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Research Article

The Effects of Water Deficit Stress on Physiological and Biochemical Changes of Medicinal Plants *Ocimum basilicum L.* under Climatic Conditions in Ardabil, Iran

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ABSTRACT

Basil (*Ocimum basilicum L.*) is an important medicinal and aromatic plant which is cultivated throughout the world and investigation the effects of important agronomic factors on its quantitative and qualitative yield are necessary.This research was conducted in the years 2013-2014 in agricultural farm of Mr. Abedini in the village Mahmoud Abad of Namin city functions, (Ardabil, Iran), as factorial experiment in a completely randomized block design by 3 replications. The experimental treatments were water deficit stress (D_1=70, D_2=140 and D_3= 210 mm evaporation from pan class A) as category factors and three levels of nitrogen fertilizer in the form of urea (N_1= 0, N_2 = 50, N_3 = 100 kg/ha).The results showed that the most ratio of the essential oils of basil obtained in the first and the second harvests (2.37% and 1.81%, respectively) in the treatment of without nitrogen fertilizer and the lowest ratio of the essential oils of basil was in the first and second harvests (1.43% and 1.69%, respectively) in the treatment of 100 kg/ha nitrogen fertilizer. Interactions of water deficit stress and nitrogen fertilizer on dry matter yield per unit area was significant at total of two harvests (total yield); and the highest dry matter yield was observed in treatments of D_1= 70 mm evaporation and 100 kg/ha nitrogen fertilizer (1000.01 kg of dry matter per hectare), and the lowest ratio was achieved in treatments of D3=210 mm evaporation without the use of fertilizer (516.43 kg of dry matter per hectare). On the other hand the results indicated that interactions of water deficit stress and nitrogen fertilizer on essential oil yield per unit area was not significant at the first harvest, the second harvest and total yield. However, the highest essential oil yield was obtained at the first harvest, the second harvest and total yield, (9.08, 7.54 and 15.21 l/ha, respectively) in the treatments of D_1=70 mm evaporation and nitrogen fertilizer of 100 kg/ ha as well as the lowest essential oil yield (5.01, 5.1 and 9.03 l/ha, respectively) in the treatments of D_3=210 mm evaporation and failure to use of nitrogen fertilizer.

Keywords: Water Deficit Stress; Physiological and Biochemical Characteristics; Essential Oil; Ocimum basilicum L.

INTRODUCTION

Basil is a plant of the genus Ocimum belonging to the family of mint which its ecotypes have a large variety of morphology (Maruti et al., 1996).This medicinal plant is important in the treatment of diseases related to lung, chest, appetizing effect, antiparasitic, antipyretic, expectorant enhancers, carminative, ear pain, skin diseases, sexual desire, asthma, convulsive, headache, dizziness, nausea, cough, anthrax, angina, diabetes, colds, constipation, dysentery as well as it is useful in the treatment of cancer, gonorrhea and neurasthenia (Omidbaigi, 2000). In a study, Afkari in 2013 stated that the highest dry matter yield per unit area have been at the first harvest in treatments of 80 plants per square meter and nitrogen fertilizers of 100 kg/ha (32.617 kg/ha) and the lowest ratio was in treatment s of 40 plants per square meter without fertilizer (71.2250 kg/ha). On the other hand, maximum yield of dry matter per unit area has obtained in the second harvest with density of 80 plants per square meter and 100 kg of nitrogen fertilizer per hectare (21.437 kg of dry matter per hectare) and the lowest ratio has achieved with density of 40 plants per square meter without application of nitrogen fertilizer (21.223 kg of dry matter per hectare). Greatest amount of essential oils of basil was at the first harvest in density of 60 plants per square meter (98.2%) and the lowest was in density of 40 plants per square meter (1.29%), also in the second harvest, the highest essential oil was taken in density of 60 plants per square meter (3.2%) and the lowest was in density of 40 plants per square meter (1.1%). Dadvand et al., in 2008 reported their highest results including: 4197.5 kg/ha the yields of fresh matter of basil, dry matter (1078.6 kg/ha), leaf (671.1 kg/ha), the amount of essential oil (0.826% based on dry weight) and essential oil yield (5.164 kg/ha) under conditions at 20 × 20 cm planting and consumed 5 kg of nitrogen fertilizer per hectare.Elham et al., in 2005 indicated that the highest percentage of essential oils was achieved from the herb valerian with density of 40000 plants per hectare. Akbarinia et al., in 2005 in a study on coriander plant showed that the density had significant effect on all traits; the most seed and essential oil yields have been in a density of 30 plants per square meter. Ebadi et al., in 2009 investigated the effects of seeding rate on yield, the percentage of essential oil and chamazulene in modified German Chamomile Presov and stated that the ratio of required seed had significant effect only on the number of plants per plot and essential oil yield; consumed 0.4 grams of seeds produced the highest essential oil and chamazulene (respectively percent by weight based on the weight of the 0.63 and 5.9 dry) and the most essential oil yield (0.97 grams per square meter). Dumel and Ceylon in 1977 reported that the maximum yield of fresh matter of basil (1551 kg per hectare) obtained in rows culture of 15 cm. Jill et al, in 2000 obtained the greatest amount of essential oils in plant density of 30 × 30 cm² and maximum performance in plant density of 20 × 40 cm². Heydariet al., in 2009 found that in *Mentha piperita L.* plant density increased only dry yield and essential oil yield int he first and second harvests andhad no significant effect on the percentage of leaves essential oil and oil producing glands as well as the highest oil yield per hectare was achieved by increasing plant density. Omidbaigi, in 2000 concluded that 40 plants of coriander per square meter had the highest percentage of essential oils with the 0.4554 percent.Basil essential oil content varies according to the climatic conditions of growth between 0.5 and 1.5 percent. Constituents of the essential oil are different.In general, the components of basil oil are linalool, citral, eugenol, cineole, geraniol, camphor and methyl cinnamate (Simon et al., 1990, Ozok et al., 1995). Afkari, in 2013 obtained the greatest dry matter yield and essential oil yield in density of 80 plants per square meter and consumed 100 kg of nitrogen fertilizer which were also known as the best treatment. Gill and Randhava in 2000 reported the highest essential oil can be achieved in plant density of 30 × 30 cm². Algenerie et al., in 2001 in an experiment with 4 spacing 15, 25, 35, 40 cm reported that the number of branches, leaves and dry weight enhanced by increasing plant space, while plant height was reduced and the highest essential oil was obtained by the spacing of 35 cm.

MATERIAL AND METHODS

Factorial experiment in a completely randomized block design by 3 replications was conducted to study the effects of water deficit stress and nitrogen on agronomic and nutritional characteristics of Basil (*Ocimum basilicum L.*). The experimental treatments were water deficit stress (D_1=70, D_2=140 and D_3= 210 mm evaporation from pan class A) as category factors and three levels of nitrogen fertilizer in the form of urea (N_1= 0, N_2 = 50, N_3 = 100 kg/ha).Seed sown on 12 May 2013 for the cluster and 4-leaf seedlings were thinned.Seed planting depth was considered one centimeter. Half the amount of determined nitrogen at the same time planting and the rest of nitrogen were given to the farm on the stage of 8-leaf plants. At the first harvest, basil was collected from a distance of 10 cm from the soil surface.The final harvest (second harvest) coincided with the full flowering. Parameters measured included leaf area index, the amount of essential oil (based on percentage of dry matter), essential oil yield per hectare, dry matter yield in the first, the second and total harvest (sum of two harvests); these two harvests were done in stage of full flowering. During periods of stress treatment, soil moisture was repeatedly measured using TRD to achieve more accuracy. Only two lines were harvested from each plot in order to determine the quantitative and qualitative performance. After collecting plant samples, the samples were dried at room temperature and away from light. The extraction of essential oil was done by hydro-distillation (by Clevenger apparatus) for three hours.Data obtained from this study was analyzed by statistical software MSTAT-C and SAS and the comparison of means was calculated by Duncan method.

Table 1- The profile of research farm

EC (ds / m)	Acidity (PH)	The average annual Rainfall (mm)	Longitude	latitude	Height above sea level (m)	The average annual temperature (° C)
1.1	6.3	352	46º and 48'	59º and 33'	1678	19.21

Table2-Physical and chemical properties of soil of research farm

Sp (%)	Texture			$CaCO_3$	K	P	N	OM	O.C
	Clay (%)	Silt (%)	Sand (%)	ppm	ppm	ppm	(%)	(%)	(%)
29.22	23.3	19.92	55.1	3.62	112	8.8	0.61	1.35	7.04

RESULTS AND DISCUSSION

The effect of water deficit stress on dry matter yield. The results showed that water deficit stress had a significant effect on dry matter yield (p <0/01).The highest and lowest dry matter yield per unit area (in the first, second and total harvests) obtained in D_1 = 70mm and D_3 =210 mm evaporations, respectively (Table 3 and 4).It is clear that the weight of each plant was reduced by increasing water deficit stress and the minimum weight was obtained from the treatment plant of D_3 = 210 mm evaporation.The results of this study were consistent with the findings of Singh et al., in 1989, Anvar et al., in 2005 that the performance of the branches, number of leaves, pigments of leaves and the ratio of leaf to stem and leaf area index had increased taking 100 kg/ha nitrogen fertilizer.

The interactions of water deficit stress and nitrogen fertilizer on dry matter yield

The results showed that the interactions of water deficit stress and nitrogen fertilizer on dry matter yield per unit area in the first harvest was significant (p <0/01) and the highest dry matter yield per unit area had been obtained in the first harvest in treatment of D_1 =70 mm evaporation and 100 kg nitrogen fertilizers per hectare (550.12 kg/ha) and the lowest had been achieved in treatment of D_2 =140 mm evaporation and treatment without fertilizer (231.09 kg/ha) (Tables 3 and 6).Although the interaction water deficit stress and nitrogen fertilizer on dry matter yield per unit area in the second harvest was not significant (Table 3), but the greatest dry matter yield per unit area was in the second harvest in treatment of D_1 =70 mm evaporation and 100 kg nitrogen fertilizers per hectare (427.01 kg/ha) and the minimum was in treatment of D_3 =210 mm evaporation and treatment without fertilizer (271. 9 kg/ha).Therefore, in the second harvest like the first one, the highest dry matter yield per unit area was in the minimum levels of water deficit stress and maximum levels of nitrogen fertilizer (Table 6). Also, the results showed that the interaction of water deficit stress and nitrogen fertilizer on dry matter yield per unit area was statistically significant in total of two harvests (total yield) (Table 3) and the most dry matter yield was in treatment of D_1 =70 mm evaporation and 100 kg nitrogen fertilizers per hectare (1000.01 kg/ha) and the minimum was in treatment of D_3 =210 mm evaporation and treatment without fertilizer (516.43 kg/ha) (Table 6).However, this study showed that the average water stress and nitrogen fertilizer can be enough to achieve maximum dry matter production.

The effect of water deficit stress onratio of basil essential oil

Although the deficit stress per unit area had no significant effect on the ratio of basil essential oil in the first and second harvests (Table 3), but most of basil essential oil was in the first harvest treatment of D_1 = 70mm evaporation, 1.52%, and lowest ratio was in treatment of D_3 = 210mm evaporation, 1.12% (Table 4). Also in the second harvest, the most basil essential oil in D_2 = 140mm evaporation and the lowest in D_3 = 210 mm evaporation were obtained (Table 4). These results were in line with the findings of Nobahar and Pazuki in 2010 as well as Marouti et al in 1996 who stated that the amount of essential oil was decreased by increasing of water deficit stress than control.

The effect of nitrogen fertilizer onratio of basil essential oil

The results showed a significant effect of nitrogen on the basil essential oil (Table 3) and the most basil essential oil was in the first and the second harvests (2.37% and 1.81%, respectively) in the treatment of without nitrogen fertilizer. In other words, the essential oil is inversely related to increasing of nitrogen fertilizer and the least amount of essential oil of basil obtained in the first harvest and second harvest (1.43% and 1.69%, respectively) in the treatment of 100 kg nitrogen fertilizer per hectare (table 5). However**,** the reasons can be less maturity of plants growing in the fertilizer treatmentsthat the results were similar to the research of Singh et al. in 1989 and Arabasi and Bayram in 2004 who stated that the essential oil reduced in the presence of nitrogen in comparison with treatment without fertilizer.

The effect of water deficit stress on essential oil yield

The results showed that water deficit stress had significant effects on the yield of the essential oil of basil in the first harvest, the second harvest and total yield per unit area (Table 3). And by reducing stress because of increasing dry matter yield per unit area, essential oil yield per unit area in the first harvest, the second harvest and total yield (7.00, 5.22 and 12.01 liters per hectare, respectively) obtained in D_1 =70 mm evaporation and the lowest was (4.69, 4.19, 8.88 liters per hectare, respectively) in the treatment of D_3 =210 mm evaporation (table 4). So the intensity of light in Ardabil is enough for basil as if it is possible to achieve the desired quantitative and qualitative yield, even in the treatment D_1 =70 mm evaporation and the absence of significant difference in the percentage of oil also emphasized this issue. Naghdi et al, 2002, in a study on thyme have been reported similar results.

The effect of nitrogen fertilizer on essential oil yield

Nitrogen fertilizer has a significant effect on basil essential oil yield in the first harvest, the second harvest and total yield per unit area (Table 3). The highest essential oil yield per hectare obtained at the first harvest, second harvest, and total yield (7.21, 7.17 and 13.83 liters per hectare, respectively) with 100 kg of nitrogen fertilizer per hectare and the lowest (5.93, 5.57 and 12.03 liters per hectare, respectively) achieved in the treatment of without nitrogen fertilizer (table 5). By increasing of nitrogen, essential oil yield has increased per unit area (Table 5). This increase is due to the positive impact of nitrogen fertilizer on the dry matter yield per unit area. Although application of nitrogen reduced the amount of essential oil, but this decline was offset by increased production of dry matter per hectare. In the study by Zheljazkov et al., in 1997 conducted to evaluate the effect of nitrogen fertilizer on yield of peppermint essential oil was shown that the essential oil yield per hectare increased with the consumed 151 kg of nitrogen per hectare. Arabasy and Bayram in 2004 reported that essential oil yield of basil increased in the presence of nitrogen fertilizer

The interactions of water deficit stress and nitrogen fertilizer on ratio of basil essential oil in first and second harvests

The results indicated that the interactions of water deficit stress and nitrogen fertilizer on ratio of the basil essential oil in the first harvest and second harvest were not statistically significant (Table 3). The highest essential oil content of basil were in the first harvest in treatment of D_1=70 mm evaporation and lack of fertilizer nitrogen, 2.07%, and the second harvest in treatment of D_2 =140 mm evaporation without nitrogen fertilizer, 1.87% (Table 6). Pirzad et al., in 2008 in a study on German chamomile have reported similar results.

The interactions of water deficit stress and nitrogen fertilizer on essential oil yield

The results indicated that the interaction of water deficit stress and nitrogen fertilizer on essential oil yield per unit area was not significant in the first harvest, the second harvest and total yield (Table 3). However, the highest essential oil yield in the first harvest, the second harvest and total yield (9.08, 7.54 and 15.21 liters per hectare, respectively) was in the treatment of D_1=70 mm evaporation and 100 kg/ha nitrogen fertilizer and the least yield of essential oil (5.01, 5.1 and 9.03 liters per hectare, respectively) obtained in the treatment of D_3 =210 mm evaporation and lack of nitrogen fertilizer (table 6). Dadvand, sarab. in 2008 in a research on basil have also reported similar results.

Table 3- Analysis of variance related to measured traits

Mean squares									
Essential Oil Yield			The Amount of Essential Oil			Dry Matter Yield		Degrees of freedom	Sources of changes
Total yield	Second harvest	First harvest	Second harvest	First harvest	Total yield	Second harvest	First harvest		
0.341**	0.43**	0.103 ns	0.0812**	0.0091 ns	1254.11**	310.01**	196.21ns	3	Repeat
49.371**	8.671**	17.211**	0.0021 ns	0.0012 ns	199832.49**	39871.83**	61138.57**	2	Water stress
4.211**	5.231**	7.204**	0.0937**	0.710**	10094.61**	2019.78**	80536.1**	2	Nitrogen
0.051 ns	0.049 ns	0.019 ns	0.00296 ns	0.0110 ns	517.20*	12.17**	497.54**	4	D × N
7.101	2.121	4.101	13.205	16.190	15.092	9.16	10.206	24	Error

ns, *, ** indicate non significant and significant in the levels of five percent and one percent, respectively

Table 4- The effects of water deficit stress on dry matter yield, essential oil content and essential oil yield in different harvests

treatments	Dry Matter Yield (Kg / ha)			The Amount of Essential Oil (%)		Essential Oil Yield(l / ha)		
	First harvest	Second harvest	Total yield	First harvest	Second harvest	First harvest	Second harvest	Total yield
D_1	459.013a	390.32a	847.12a	1.52ab	1.47a	7.0a	5.22a	12.01a
D_2	371.67b	315.828b	686.211b	1.55a	1.59ab	5.79b	4.9a	10.69b
D_3	313.477c	267.416c	578.565c	1.12b	1.31c	4.69c	4.19c	8.88c

D_1 =70mm evaporation from pan, D_2 = 140mm evaporation from pan, D_3 =210mmevaporation from pan. *Same letters in each column represent no significant difference.

Table 5- The effects of nitrogen fertilizer on dry matter yield, essential oil content and essential oil yield in different harvests

treatments	Dry Matter Yield (Kg / ha)			The Amount of Essential Oil (%)		Essential Oil Yield(l / ha)		
	First harvest	Second harvest	Total yield	First harvest	Second harvest	First harvest	Second harvest	Total yield
N_1	297.370c	279.40c	682.103c	2.37c	1.81a	5.93c	5.57c	12.03bc
N_2	368.471b	371.08b	751.92b	1.77b	1.76ab	6.48b	6.31b	12.14b
N_3	392.037a	401.12a	891.69a	1.43a	1.69b	7.21a	7.17a	13.83a

N_1:control treatment (without nitrogen fertilizer, N_2: Treatment of 50 kg/ha nitrogen fertilizer, N_3:Treatment of 100 kg/ha nitrogen fertilizer.

*Same lettersin each columnrepresentnosignificantdifference.

Table 6- The interactions of water deficit stress and nitrogen fertilizer on dry matter yield, essential oil content and essential oil yield in different harvests

treatments	Dry Matter Yield (Kg / ha)			The Amount of Essential Oil (%)		Essential Oil Yield(l / ha)		
	First harvest	Second harvest	Total yield	First harvest	Second harvest	First harvest	Second harvest	Total yield
$N_1 \times D_1$	387.08d	384.31c	763.11d	2.07ab	1.87abc	7.45c	7.01c	13.13c
$N_2 \times D_1$	451.24b	410.19b	881.02b	1.82c	1.76bcd	8.32b	7.32b	14.33b
$N_3 \times D_1$	550.12a	427.01a	1000.01a	1.77de	1.63d	9.08a	7.54a	15.21a
$N_1 \times D_2$	231.09e	331.06e	608.2f	2.47a	1.89ab	6.4d	6.09e	11.09e
$N_2 \times D_2$	3.71.16d	339.9d	700.09e	1.90c	1.79abc	6.78d	6.05d	11.83e
$N_3 \times D_2$	461.71b	341.18d	801.07c	1.75cde	1.71cd	6.39c	5.77d	13.01d
$N_1 \times D_3$	257.33f	271.9g	516.43h	1.94b	2.01a	5.01e	5.01h	9.03h
$N_2 \times D_3$	321.21e	289.29f	589.50c	1.69cd	1.75bcd	5.41e	4.22g	9.83g
$N_3 \times D_3$	410.60c	296.04f	692.04e	1.67e	1.74bcd	6.68d	5.91f	10.03f

D_1 =70mm evaporation from pan, D_2 = 140mm evaporation from pan, D_3 =210mmevaporation from pan. N_1:control treatment (without nitrogen fertilizer, N_2: Treatment of 50 kg/ha nitrogen fertilizer, N_3:Treatment of 100 kg/ha nitrogen fertilizer.

*Same letters in each column represent no significant difference.

CONCLUSION

The results showed that water deficit stress and nitrogen fertilizer had significant impacts on quantitative and qualitative yields of basil (*Ocimum basilicum L.*). Considering that the maximum dry matter yield and essential oil had been achieved in treatment of D_1 =70 mm evaporation with the consumed 100 kg of nitrogen fertilizer per hectare, more researches are recommended to investigate the effect of higher levels of the treatments. In addition, with regard to the amount and duration of sunlight under climatic conditions in Iran under as well as their crucial roles in producing of high quality basil can be extended the cultivation of medicinal herbs such as basil to meet the needs of the pharmaceutical industries.

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