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How Temperature Stress, Fertilizer and Cultivar Affect the Essence of Savory Plant (*Satureja hortensis* L.)

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ABSTRACT

To investigate the mutual effects of temperature stress, fertilizer and cultivar on the essence of savory, a study was carried out in Isfahan (2018) as a split plot experiment in the form of randomized complete blocks design with three replications. Main plots were fertilizer in four levels (gibberellic acid, micro fertilizer, amino acid and normal water as control) whereas subplots were three cultivars (Mutica, Bakhtiari and Khouzestani). The planting dates of April 20th, June 20th and May 20th were implemented as three temperature conditions separately, and finally combined analysis was carried out on all three dates. Measured traits were essential oil efficiency, alpha-pinene, alpha terpinene, and gamma-terpinene. According to results, treatments affected plant traits significantly and the combination of Mutica cultivar, micro fertilizer and low temperature showed produced the highest essential oil and its compounds.

Keywords: Savory, Temperature stress, Cultivar, Fertilizer, Essence.

INTRODUCTION

Medicinal plants, especially exclusive and indigenous species, are among the most valuable sources of Iran's natural resources, which can play an important role in society's health, job creation, and non-oil exports if scientifically recognized and properly exploited. Savory is an aromatic plant from the mint family (Lamiaceae) with many straight stems and leaves which have secretory glands containing essential oils. The flowering branches of this plant are used in health, food and pharmaceutical industries (Akbari *et al*, 2013). Savory plant is widely used as antioxidant and antimicrobial agent in food and pharmaceutical industries (Hadian *et al*, 2010). Finding the optimal planting temperature is one of the important factors to achieve maximum crop yield. The temperature of planting time affects the establishment and growth as well as the quantity and quality of the crop (Padid, 2021). There are also several reports on the effects of this factor on the amount of active ingredients of medicinal plants (Mohammadpour 2018). In addition to weather condition, nutritional elements are also very important for medicinal plants, because these elements affect the quality and quantity of essential oils via affecting the growth of plants (Moradi, 2010).

Plants need a small amount of micronutrient elements, but lack of these elements can sometimes act as a limiter for the absorption of other nutrients and growth. Foliar application of micronutrients can improve the plant growth (Mohdi Dehnavi, 2009). Regarding the adverse effect of temperature on quantitative and qualitative yield of savory, it is necessary to apply combined treatments of fertilizers and growth hormones to eliminate these effects. Current research was carried out to show the best combination of temperature, fertilizer and cultivars for increasing essence oil.

MATERIALS AND METHODS

The experiment was carried out to investigate the mutual effects of temperature stress, fertilizer and cultivar on the essence of savory, in a farm located 12 km north East of Isfahan (32˚26′ north latitude and 51˚42′ east longitude at an altitude of 1550 meters). The region has dry climate with hot summers based on the Koppen classification. The long-term average annual rainfall and temperature are 120 mm and 16°C, respectively.

The study was carried out as a split plot experiment in the form of randomized complete blocks design with three replications. Main plots were fertilizer in four levels (gibberellic acid, micro fertilizer, amino acid and normal water as control) whereas subplots were three cultivars (Mutica, Bakhtiari and Khouzestani). The planting dates of April 20th, June 20th and May 20th were implemented as three temperature conditions separately, and finally combined analysis was carried out on all three dates.

Fertilizer characteristics:

1. Pure gibberellic acid (Merck, Germany, CAS 77-06-5), sprayed at concentration of 2/1000.

2.Microbiomin 464 SP fertilizer (JHBiotech, USA) containing micronutrient elements (6% by weight of organic nitrogen, 10 % by weight of soluble sulfur (S), 4.5 % by weight of iron (Fe), 6 % by weight of manganese (Mn), 4.5% by weight of zinc (Zn), 2% of copper (Cu), 0.8

% of solution (B), 0.1% of soluble molybdenum (Mo), 0.0005% of cobalt (Co)), with acid composition Amino glycine, inorganic sulfate, iron chelate, manganese chelate, zinc chelate, copper chelate, bar complex, molybdenum complex, sprayed at concentration of 2/1000.

3. Amino acid fertilizer ((Futureco, Spain) soluble in water, with a composition of 45% by weight of free amino acid (free amino acid includes: 4% aspartic acid, 9% glutamic acid, 1% histidine, 5% serine, 2% glycine, 3% threonine) , 5% arginine, 3% alanine, 1 % tyrosine, 2% valine, 1% methionine, 1% phenylalanine, 5% isoleucine, 1% leucine, 2% lysine, and 4% proline), 18 % by weight of total nitrogen, 8% by weight of organic nitrogen, 10% by weight of ammonia nitrogen, with 4.5 pH with 1% dilution, sprayed at 2 mM concentration.

Seeds with vigors above 80% were obtained from Isfahan Agricultural Researches Center. In order to produce seedlings, four seeds were sown in seedlings trays filled by peat moss (DOMOFLOR MIX brand). The main land (65*30 meters) was prepared using plow and disc. Fertilization was done based on the soil test results. Cultivation was done with a distance of 40 cm between plants and 60 cm between rows. Fertilizers were sprayed every 15 days in total four times (September, October, November and December 2018).

Irrigation was carried out in flood and depending on the needs of the plant approximately once every seven days.

Narrow-leaf weeds were controlled by Super Gallant herbicide (1.5/1000 concentration) and broad-leaf weeds were controlled by hand weeding.

Measured traits were essential oil efficiency, alpha-pinene, alpha terpinene, and gamma-terpinene.

Obtained data were analyzed using SAS 9.1.3 and MSTATC 1.2 programs and means were compared using Duncan's multi-range test at the 5% probability level.

RESULTS AND DISCUSSION

The interaction effect of treatments on the yield of essential oil was significant (Table1). The highest rate belonged to control and Mutica cultivar at low temperature, while Control and Bakhtiari cultivar at high temperature had the lowest efficiency (Table2). Low temperature was found to be the most suitable condition for this trait due to the favorable weather conditions for increasing essential oil biosynthesis. Experiments conducted under controlled conditions have shown that changes in environmental factors such as temperature, radiation and day length can affect the amount of essential oil and its quality (Mohammedpour, 2018). Padid *et al*. (2021) stated that high temperature during the formation of flowering branches had a bad effect on the process of essential oil production and reduced the percentage of essential oil. Dzida *et al*. (2015) reported similar results regarding the effect of temperature stress on the amount of essential oil in their study on summer savory. Chiane *et al*. (2023) reported that the amount of essential oil was increased by amino acid. Makizadeh *et al*. (2013) reported the lack of effect of biofertilizers on essential oil. Noushkam *et al*. (2014) reported no effect of fertilizer on the amount of essential oil. The interaction effect of treatments on alpha-pinene was significant. The highest amount of this trait belonged to Control and Mutica cultivar at low temperature. Apparently, the cold was more suitable for the production of this substance, but the fertilizer has not affected its synthesis. The studies of El-Gohary *et al*. (2015) showed that the amount of alpha-pinene was affected by different concentrations of amino acids. Noushkam *et al*. (2014) reported no effect of fertilizer on the amount of alpha-pinene. Mean comparison results of treatments interaction effects on of alpha terpinene indicated a significant difference between the means. The highest amount of alpha terpinene belonged to Micro fertilizer and Mutica cultivar at low temperature whereas Micro and Bakhtiari cultivar at high temperature had the lowest amount. Mohammadpour (2018) reported that the effect of temperature on the amount of alphaterpinene was not significant. The studies of El-Gohary *et al*. (2015) showed that the amount of alphaterpinene was affected by different concentrations of amino acids. Chianeh *et al*. (2023) reported that the amount of alpha terpinene increased by amino acid. Makizadeh *et al*. (2012) reported the increase of alphaterpinene by using biofertilizers. NoushKam *et al*. (2014) reported no effect of fertilizer on the amount of alphatripenen.

Table 1. Variance analysis results of some measured traits

Source of Variation	Degrees of freedom	Means of squares			
		Essential oil	Alpha Pinene	Alpha Terpinene	Gamma Terpinene
Heat Stress	2	0.004	3×10^{-4ns}	002^{ns}	0.014^{**}
Rep (Heat Stress)	6	0.001	3×10^{-4}	4×10^{-4}	7×10^{-4}
Fertilizer	3	8×10^{-4ns}	0.001^{*}	0.001^{ns}	0.003^{ns}
Heat Stress× Fertilizer	6	2×10^{-4ns}	0.001^{*}	0.001^{ns}	0.003^{ns}
Error a	18	8×10^{-4ns}	3×10^{-4}	5×10^{-4}	0.001
Cultivar	2	0.054^{*}	0.097^{**}	0.038^{**}	0.616^{**}
Heat Stress× Cultivar	4	0.002^{*}	0.001^{*}	7×10^{-4}	0.003^{ns}
Fertilizer ×Cultivar	6	0.001^{*}	3×10^{-4ns}	6×10^{-4}	0.001^{ns}
Heat Stress× Fertilizer ×Cultivar	12	0.001^{*}	0.001^{*}	0.002^{ns}	0.001^{ns}
Error b	48	5×10^{-4ns}	5×10^{-4ns}	9×10^{-4}	0.002
CV (%)		2.97	3.17	4.05	3.19

^{ns}, * and ** are non-significant. significant at 5% and 1% probability levels, respectively

Table 2. Mean comparison results of treatments interaction on measured traits

Temperature Stress	Fertilizer	Cultivar	Essential oil (%)	Alpha Pinene (%)	Alpha Terpinene (%)	Gamma Terpinene (%)
Cold	Micro	Mutica	2.842abc	1.279abc	2.336a	21.572a
		Bakhtiari	1.662g-j	1.162a-e	0.788h-l	9.108fgh
		Khouzestani	3.123ab	0.016f	1.48b-g	2.395jk
	Amino acid	Mutica	3.102ab	1.109a-e	1.508b-g	17.084a-d
		Bakhtiari	1.988e-j	0.959b-e	0.859g-l	9.884fgh
		Khouzestani	2.747a-d	0.005f	1.655a-f	2.812ijk
	Gibberellic Acid	Mutica	1.928f-j	1.202a-d	2.069abc	20.262ab
		Bakhtiari	1.918f-j	1.132a-e	0.862g-l	10.191fgh
		Khouzestani	2.848abc	0.01f	1.378d-i	2.236jk
	Control	Mutica	3.308a	1.336abc	2.134ab	21.225a
		Bakhtiari	1.623g-j	1.564a	0.999f-l	10.724e-h
		Khouzestani	2.446b-f	0.009f	1.512b-g	2.488jk

There is no significant difference between numbers with at least one common letter in each column, according to Duncan's test at 5% probability level

Table 2 (continued)- Mean comparison results of treatments interaction on measured traits

Temperature Stress	Fertilizer	Cultivar	Essential oil (%)	Alpha Pinene (%)	Alpha Terpinene (%)	Gamma Terpinene (%)
Normal	Micro	Mutica	2.816a-d	1.154a-e	1.654b-f	15.711b-e
		Bakhtiari	1.406ijk	1.001b-e	0.588kl	6.823h-k
		Khouzestani	2.292c-g	0f	1.413c-h	2.162jk
	Aminoacid	Mutica	2.683a-e	1.404ab	1.931a-d	16.868a-d
		Bakhtiari	1.418ijk	1.167a-e	0.667jkl	7.857ghi
		Khouzestani	2.84abc	0f	1.156e-k	1.66jk
	Gibberellic Acid	Mutica	2.503b-f	1.218a-d	1.662b-f	16.61a-d
		Bakhtiari	1.313jk	1.155a-e	0.742i-l	9.744fgh
		Khouzestani	2.407b-f	0.017f	1.373d-i	2.065jk
	Control	Mutica	2.718a-e	0.779de	1.06f-l	10.431e-h
		Bakhtiari	1.526h-k	0.99b-e	0.578kl	7.783ghi
		Khouzestani	2.553b-f	0.013f	1.292d-j	2.046jk

There is no significant difference between numbers with at least one common letter in each column, according to Duncan's test at 5% probability level

Table 2 (continued)- Mean comparison results of treatments interaction on measured traits

Temperature Stress	Fertilizer	Cultivar	Essential oil (%)	Alpha Pinene (%)	Alpha Terpinene (%)	Gamma Terpinene (%)
Warm	Micro	Mutica	2.384b-f	1.115a-e	1.476b-g	14.448c-f
		Bakhtiari	1.533h-k	0.703e	0.481l	7.116hij
		Khouzestani	2.237c-h	0f	1.025f-l	1.353k
	Aminoacid	Mutica	2.388b-f	0.869cde	1.212e-k	12.893d-g
		Bakhtiari	1.391ijk	1.038b-e	0.66jkl	8.975fgh
		Khouzestani	2.464b-f	0f	1.069f-l	1.839jk
	Gibberellic Acid	Mutica	2.217c-h	1.004b-e	1.554b-f	17.83a-d
		Bakhtiari	1.434ijk	1.265a-d	0.778h-l	11.038e-h
		Khouzestani	2.09d-i	0f	1.328d-j	2.325jk
	Control	Mutica	2.572b-f	1.304abc	1.809a-e	18.586abc
		Bakhtiari	0.872k	1.065b-e	0.701jkl	11.045e-h
		Khouzestani	2.392b-f	0f	1.089f-l	2.069jk

There is no significant difference between numbers with at least one common letter in each column, according to Duncan's test at 5% probability level

The highest amount of gamma-terpinene belonged to Micro fertilizer and Mutica cultivar at low temperature, while the same fertilizer and Khuzestani cultivar produced the lowest amount of gamma-terpinene at high temperature. Apparently, the cold was more suitable for the production of this substance, but micro fertilizer has shown contradictory behavior. Mohammadpour (2018) reported that the effect of temperature on the amount of gamma-terpinene was not significant. The results of El-Gohary *et al*. (2015) showed that the amount of gamma-terpinene was affected by different concentrations of amino acids. RezaeiChianeh *et al*. (2023) reported that the amount of gamma-terpinene was increased by amino acid. Makizadeh *et al*. (2012) reported the increase in amino acid content by using biofertilizers. Noushkam *et al*. (2014) reported no effect of fertilizer on the amount of gamma-terpinene.

CONCLUSION

In general, the combination of Mutica cultivar, micro fertilizer and low temperature seems reasonable for better production of essential oil and its compounds in the same conditions as this study, although, micro fertilizer has shown contradictory behavior in some cases.

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