress as well. In a study on the effects of plant growth regulators in milk thistle, reported that foliar spraying with these regulators at the stage of rosette, increased the total silymarin content by raising grain yield in area unit [16]. As a result, No exogenous application at the level 100 µM SNP improved grain, silymarin and oil yield under stress.

Furthermore, the concentration of 100 µM SNP is more efficient than level 200 µM and NO foliar application in Sari ecotype displayed more efficiency under drought stress. The positive and significant correlation between grain yield with oil and silymarin yield under farming conditions indicates the positive influence of NO in silymarin yield, even under stress. As a result, the cultivation of this medical plant can be considered as an economic crop in the medicinal and oil extraction industry.

REFERENCES

1. Abreu, I. N. and Mazzafera, P. (2005). Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. Plant Physiology and Biochemistry, 43, 241–248.
2. Ahmed, ME, Mahmoud, FA. (2010). Effect of irrigation on vegetative growth, oil yield and protein content of two sesame (*Sesamum indicum* L.) cultivars. Res J Agric Biol Sci 6 (5): 630-636.
3. Aliabadi Farahani, H., Valadabadi,S.A., Daneshian, J and Khalvati,M.A. (2009). Evaluation changing of essential oil of balm (*Melissa officinalis* L.) under water deficit stress conditions. Journal of Medicinal Plant Research*.* 3: 329-333.
4. Alkire, B. H., Simon, J. E., Palevtich, D. and Putievsky, E. (1993). Water management for Midwestern peppermint (*Mentha piperita* L.) growing in highly organic soils, Indiana, USA. Acta Horticulturae, 344,544-556.
5. Arasimowicz, M. and Floryszak-Wieczorek, J.(2007). Nitric oxide as a bioactive signaling molecule in plant stress responses. Plant Science, 172: 876–887.
6. Belitz,A.R. and Sams,C.E.(2007). The effect of water stress on the growth, yield, and flavonoligana content in milk thistle (*Silybum marianum*). Proceeding of International Symposium on Medicinal and Nutraceutical Plants.Ed:A.K.Yadav.Acta Hort.,p756.ISHS.
7. Besson-Bard ,A., Pugin .A.and Wendehenne .D. (2008). New insights into nitric oxide signaling in plants. Annual Review of Plant Biology, *59*: 21–39.
8. del-Rı´o,L., Corpasa, F. and Barroso,J . (2004). Nitric oxide and nitric oxide syntheses activity in plants. Phytochemistry, 65: 783–792.
9. Egilla, J. N., Davies ,Jr. and Boutton, T.W.(2005). Drought stress influences leaf water content, photosynthesis, and water-use efficiency of *Hibiscus rosa-sinensis* at three potassium concentrations. Photosynthetica, 43: 135–140.
10. Fan,H.,Li,T.,Guan,L.,Li,Z.,Cai,Y. and Lin,Y.(2012).Effect of exogenous nitric oxide on antioxidant and DNA methylation of *Dendrobium huoshanense* grown under drought stress. Plant Cell Tissue Organs Culture, 109: 307-314.
11. Faroog, M., Basra,S.M.A., Wahid,A. and Rehman,H.(2009). Exogenously applied nitric oxide enhances the drought tolerance in fine grain aromatic rice. Journal of Agronomy & Crop Science, 195: 254-261.
12. Flora, K., Hahn.M., Rosen.H. and Benner.K. (1998). Milk thistle (*silybum marianum*) for the therapy of liver disease. Am.J.Gastroenterol. 93:139-143.

13. Fu,J. and Huang,B.(2001).Involvement of antioxidant and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environmental and Experimental Botany, **45**: 105-114.
14. Garcia-Mata,C. and Lamattina,L. (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiology, **126**:1196-1204.
15. Garg,B.K.(2003). Nutrient uptake and managment under drought: nutrient-moisture interaction . Curr. Agric.27: 1-8.
16. Geneva, M., Zehirov.G., Stancheva.I., Iliev.L . and Georgiev.G.(2008). Effect of soil fertilizer, foliar fertilizer , and growth regulator application on milk thistle development , seed yield, and silymarin content. Communications in soil science and plant analysis. 39:17-24.
17. Hammouda,F.M., Ismail.S.I., Hassan.N.M., Zaki.A.K. and Kamel.A.(1993). Evaluation of the silymarin content in silybum marianum Gaertn. Cultivated under different agriculture conditions. Phytotherapy research. 7 : 90-91.
18. Hendawy , S.F., Hussein.M.S., Youssef.A.A. and EL-Mergawi.R.A.(2013). Respnse of silybum marianum plant to irrigation intervals combined with fertilization. Nusantara Bioscience 5:22-29.
19. Huaifu,F.,Shirong , G., Yansheng,J.,Runhua,Z. and Juan,L.(2007). Effect of exogenous nitric oxide on growth , active oxygen species metabolism, and photosynthetic characteristics in cucumber seedlings under Nacl stress. Front.Agric.China.1,303-314.
20. Kim, J. Y., Mahe´, A., Brangeon, J. and Prioul, J. L. (2000).A maize vacuolur invertase, IVR2, is induced by water stress.Organ/tissue specificity and diurnal modulation of expression. Plant Physiology, **124**: 71–84.
21. Kvasnicka, F., Biba.B., Sevick.R., Voldrich.M. and Kratka.J. (2003). Analysis of the active components of silymarin. J.Chromatogr. A.990, 239-245.
22. Lamattina,L.,Garcia-Mata,C.,Graziano,M.and Pagnussat,G.(2003).Nitrix oxide: The versatility of on extensive signal molecule.Ann Rev Plant Biol.54:109-136.
23. Laribi, B., Bettaieb, I ., Kouki, K ., Sahli, A., Mougou, A and Marzouk,B .(2009). Water deficit effects on caraway (*Carum carvi* L.) growth, essential oil and fatty acid composition. Indian Crop Production. 30: 372-379.
24. Leshem ,Y.Y. and Haramaty ,E .(1996). The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* L. foliage. Journal of Plant Physiolgy, 148: 258-263.
25. Leshem, YY., Wills, RBH.and Ku, VVV. (1998). Evidence for the function of the free radical gas-nitric oxide (NO) as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiological Biochemistry, 36: 825–833
26. Loggini,B.,Scartazza,A.,Brugnoli,E. and Narari, F.(1999).Antioxidant defense system, pigment composition and photosynthesis efficiency in two wheat cultivars subjected to drought. Plant Physiology, 119: 1091-1099.
27. Lovisolo,C. and Schubert.A.(1998). Effects of water stress on vessel size and xylem specific hydraulic conductivity in vitis vinifera. J. Exp. Bot. 49:693-700.
28. Malekzadeh,M., Mirmazloum,S.I., Rabbi Anguorani,H., Mortazavi,S.N. and Panahi,M. (2011).The physicochemical properties and oil constituents of milk thistle (*Silybum marianum*) under drought stress. Journal of Medicinal Plants Research, 5(8)**:** 1485-1488.
29. Misra,A.N.,Misra,M.and Singh,R.(2011). Nitric oxide ameliorates stress responses in plants.Plant Soil Environment.57(3): 95-100.
30. Morazzoni,P. and Bombard .E.(1995). Silybum marianum (Carduus marianus). Fitoterapia. 66 : 3-42.
31. Munns,R.(2002). Comparative physiology of salt and water stress. Plant Cell Environment, **25**:239-250.
32. Moussavi,S.M., Salari.M. and Mobasser. H.R.(2011). The effect of different irrigation intervals and mineral nutrition on seed yield of Ajowan (Trachy spermum ammi). Ann Biol Res . 2 (6) : 692-698.
33. Neill,S.,Barros,R.,Bright,J.,Desikan,R.,Hancock,J.,Harrison,J.,Morris,P.,Rieeiro,D.and Wilson,I.(2008).Nitic oxide, stomatal closure and abiotic stress.J.Exper.Bot.59,165-176.
34. Sharma, P. and Dubey,R.S. (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings.Plant Growth Regul., 46: 209-221.
35. Selmar, D.(2008). Potential of salt and drought stress to increase pharmaceutical significant secondary compounds in plants. Agronomy Forest Research. 58: 139-144.
36. Simpson, G.G. (2005) **.** NO flowering. Bioessays, **27**: 239-241.
37. Stoiljkovic, Z., Petrovic.S.D. and Ilic.B.S.(2007). Examination of localization of silymarin and fatty oil in silybum marianum (L.) Gaertn. Fruit. CI & CEQ .13(2) .55-59.
38. Tan,J.,Zhao,H.,Hong.J.,Han,Y. and Zhao,W.(2008). Effects of exogenous nitric oxide on photosynthesis, antioxidant capacity and proline accumulation in wheat seedlings subjected to osmotic stress. World Journal of Agricultural Sciences, 4 (3): 307-313.
39. Turtola, S., Manninen, A., Rikala, R., Kainulainen, P .(2003). Drought stress alters the concentration of wood terpenoids in scots pine and Norway spruce seedling. J. Chem. Ecol. 29: 1981-1995.
40. Wahid, A., M. Parveen, S. Gelani, and S. M. A. Basra.(2007). Pretreatment of seeds with H2O2 improves salt tolerance of wheat seedling by alleviation of oxidative damage and expression of stress proteins. J. Plant Physiol. 164, 283–294.
41. Wahid, A. and Rasul, E. (2005). Photosynthesis in leaf, stem, flower and fruit. In: M. Pessarakli ed. Handbook of Photosynthesis, 2nd edn. pp. 479–497. CRC Press, Boca Raton, FL.
42. Wilson,I., Ribeiro,D., Bright,J., Confraria,A., Harrison,J., Barros,R., Desikan,R., Neill,N. and Hancock,J .(2009). Role of nitric oxide in regulating stomatal apertures. Plant Signaling & Behavior, 4**:5**: 467-469;

43. Zeng,C., Liu,L.,Wang,B., Wu,X. and Zhou,Y. (2011). Physiological effects of exogenous nitric oxide on *Brassica juncea* seedlings under NaCl stress. Biologia Plantarum, **55**: 345-348.
44. Zhu, J.K. (2002). Salt and Drought stress signal transduction in plants. Annual Review of Biology, **53**: 247-273.