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The Evaluation of Organic and Chemical Fertilizers on Growth Characteristics and Essential Oil of Sage (*Salvia Officinalis* L.) Under Water Stress

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Abstract: *Sage (Salvia officinalis* L.*) is a popular medicinal plant which is widely used in food and pharmaceutical industries. In order to study of vermicompost, biofertilizer and nitrogen on quantity and quality yield of Sage under different moisture conditions, a field experiment in split plot design with 15 treatments and three replications was conducted at Tarbiat Modares University in two successive seasons (2011-2012 and 2012-2013). Treatments included three irrigation levels (irrigation after depletion of 40% available water, irrigation after depletion of 60% available water, irrigation after depletion of 80% available water) and five different soil fertility systems (control (no fertilizer) (F0), chemical fertilizer (urea) (Ur), nitrogen fixing bacteria (NFB), vermicompost (V) vermicompost + nitrogen fixing bacteria (V+NFB)) will be allocated to main plots and sub plots respectively. Results showed thatthe highest plant height, canopy diameter, leaf area index and dry matter yield were obtained at no stress and V+NFB at all three harvest. Also, the maximum essential oil content and essential oil compounds were obtained at moderate stress and V+NFB at all three harvest. Among the three times harvest, the highest plant height (28.84 cm), canopy diameter (29.58 cm), leaf area index (1.77) and dry matter yield (2.964 t ha-1) were observed at third harvest time. The maximum essential oil content (1.90%) was obtained at second harvest time and the highest α-thujone (32.73%), 1, 8- cineol (10.91%) and camphor (32.47%) were obtained at third harvest time.*

Keywords: *Organic fertilizer, Biofertilizer, Chemical fertilizer, drought stress, essential oil.*

1. Introduction

Sage is a popular medicinal plant which is widely used in food and pharmaceutical industries. Vermicompost rich source of macronutrients, micronutrients, vitamins, enzymes and hormones of plant growth promoters. Therefore, the use of vermicompost in sustainable agriculture, in addition to increasing population and activity of beneficial soil microorganisms, causes the rapid growth of medicinal plants [1].The uses of bacteria (Azotobacter, Azospirillum and Pseudomonas) as a biofertilizer have causes increasing the efficiency of nitrogen and phosphorus fertilizers and improving the growth of several crops [2]. Drought stress is one of the most important environmental stresses that affect the growth and yield of plants [3]. Management of nutrients for plants, especially during drought conditions and to assess the effect of such management on the quantity and quality of sage medicinal plant is a special place. Limited information are available about the response of sage under water deficiency conditions in different fertilizing systems, so the aim of this research was to study the growth, yield and essential oil production of these species under these conditions.

2. Materials and Methods

Field experiments were carried out at the field research station of the Faculty of Agriculture of Tarbiat Modares University in Tehran, Iran during two successive seasons (2011-2012 and 2012-2013).

The experiment was laid out as a randomized complete block design arranged in split plots with three replications. Three irrigation levels, irrigation after depletion of 40% available water (I1), irrigation after

depletion of 60% available water (I2), irrigation after depletion of 80% available water (I3) and five different soil fertility systems, control (no fertilizer) (F1), chemical fertilizer (urea=150 kg ha-1) (F2), nitrogen fixing bacteria(*Azotobacter+Azospirillum+Pseudomonas*) (F3), vermicompost (8 t ha-1) (F4) vermicompost + nitrogen fixing bacteria (F5) were allocated to the main plots and sub plots, respectively. The vermicompost is broadcasted by hand uniformly 3 days before planting and incorporated to the soil by a rototiller. ½ of the urea (75 kg ha^{-1}) was applied at the planting time and ½ in one month later.Nitrogen fixing bacteria (*Azotobacter+Azospirillum+Pseudomonas*) before planting were inoculated with the seedlings roots (as adrift). Seedlings planted by hand on 5 may 2012 by density of 55555 plants ha^{-1} (60×30 cm). A TDR probe (Time-Domain Reflectometry, Model TRIME-FM, England) was applied to measure soil water content.Essential oil extraction was subjected to conventional hydrodistillation using a Clevenger-type apparatus in accordance. Essential oil analysis was performed using a TRACE GC (ThermoQuest-Finnigan) equipped with a DB-5 fused silica column (30 m × 0.25 mm, 0.25 μm film thickness). GC–MS analyses were carried out on a TRACE MS (ThermoQuest-Finnigan).

3. Results

Results showed that the highest plant height, canopy diameter, leaf area index and dry matter yield were obtained at no stress and V+NFB at all three harvest (Table 1).Also, the maximum essential oil content and essential oil compounds were obtained at moderate stress and V+NFB at all three harvest (Table 2). Results indicated that among the three times harvest, the highest plant height (28.84 cm), canopy diameter (29.58 cm), leaf area index (1.77) and dry matter yield (2.964t.ha^{-1}) were observed at third harvest time (Table 3). Also, the maximum essential oil content (1.90%) was obtained at second harvest time and the highest α-thujone (32.73%), 1, 8- cineol (10.91%) and camphor (32.47%) were obtained at third harvest time(Table 3).

4. Discussion

Reduction in the morphological characteristics and dry matter by increasing drought stress could be the result of a preferential allocation of biomass production to the roots [4] or a reduction in chlorophyll content and, consequently, photosynthesis efficiency[5]. Positive and synergistic effects between vermicompost and bacteria can increase bacterial activity in the soil and bacteria in the rhizosphere through mechanisms such as production of growth hormone which increased root growth [6]. Moreover, due to their positive effect on growth bed, increasing of root surface and more absorbtion of nutrients, they increase the activity of photosynthesis, growth and yield of the plant [7]. The increase in essential oil concentration under water stress could be due to the fact that plants produce high terpene concentrations under water stress conditions due to a low allocation of carbon to the growth, suggesting a trade-off between growth and defense [8].Vermicompost and nitrogen fixing bacteria, by providing more uptake of phosphorus and nitrogen, increase the essential oil content.

TABLE I:Means comparison of some measured characters of sage under different irrigation regimes and fertilizer treatments

	Plant height(cm)			Canopy diameter (cm)			Leaf area Index			Dry matter yield (t.ha^{-1})		
Treatment	September 2012	May 2013	September 2013	September 2012	May 2013	September 2013	September 2012	May 2013	September 2013	September 2012	May 2013	September 2013
Irrigation	***	***	***	***	***	***	***	***	***	***	***	***
I_1	29.06a	25.14a	35.58a	33.24a	31.15a	37.89a	1.37a	1.08a	2.37a	2.336^{a}	2.179^{a}	3.971^{a}
I_2	23.07b	19.19b	29.06b	27.09b	25.18b	31.44b	0.83b	0.73b	1.84b	1.335^{b}	1.222^{b}	3.090^{b}
I_3	16.41c	14.09c	21.11c	16.49c	13.69c	19.40c	0.36c	0.32c	1.09c	0.600^{c}	0.528^{c}	1.833^{c}
Fertilizer	***	***	***	***	***	***	***	***	***	***	***	***
F_0	17.68e	14.96e	23.22e	17.21d	15.30d	21.71d	0.59e	0.50e	1.43d	0.945^{e}	0.831^{e}	2.389^{d}
Ur	22.83c	19.09c	28.22c	26.31c	23.58c	29.10c	0.81c	0.71c	1.76c	1.447^{c}	1.191^{c}	2.955^{c}
V	24.74b	21.02b	30.46b	27.70b	26.00b	32.30b	0.95b	0.77b	1.87b	1.582^{b}	1.268^{b}	3.138^{b}
NFB	21.88d	18.34d	27.56d	26.28c	24.12c	29.37c	0.75d	0.63d	1.75c	1.219^{d}	1.052^{d}	2.931^{c}
V+NFB	27.11a	23.94a	33.39a	30.54a	27.70a	35.40a	1.17a	0.94a	2.04a	1.925^{a}	1.571^{a}	3.409^{a}
Irrigation× Fertilizer	***	***	***	***	***	***	***	***	***	***	***	***
I_1×F_0	23.97ef	20.20d	30.27e	23.33e	20.83g	29.67f	0.97f	0.77e	1.89ef	1.583^{f}	1.287^{e}	3.171^{ef}
I_1×Ur	29.07c	24.83c	35.17c	34.67b	32.60c	38.07c	1.37c	1.24b	2.46b	2.686^{b}	2.065^{b}	4.118^{b}
I_1×V	31.40b	26.97b	37.53b	35.53b	34.13b	40.67b	1.50b	1.07c	2.48b	2.461^{c}	1.775^{c}	4.152^{b}
I_1×NFB	27.90d	24.10c	33.97d	34.67b	32.40c	37.50c	1.26d	0.95d	2.36c	2.078^{d}	1.588^{d}	3.953^{c}
I_1×V+NFB	32.98a	29.60a	40.97a	38.00a	35.77a	43.53a	1.76a	1.37a	2.66a	2.872^{a}	2.272^{a}	4.460^{a}
I_2×F_0	17.80i	14.30h	23.63i	16.97h	15.17i	23.00h	0.60j	0.54g	1.55h	0.907^{i}	0.896^{g}	2.587^{h}
I_2×Ur	23.13f	19.17e	28.97f	28.47d	25.80f	30.80ef	0.80h	0.67f	1.85f	1.208^{g}	1.131^{f}	3.093^{f}
I_2×V	24.63e	20.61d	31.03e	28.93d	28.40e	34.60d	0.91g	0.79e	1.92e	1.527^{f}	1.323^{e}	3.218^{e}
I_2×NFB	21.80g	17.63f	27.50g	28.67d	26.40f	31.33e	0.73i	0.70f	1.79g	1.173^{g}	1.177^{f}	2.990^{g}
I_2×V+NFB	28.00cd	24.23c	34.17d	32.43c	30.13d	37.47c	1.11e	0.95d	2.13d	1.857^{e}	1.585^{d}	3.560^{d}
I_3×F_0	11.27k	10.37j	15.77k	11.33j	9.90l	12.47k	0.21m	0.19i	0.84m	0.344^{l}	0.309^{j}	1.410^{m}
I_3×Ur	16.30j	13.27i	20.77j	15.80hi	12.33k	18.43j	0.27l	0.23i	0.99l	0.449^{k}	0.379^{ij}	1.654^{l}
I_3×V	18.20i	15.50g	22.80i	18.63g	15.47i	21.63i	0.45k	0.47h	1.22j	0.757^{j}	0.705^{h}	2.043^{j}
I_3×NFB	15.93j	13.30hi	21.20j	15.50i	13.57j	19.27j	0.24lm	0.23i	1.11k	0.405^{kl}	0.392^{i}	1.851^{k}
I_3×V+NFB	2037h	18.00f	25.03h	21.20f	17.20h	25.20g	0.62j	0.51gh	1.32i	1.046^{h}	0.856^{g}	2.206^{i}

Mean values of the three recollections and each recollection date. Different letters express significant differences at P <0.05 within each column.

Irrigation:Irrigation after depletion of 40% available water (I_1), Irrigation after depletion of 60% available water (I_2), Irrigation after depletion of 80% available water (I_3)

Fertilizer regimes:Control (no fertilizer) (F0), Urea(Ur), Nitrogen fixing bacteria (NFB), Vermicompost (V) Vermicompost + Nitrogen fixing bacteria (V+NFB)

*** Indicate significance at $P < 0.001$.

TABLE II:Means comparison of some measured characters of sage under different irrigation regimes and fertilizer treatments

	Essential oil content (%)			α-Thujone (%)			1, 8-Cineole (%)			Camphor (%)		
Treatment	September 2012	May 2013	September 2013	September 2012	May 2013	September 2013	September 2012	May 2013	September 2013	September 2012	May 2013	September 2013
Irrigation	***	***	***	***	***	***	***	***	***	***	***	***
I_1	0.49^{c}	1.12^{c}	1.07^{c}	27.93^{c}	23.69^{c}	29.18^{c}	8.66^{c}	7.38^{c}	8.58^{c}	18.72^{c}	16.48^{c}	19.77^{c}
I_2	0.97^{a}	2.42^{a}	2.19^{a}	34.12^{a}	31.77^{a}	35.78^{a}	12.97^{a}	11.38^{a}	13.34^{a}	24.81^{a}	22.14^{a}	26.69^{a}
I_3	0.78^{b}	2.18^{b}	1.95^{b}	29.06^{b}	26.73^{b}	33.23^{b}	10.37^{b}	10.25^{b}	10.79^{b}	23.88^{b}	20.40^{b}	23.96^{b}
Fertilizer	***	***	***	***	***	***	***	***	***	***	***	***
F_0	0.66 ^{d}	1.60 ^{e}	1.42^{e}	29.65^{d}	27.41^{a}	32.68^{b}	10.23^{e}	9.66^{c}	10.85^{a}	22.16^{e}	19.65^{c}	23.42^{d}
Ur	0.73^{c}	1.85^{d}	1.65^{d}	30.28^{c}	27.27^{b}	32.75^{a}	10.31^{d}	9.68^{ab}	10.93^{b}	22.33^{d}	19.67^{b}	23.48^{b}
V	0.77^{b}	2.01^{b}	1.86^{b}	30.67^{b}	27.42^{a}	32.75^{a}	10.89^{b}	9.67^{bc}	10.93^{b}	22.60^{b}	19.68^{ab}	23.49^{b}
NFB	0.74^{c}	1.90^{c}	1.75^{c}	30.25^{c}	27.42^{a}	32.70^{b}	10.74^{c}	9.66^{c}	10.87^{g}	22.56^{c}	19.66^{bc}	23.47^{c}
V+NFB	0.84^{a}	2.15^{a}	2.01^{a}	31.00^{a}	27.45^{a}	32.77^{a}	11.17^{a}	9.70^{a}	10.95^{a}	22.71^{a}	19.69^{a}	23.51^{a}
Irrigation× Fertilizer	***	***	***	***	***	***	***	***	***	***	***	***
I_1×F_0	0.37^{j}	1.01^{m}	0.94 ^{l}	26.34^{i}	23.75^{c}	29.13^{h}	7.48j	7.35f	8.54^{h}	18.63^{l}	16.45^{g}	19.72^{i}
I_1×Ur	0.49^{i}	1.09^{l}	1.06^{ik}	28.45^{gef}	23.33^{d}	29.19^{fg}	8.75i	7.41de	8.60^{g}	18.78^{j}	16.50^{e}	19.79^{g}
I_1×V	0.51^{i}	1.15^{k}	1.13^{hi}	28.20^{gh}	23.76^{c}	29.20^{f}	8.77i	7.39de	8.60^{g}	18.7^{1k}	16.47^{f}	19.78^{gh}
I_1×NFB	0.50^{i}	1.11^{kl}	1.03^{k}	28.19^{h}	23.78^{c}	29.15^{gh}	8.76i	7.37ef	8.56^{h}	18.69^{k}	16.48^{ef}	19.76^{h}
I_1×V+NFB	0.55^{h}	1.24^{j}	1.19^{h}	28.47^{ef}	23.81^{c}	29.21^{f}	9.56^{g}	7.42^{d}	8.61^{g}	18.79^{j}	16.50^{e}	19.80^{g}
I_2×F_0	0.89^{d}	1.97^{h}	1.73^{f}	34.01^{b}	31.75^{a}	35.73^{b}	12.67^{d}	11.37^{b}	13.27^{c}	24.60^{e}	22.12^{b}	26.64^{c}
I_2×Ur	0.93^{c}	2.38^{d}	2.11^{d}	34.07^{b}	31.77^{a}	35.80^{a}	12.74^{c}	11.39^{ab}	13.37^{a}	24.89^{b}	22.13^{b}	26.70^{b}
I_2×V	1.00^{b}	2.57^{b}	2.32^{b}	34.11^{ab}	31.77^{a}	35.81^{a}	13.26^{a}	11.37^{ab}	13.38^{a}	24.83^{c}	22.14^{b}	26.70^{b}
I_2×NFB	0.93^{c}	2.45^{c}	2.22^{c}	34.09^{ab}	31.75^{a}	31.75^{b}	12.89^{b}	11.36^{b}	13.30^{b}	24.73^{d}	22.13^{b}	26.68^{b}
I_2×V+NFB	1.10^{a}	2.71^{a}	2.56^{a}	34.32^{a}	31.79^{a}	35.83^{a}	13.29^{a}	11.41^{a}	13.39^{a}	25.00^{a}	22.16^{a}	26.73^{a}
I_3×F_0	0.70^{g}	1.84^{i}	1.59^{g}	28.61^{e}	26.73^{b}	33.18^{e}	10.54^{f}	10.25^{c}	10.74^{f}	23.24^{i}	20.38^{d}	23.91^{f}
I_3×Ur	0.77^{f}	2.08^{g}	1.79^{f}	28.32^{ghf}	26.72^{b}	33.25^{cd}	9.43^{h}	10.25^{c}	10.82^{de}	23.31^{h}	20.38^{d}	23.96^{e}
I_3×V	0.80^{e}	2.31^{e}	2.13^{d}	29.70^{d}	26.74^{b}	33.25^{cd}	10.65^{e}	10.26^{c}	10.81^{e}	24.25^{g}	20.43^{c}	23.98^{de}
I_3×NFB	0.78^{f}	2.15^{f}	1.98^{e}	28.47^{ef}	26.73^{b}	33.21^{de}	10.57^{f}	10.24^{c}	10.76^{f}	24.27^{g}	20.38^{d}	23.97^{de}
I_3×V+NFB	0.87^{d}	2.50^{c}	2.26^{bc}	30.20^{c}	26.76^{b}	33.27^{c}	10.66^{e}	10.27^{c}	10.84^{d}	24.23^{f}	20.42^{c}	23.99^{d}

Mean values of the three recollections and each recollection date. Different letters express significant differences at P <0.05 within each column.

Irrigation:Irrigation after depletion of 40% available water (I_1), Irrigation after depletion of 60% available water (I_2), Irrigation after depletion of 80% available water (I_3)

Fertilizer regimes:Control (no fertilizer) (F0), Urea(Ur), Nitrogen fixing bacteria (NFB), Vermicompost (V) Vermicompost + Nitrogen fixing bacteria (V+NFB)

*** Indicate significance at $P < 0.001$.

TABLE III:Means comparison of some measured characters of sage under different irrigation regimes and fertilizer treatments in different harvest times

Treatment	Plant height(cm)	Canopy diameter (cm)	Leaf area Index	Dry matter yield ($t.ha^{-1}$)	Essential oil content (%)	α-Thujone (%)	1, 8-Cineole (%)	Camphor (%)
Irrigation	***	***	***	***	***	***	***	***
I_1	29.93^{e}	34.09^{a}	1.61^{a}	2.702^{a}	0.89^{c}	26.93^{c}	8.21^{c}	18.32^{c}
I_2	23.77^{b}	27.90^{b}	1.12^{b}	1.882^{b}	1.86^{a}	33.89^{a}	12.56^{a}	24.56^{a}
I_3	17.20^{c}	16.53^{c}	0.59^{c}	0.987^{c}	1.64^{b}	29.68^{b}	10.47^{b}	22.75^{b}
Fertilizer	***	***	***	***	***	***	***	***
F_0	18.62^{e}	18.07^{d}	0.84^{e}	1.388^{e}	1.23^{e}	29.91^{d}	10.25^{e}	21.74^{b}
Ur	23.41^{c}	26.33^{c}	1.10^{c}	1.865^{c}	1.41^{d}	30.10^{c}	10.31^{d}	21.83^{b}
V	25.41^{b}	28.67^{b}	1.20^{b}	1.996^{b}	1.55^{b}	30.28^{b}	10.50^{b}	21.92^{ab}
NFB	22.59^{d}	26.59^{c}	1.04^{d}	1.730^{d}	1.46^{c}	30.12^{c}	10.42^{c}	21.90^{ab}
V+NFB	28.15^{a}	31.21^{a}	1.38^{a}	2.302^{a}	1.66^{a}	30.41^{a}	10.61^{a}	21.97^{a}
Irrigation× Fertilizer	***	***	***	***	***	***	***	***
$I_1 \times F_0$	24.81^{e}	24.61^{g}	1.21^{e}	2.014^{fg}	0.78^{l}	26.41^{h}	7.79k	18.27^{m}
$I_1 \times Ur$	29.69^{c}	35.11^{c}	1.69^{b}	2.956^{b}	0.88^{k}	26.99^{g}	8.25i	18.36^{k}
$I_1 \times V$	31.97^{b}	36.78^{b}	1.68^{b}	2.796^{c}	0.93^{j}	27.05^{fg}	8.25i	18.32^{l}
$I_1 \times NFB$	28.66^{d}	34.86^{c}	1.53^{c}	2.540^{d}	0.88^{k}	27.04^{fg}	8.23j	18.31^{l}
$I_1 \times V+NFB$	34.51^{a}	39.10^{a}	1.93^{a}	3.20^{a}	0.99^{i}	27.16^{f}	8.53h	18.36^{k}
$I_2 \times F_0$	18.58^{i}	18.38^{i}	0.89^{g}	1.463^{j}	1.53^{g}	33.83^{b}	12.44d	24.45^{e}
$I_2 \times Ur$	23.76^{f}	28.36^{f}	1.11^{f}	1.811^{h}	1.81^{d}	33.88^{b}	12.50c	24.57^{b}
$I_2 \times V$	25.42^{e}	30.64^{e}	1.21^{e}	2.023^{f}	1.96^{b}	33.90^{ab}	12.67b	24.56^{c}
$I_2 \times NFB$	22.31^{g}	28.80^{f}	1.07^{f}	1.780^{hi}	1.87^{c}	33.86^{ab}	12.52c	24.51^{d}
$I_2 \times V+NFB$	28.80^{cd}	33.34^{d}	1.40^{d}	2.334^{e}	2.12^{a}	33.98^{a}	12.70a	24.63^{a}
$I_3 \times F_0$	12.47^{k}	11.23^{k}	0.41^{k}	0.688^{n}	1.38^{h}	29.51^{d}	10.51f	22.51^{j}
$I_3 \times Ur$	16.78^{j}	15.52^{j}	0.49^{j}	0.827^{m}	1.54^{g}	29.43^{d}	10.17g	22.55^{i}
$I_3 \times V$	18.83^{i}	18.58^{i}	0.71^{i}	1.168^{l}	1.75^{e}	29.90^{c}	10.57e	22.89^{g}
$I_3 \times NFB$	16.81^{j}	16.11^{j}	0.53^{j}	0.883^{m}	1.64^{f}	29.47^{d}	10.52f	22.87^{h}
$I_3 \times V+NFB$	21.13^{h}	21.20^{h}	0.82^{h}	1.369^{k}	1.88^{c}	29.08^{e}	10.59e	22.91^{f}
Harvest time	***	***	***	***	***	***	***	***
September 2012	22.85^{b}	25.61^{b}	0.86^{b}	1.424^{b}	0.75^{c}	30.37^{b}	10.67^{b}	22.47^{b}
May 2013	19.47^{c}	23.34^{c}	0.71^{c}	1.183^{c}	1.90^{a}	27.40^{c}	9.67^{c}	19.67^{c}
September 2013	28.84^{a}	29.58^{a}	1.77^{a}	2.964^{a}	1.74^{b}	32.73^{a}	10.91^{a}	23.47^{a}

Mean values of the three recollections and each recollection date. Different letters express significant differences at $P < 0.05$ within each column.
Irrigation:Irrigation after depletion of 40% available water (I_1), Irrigation after depletion of 60% available water (I_2), Irrigation after depletion of 80% available water (I_3)
Fertilizer regimes:Control (no fertilizer) (F0), Urea(Ur), Nitrogen fixing bacteria (NFB), Vermicompost (V) Vermicompost + Nitrogen fixing bacteria (V+NFB)
*** Indicate significance at $P < 0.001$.

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