THE EFFECT OF PLANTING DENSITY AND NUMBER OF HAND WEEDING TIMES ON WEEDS CONTROL AND RELATIONSHIP BETWEEN MORPHOLOGICAL CHARACTERISTICS WITH CONTENT AND YIELD OF ESSENTIAL OIL OF *SATUREJA HORTENSIS* L.

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Abstract

The aim of this study was to evaluate the effect of planting density and number of weeding times on weeds control and the relationship between morphological characteristics with the content and yield of *Satureja hortensis* essential oil (EO). *S. hortensis* planting density at four levels (50, 75, 100 and 125 plants per m^2) was the first factor and hand weeding in 4 times (2, 4, 6 and 8 weeks after planting- WAP), 3 times (2, 4 and 8 WAP), 2 times (4 and 8 WAP) and weedy check as second factor. Dominant weeds were included redroot pigweed (*Amaranthus retroflex* L.), black nightshade (*Solanum nigrum* L.) and lambsquarters (*Chenopodium album* L.) in this field. Increasing *Satureja hortensis* density under densities of 50, 75 and 100 plants per m^2 with weeding especially four times, significantly increased the dry weight and content of EO and yield of this plant. But the delay in the weeding times, such as twice weeding, significantly reduced this trait. This indicates that weeding in *S. hortensis* should be done at the beginning of the growing season due to its slow growth. Also, increasing the density of *S. hortensis* from 100 to 125 plants per m^2 intensifies competition, so that its EO yield content decreased despite increasing the number of weeding times compared to the weedy check. Overall, due to the favorable effect of weed control under three or four weeding times on dry weight and content and EO yield of *S. hortensis*, weeding at the mentioned times is recommended in Gonbad Kavous area and areas with similar conditions to Gonbad Kavous.

Keywords: *Satureja hortensis,* planting density, hand weeding times*,* morphological characteristics, weeds control, content and yield of essential oil

INTRODUCTION

Weeds are one of the most important problems in organic farming. The costs of weed control in organic farming are so high that synthetic herbicides uses are prohibited as compared with permitted herbicides in conventional agriculture (Abouziena *et al.*, 2016). Weeds have a detrimental effect on medicinal plant production. The presence of weeds in the field of medicinal plant reduces the yield by 25 to 95% depending on the growing condition, content and quality of active metabolites in plants (Carrubba and Militello, 2013).

There are some safe methods that can be used in organic farming of medicinal plants to control weeds, such as soil solarization, mulching, natural herbicides, warm water, and agronomic practices that have been successfully adopted in many countries as safe methods (Abouziena *et al.*, 2016).

The critical period of weed interference (CPWI) can be defined as the stage in the growth cycle of any crop during which weeds must be controlled to prevent unrecoverable yield losses. CPWI knowledge also contributes to minimize yield losses that this crop can exhibit due to weed infestation (Safdar *et al.*, 2016); determining CPWI is an indispensable tool for suggestion effective weed management strategies in any crop production system (Tursun *et al.*, 2016).

The intensity and duration of weed interference are factors that determine the extent of yield losses (Swanton *et al.*, 2015). Several studies (Kumar *et al.*, 2015; Tursun *et al.*, 2015; Yadav *et al.*, 2018) reported that weed interference outside the critical period of weed control had a negligible effect on crop yield. Hence, determination of this critical period is essential for better weed management. In fact, it is the first step in an integrated weed management (IWM) strategies. Therefore, it helps to decide on weeds control at the right time, efficient use of herbicide and higher productivity (Kumar *et al.*, 2015; Yadav *et al.*, 2018). However, weed interference not only affects crop yield but also this causes change in content of secondary metabolites in plants, which accomplish important functions within the plants (Olivoto *et al.*, 2017).

The first and most important step in weed management is to identify the species and understanding their distribution, so that they can be managed with an open vision and careful planning (Pourheidar Ghafarbi and Hassennszhad, 2013). It has been reported that any increase in crop density up to a certain range will result in a competition for crop yield (Giti *et al.*, 2013). Several studies have shown that increasing weed suppression by decreasing crop row distance or increasing crop density can also be higher in a more uniform pattern even without weed pressure. Narrower rows to suppress wheat weeds (Drews *et al.*, 2009) and rice has been reported (Chauhan and Johnson, 2011).

Satureja is a genus of aromatic plants belonging to Lamiaceae family. *Satureja hortensis* L. represents an annual herbaceous crop species, strongly branched with linear leaves. The flowers are also bisexual in purple or violet white with a red peeled on the inside grouped at the upper nodes of the branches (Bojor, 2003). The leaf, on the surface, has small spots called tubers with essential oils.

Identifying the dominant weeds in the cultivation of medicinal plants will be useful for growers. Therefore, due to the side effect of synthetic herbicides on the effective materials, optimal agronomic practices of plants can be a good option for controlling weeds. Far too little attention has so far been paid to study the effect of planting density and hand weeding times to control weed populations in medicinal plant of *S. hortensis*; however, such studies are essential to determine optimal planting density and weeds control in this plant. Therefore, the main objective of this study was to determine the effect of plant density and different number of hand weeding times on weeds control and the relationship between morphological characteristics with content and EO yield of *S. hortensis*.

MATERIALS AND METHODS

The field experiment was carried out in Shahabi Agriculture and Animal Husbandry complex located at Gonbad Kavous road to Dashliboron in Golestan province, Iran with a longitude 55° 9' 30'' and a latitude of 37° 23' 36'' east, 50 m above sea level and with an average long-term annual rainfall of 300 mm in growing season 2018–2019.

The soil physical and chemical properties of the studied place was measured before doing the experiment. In so doing, six points of soil sample at a depth of 0 to 30 cm were randomly collected by auger each point of which contained approximately 3 kg of soil. The soil of six points was then mixed with each other well. Finally, a mixture of 4 kg of soil was prepared and transferred to Gonbad Kavous Soil Laboratory for physical and chemical analysis. Chemical properties of the topsoil layer of the experimental place were: pH (7.38), electrical conductivity (7.8 mm cm^{-1}), organic carbon (0.35%), total nitrogen (0.04%), soluble phosphorus (6 ppm) and soluble potassium (474 ppm). The soil texture containing 15, 24 and 64% clay, sand and silt was loamy silt respectively. The size of the plots was 4 × 5 m and the distance between the plots was one meter.

This experiment was performed as a factorial experiment base on the randomized complete block design in three replications. Planting density of *S. hortensis* at four levels (50, 75, 100 and 125 plants per m^2) was the first factor and hand weeding in 4 times (2, 4, 6 and 8 weeks after planting- WAP), 3 times of weeding (2, 4 and 8 WAP), 2 times of weeding (4 and 8 WAP) and weedy check in during the growing season as second factor.

First, the land was selected and then non-plant remains such as rocks, metal and plastic parts were removed. Plowing was firstly done to prepare the seedbed in relatively deep manner with the help of a reversible plow in March and April 2019. Additional operations such as crushing the mulch, softening the soil and leveling the ground were then performed using a disc and a trowel.

100 kg/ha Urea (46% N) and 100 kg/ha Triple Super Phosphate (46% P$_2$O$_5$) were used as fertilizer source for nitrogen and phosphorus respectively. The entire rate of basic fertilizer of phosphorus and half of nitrogen fertilizers were applied when planting was simultaneously done with secondary

plowing at a depth of 10 cm. The remaining half of nitrogen was applied at the time of flowering as a top dressing. Potash fertilizer was not used due to the richness of the soil.

Certified seeds of *S. hortensis* were obtained from Pakan Bazr Isfahan Company, located Iran. *S. hortensis* seeds were planted by hand on April 4, 2019. The distance between planting rows and planting depth of *S. hortensis* seeds were 30 and 5 cm, respectively. After seedlings growth, additional seedlings was removed to create the desired density according to the design plan.

Measurement of Density and Dry Weight of Weeds

To evaluate weed density, three 0.5 × 0.5 m quadrats were encompassed, and the weeds were identified and counted separately based on genus and specie. Then, the average plant density of each weed and their total in terms of number per m² was reported. To measure the dry weight of each weed, after separating the species of each weed in each quadrate, they were removed from the soil surface. Then, they were half withered in indirect sunlight and finally dried with the help of oven at 60 °C until a constant weight is reached (10% on the base weight (Caceres, 2000)) (Martinov *et al.,* 2007). Then it was weighed with a digital lab scale (BEST Company, Model of MT-200) with an accuracy of 0.01 g and the average of three quadrats per plot was reported.

Measurement of *S. Hortensis* Characteristics

Plant height at the beginning of *S. hortensis* flowering stage was measured in each experimental plot. For this purpose, 10 plants from each experimental plot considering the marginal effect were randomly selected and their average was reported. To measure the fresh and dry weight of *S. hortensis*, they were removed from the soil surface. Then they were dried in an oven at 40 °C until a constant weight is reached (To prevent the breakdown of allochemicals). It was then weighed with a digital lab scale (BEST Company, Model of MT-200) with an accuracy of 0.01 g and the average of three quadrats per plot was reported.

To measure leaf area, three 0.5 × 0.5 m quadrats were encompassed in each plot randomly. At the densities of 50, 75, 100 and 125 plants per m², all *S. hortensis* leaves were separated and the leaf area was then reported in the plant per mm² by Leaf Area Meter (Delta-t model).

In order to evaluate content and yield of EO, *S. hortensis* aerial parts were harvested and dried in the oven at 40 °C (to prevent the volatile allelochemicals of the EO). In so doing, A 20 g sample of *S. hortensis* aerial parts was first grounded into small pieces by a mill and then passed through sieves with 8 mesh (number of squares or sieve particles per inch). In the next step, 150 ml of distilled water was added to it and placed in a 1000 ml volumetric

balloon on the heater for 2 hours. Due to heat and increasing water vapor pressure, the essential oil droplets became liquid in contact with the cold wall of the condenser (condensate action). Subsequently, most of the EO was placed at the end of the calibrated tube due to its lower density than that of water. The resulting EO was collected by syringe and then placed in micro vials. Sodium sulfate was used in order to extract water from the EO. The vials containing the EO were weighed by a digital scale with a 0.0001 accuracy and the EO content was calculated according to the following equation:

Content of essential oil (%) =

$$= \frac{\text{Essence weight}}{\text{Sample dry weight}} \times 100. \qquad (1)$$

Then the yield of *S. hortensis* EO (kg.ha⁻¹) was obtained by multiplying the content of EO with the dry matter yield of experimental treatments.

The data normalization was tested by Minitab software (Version 14) to make the data normal. The data were then analyzed using SAS 9.3 version (SAS Institute, 2011) and mean data were compared using Least Significant Difference (LSD) test at 5% probability level.

RESULTS

Effect of Plant Density and Number of Weeding Times on Morphological Traits, Content and EO Yield of *S. Hortensis*

Planting density and number of weeding times significantly affected the plant height, fresh and dry weight, leaf area, content and EO yield of *S. hortensis* at $p < 0.01$. Also, the interaction effects of planting density and number of weeding times on all measured traits except leaf area were significant at $p < 0.01$ (Tab. I).

Plant Height

The amount of changes in the plant height of *S. hortensis* was between 45.33 and 36.33 cm. The highest plant height was obtained with the treatment of 125 plants per m² in weedy check. However, no statistically significant difference was observed with the treatment of 100 plants per m² in weedy check. Therefore, they were placed in the same group. But the lowest plant height was related to 75 plants per m² with weeding three times, but the difference with some treatments was not significant (Tab. II).

Fresh Weight

Fresh weight of *S. hortensis* under different treatments of weeding times and densities except 125 plants per m² was more than weedy check. The highest fresh weight was related to 75 plants per m² with weeding 4 times in the amount of 8140 mg per plant. The difference with 50 plants in the same number of hand weeding times was not significant.

I: *Analysis of variance (ANOVA) of the effect of planting density and number of weeding times on morphological traits, content and EO yield of S. hortensis*

S.O.V	Df	Plant height	Plant fresh weight	Plant dry weight	Leaf area	Content of essential oil	Essential oil yield
r	2	25.00 ^{ns}	244508.33^{ns}	10399.32 ^{ns}	370208.30^{ns}	0.004^{ns}	3.45^{ns}
a	3	1191.97**	7790127.78**	748520.06**	44240763.90**	1.25**	171.95**
b	3	4024.31**	10593294.44**	469734.95**	1269097.20**	0.26**	297.06**
a × b	9	2200.23**	3595496.30**	158124.49**	24282.40 ^{ns}	0.39**	67.59**
e	30	169.44	188743.89	8010.62	146208.3	0.008	5.56
CV (%)		3.29	8.25	8.58	5.42	3.49	8.83

**: indicate significant difference at 1% probability level and ns: non-significant difference, r: replication, a: plant density, b: number of weeding times, e: error, CV: Coefficient Variance

II: *Mean comparison of interaction effect of planting density and number of weeding times on morphological traits and content of EO yield of S. hortensis*

Density (plants per m²)	Number of hand weeding	Plant height (mm²)	Fresh weight (mg/plant)	Dry weight (mg/plant)	Content of essential oil (%)	Essential oil yield (kg/ha)
50	Weedy check	370.00 ± 10.00 ^{fg}	3663.30 ± 320.05^{e}	732.67 ± 64.01^{f}	2.03 ± 0.03^{g}	7.44 ± 0.26^{g}
	2 times	376.70 ± 5.80^{fg}	5860.00 ± 913.40^{d}	1183.67 ± 182.24^{cd}	2.11 ± 0.07^{g}	12.49 ± 0.34^{f}
	3 times	376.70 ± 5.80^{fg}	6116.70 ± 281.13^{b}	1185.33 ± 15.01^{cd}	2.09 ± 0.07^{g}	12.39 ± 0.67^{f}
	4 times	420.00 ± 17.30 ^{bcd}	8060.00 ± 850.70^{a}	1645.33 ± 219.15^{a}	2.77 ± 0.07^{cde}	22.79 ± 0.93^{e}
75	Weedy check	413.30 ± 15.30^{cd}	3676.70 ± 440.15^{e}	823.73 ± 89.71^{ef}	2.02 ± 0.08^{g}	12.48 ± 0.34^{f}
	2 times	373.30 ± 5.80^{fg}	6093.30 ± 107.86^{b}	1347.80 ± 30.21^{b}	2.17 ± 0.08^{g}	21.94 ± 0.58^{e}
	3 times	363.30 ± 11.50^{g}	5953.30 ± 94.52^{b}	1304.53 ± 13.77^{bc}	2.73 ± 0.08^{e}	26.71 ± 0.31^{d}
	4 times	423.30 ± 5.80^{bc}	8140.00 ± 221.13^{a}	1786.13 ± 31.57^{a}	2.98 ± 0.12^{b}	39.92 ± 0.56^{a}
100	Weedy check	440.00 ± 10.00^{ab}	4223.30 ± 136.50^{cde}	766.53 ± 25.14^{ef}	2.90 ± 0.13^{bc}	22.23 ± 0.27^{e}
	2 times	400.00 ± 20.00^{dc}	4843.30 ± 219.62^{c}	895.87 ± 31.29^{c}	2.88 ± 0.10b^{cde}	25.80 ± 0.77^{d}
	3 times	373.30 ± 11.50^{fg}	4453.30 ± 560.03^{cd}	819.88 ± 95.58^{ef}	2.90 ± 0.10^{bcd}	21.32 ± 0.25^{e}
	4 times	370.00 ± 20.00^{fg}	5743.30 ± 140.48^{b}	1068.35 ± 28.42^{d}	2.89 ± 0.02^{bcd}	30.88 ± 0.64^{c}
125	Weedy check	453.30 ± 15.30^{a}	4230.00 ± 95.39^{cde}	757.67 ± 10.72^{ef}	3.19 ± 0.14^{a}	30.21 ± 0.35^{c}
	2 times	413.30 ± 15.30^{cd}	4020.00 ± 117.89^{de}	866.60 ± 140.02^{ef}	3.23 ± 0.06^{a}	34.99 ± 0.99^{b}
	3 times	376.70 ± 15.30^{fg}	4376.70 ± 261.02^{cde}	780.40 ± 52.14^{ef}	2.75 ± 0.09^{de}	26.83 ± 0.37^{d}
	4 times	386.70 ± 11.50^{ef}	4813.30 ± 762.65^{c}	723.87 ± 29.34^{f}	2.44 ± 0.11^{f}	22.08 ± 0.50^{e}
LSD5%		21.10	729.19	150.24	0.15	3.88

Different letters in each column represent significance at 5% probability level based on least significant difference (LSD) test

Overall, the rate of increase in fresh weight of plants in densities of more than 75 plants per m² due the increasing number of weeding was more than weedy check (Tab. II).

Dry Weight

The dry weight of *S. hortensis* at 50, 75 and 100 plant densities per m² had a significant increasing trend with increasing number of weeding times compared to weedy check. The highest plant dry weight was related to 75 plant density with weeding four times (1786 mg/plant) which did not show a statistically significant difference with 50 plant density in the same number of hand weeding times, Therefore, they were placed in the same group statistically. The number of weeding times also did not show a significant effect on dry weight per plant at a density of 125 plants per m² compared to the weedy check and they had the least amount (Tab. II).

Dry weight was positively correlated with fresh weight (r = +0.974**) and EO yield (r = +0.831**). However, there were negative correlation with the leaf area (r = -0.438**) and EO content EO (r = -0.523**) (Tab. III).

III: *Correlation of morphological traits, content and EO yield of S. hortensis*

| Characteristics	Fresh weight	Dry weight	Leaf area	Content of essential oil	Essential oil yield
Fresh weight	1				
Dry weight	0.974**	1			
Leaf area	-0.356*	-0.438**	1		
Content of essential oil	-0.478**	-0.523**	0.613**	1	
Essential oil yield	0.819**	0.831**	-0.204^{ns}	0.011^{ns}	1

**: indicate significant difference at 1% probability level and ns: non-significant difference

Leaf Area

Leaf area of S. *hortensis* increased with increasing plant density from 50 to 75 plants per m². The leaf area at a density of 75 plants per m² was equal to 9200 mm². Increasing the number of plants from 100 to 125 per m² led to intense competition and shading of plants, so that the leaf area showed a sharp decline compared to other densities (Fig. 1). Also, this characteristic increased with increasing number of weeding times, but the amount of this increase was less due to the intensification of plant competition (Fig. 2).
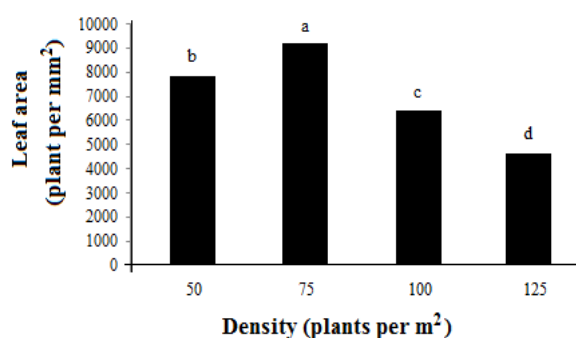
1: *The effect of different planting densities on leaf area of S. hortensis*
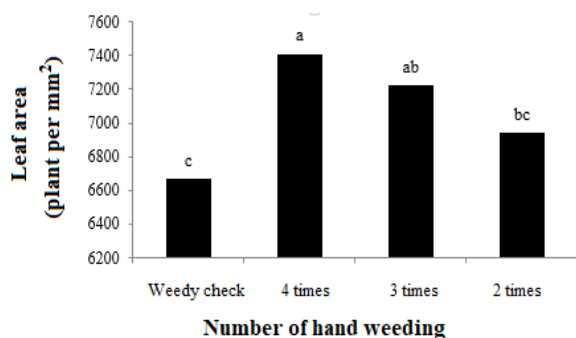
2: *The effect of number of weeding times on the leaf area of S. hortensis*

Leaf area was negatively correlated with fresh weight (r = -0.356**) and dry weight (r = -0.438**), but there were positive correlation with EO content (r = +0.613**) (Tab. III).

EO Content

EO content in density of 50 plants per m² was significantly increased in four times of weeding (36.45%) compared to weedy check. Also, this trait increased with an increase of planting density from 50 to 75 plants per m² in comparison with weedy check. The highest value was related to weeding four times in the amount of 2.98%. Increasing the number of plants per m² from 75 to 100 and 125 plants intensified the plant competition for environmental potentials and then the EO content although the application of different weeding treatments increased significantly. The highest significant content of EO was thus obtained in density of 125 plants per m² in weedy check treatment (3.19%) and twice weeding (3.23%) (Tab. II).

EO content was negatively correlated with fresh weight (r = -0.438**) and dry weight (weight (r = -0.523**) and there was positively correlated with leaf area (r = +0.613**). Also, no significant correlation was observed between content and EO yield (Tab. III).

EO Yield

Increasing the number of weeding times in densities of 50 and 75 plants per m² has increased the yield of *S. hortensis* EO. The highest EO yield was obtained in weeding four times (39.92 kg/ha). The EO yield decreased with increasing density of *S. hortensis* despite of any increase in the content of EO compared to two densities of 50 and 75 plants per m² (Tab. II).

The Effect of Planting Density and Number of Weeding Times on Density and Dry Weight of Dominant Weeds in *S. Hortensis*

The dominant weeds in the *S. hortensis* experimental field during the growing season included redroot pigweed (*Amaranthus retroflex*), black nightshade (*Solanum nigrum*) and lambsquarters (*Chenopodium album*). The effect of plant density, number of weeding times and their interaction on density and dry weight of *A. retroflex* weed was significant at 1% probability level. In contrast, the effect of these factors in different treatments were only significant on plant density *S. nigrum* at the level of 1% probability (Tab. IV).

IV: *Analysis of variance (ANOVA) of the planting density and number of weeding times on density and dry weight of each dominant weed and their total*

S.O.V	df	Density of A. retroflexus	Dry weight of A. retroflexus	Density of S. nigrum	Dry weight of S. nigrum	Density of C. album	Dry weight of C. album	Total density of weeds	Total dry weight of weeds
r	2	1.65^{ns}	0.35^{ns}	0.15^{ns}	15.89^{ns}	0.08^{ns}	0.003^{ns}	0.81^{ns}	4.22^{ns}
a	3	117.47**	106.95**	29.41**	2.70^{ns}	0.14^{ns}	0.21^{ns}	291.69**	3731.36**
b	3	26591.58**	23304.79**	10436.30**	9990.54**	7.86**	0.01^{ns}	75533.19**	1314181.70**
a × b	9	74.68**	80.01**	21.11**	1.69^{ns}	0.06^{ns}	0.0004^{ns}	182.82**	112.30^{ns}
E	30	5.98	3.19	2.57	14.42	0.17	0.13	8.46	44.09
CV (%)		7.59	7.05	9.37	24.56	28.46	30.41	5.61	3.57

**: indicate significant difference at 1% probability level and ns: non-significant difference, r: replication, a: plant density, b: number of weeding times, e: error, CV: Coefficient Variance

The Effect of Number of Weeding Times on Density of *C. Album*, Dry Weight of *S. Nigrum* and Total Dry Weight of Weeds

Density of C. Album

The density of *C. album* significantly decreased under different weeding times compared to weedy check. Weeding two and three times with one plant per m² had the lowest number. However, their difference with weeding four times were not significant (Tab. V).

Dry Weight of S. Nigrum

The range of changes in dry weight of *S. nigrum* was between 58.7 and 0.71 gram per m². The highest amount of dry weight was related to weedy check treatment. In contrast, twice weeding had the lowest amount in terms of this trait, so that the difference with three and four weeding times was not statistically significant (Tab. V).
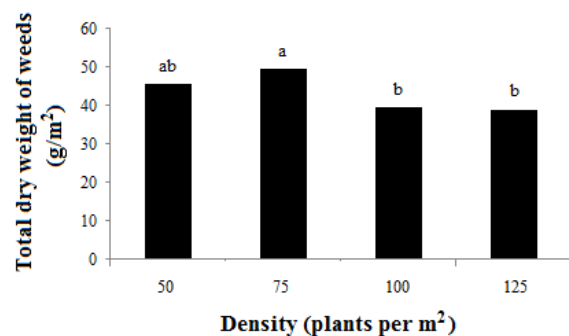
Total Dry Weight of Weeds

Total dry weight of the dominant weeds under different weeding times decreased with the same significant level (Tab. V).

The Effect of *S. Hortensis* Densities on Total Dry Weight of Different Weed Species

Total dry weight of weeds was decreased under densities of 100 and 125 plants per m² as compared to 50 and 75 plants per m² (Fig. 3).

The Effects of *S. Hortensis* Densities and Number of Weeding Times on Density and Dry Weight of *A. Retroflexus* and *S. Nigrum* Density

Density of dominant weed species such as *A. retroflexus* and *S. nigrum* decreased with any increase in the number of weeding times at certain densities of *S. hortensis* with a similar trend. *A. retroflex* with 113 plants per m² had the highest value in weedy check. The highest total weed density also belonged to weedy check at a density of 50 plants per m². However, the lowest total weed density was obtained at 125 plants per m² (7.67) in weeding four times, which did not show a statistically significant difference with some weeding treatments at certain densities (Tab. VI).

3: *The effect of S. hortensis densities on total dry weight of weed species*

V: *The effect of number of weeding times on density of Chenopodium album, dry weight of Solanum nigrum and total dry weight of weeds*

Characteristics/ Number of hand weeding	Density of C. album (plants per m²)	Dry weight of S. nigrum (g/m²)	Total dry weight of weeds (g/m²)
Weedy check	2.67a	58.74a	154.21a
4 times	1.17b	1.39b	7.80b
3 times	1.00b	1.003b	6.49b
2 times	1.00b	0.71b	4.98b
LSD 5%	0.35	3.17	6.45

Different letters in each column represent significance at 5% probability level based on least significant difference (LSD) test

The Effects of *S. Hortensis* Densities and Number of Weeding Times on Dry Weight of *A. Retroflexus*

Increasing the plant density of *S. hortensis* at all levels of weeding times decreased the dry weight of the *A. retroflexus* compared to weedy check. On the other hand, with any increase in *S. hortensis* density, the density of *A. retroflexus* decreased. On the other hand, as the density of the *A. retroflexus* decreased, its dry weight also decreased (Tab. VI).

DISCUSSION

Plant height of *S. hortensis* increased with any increase in plant density. Also, dry weight of this plant increased up to 100 plants per m² with weeding four times. Any increase in plant height under increasing planting density may be attributed to vigorous plant pressure with high competition for light, nutrients, and space in weed free environment. This result is in agreement with those of Al-Ramamneh (2009), Kizil *et al.* (2007) and Naghdi Badi *et al.* (2004). Raoufi *et al.* (2014), stated that weeds competition significantly reduced peppermint fresh weight. Also, a wide range of weeds observed in this field by shading on peppermint prevented the proper growth of this plant.

Increasing the density of *S. hortensis* from 50 plants upwards, provides a good relative weeds control which has intensified the competition in plants and weeds and thus increased EO content. However, the EO yield at 100 and 125 plants per m² especially in fewer weeding times decreased. Mohammad Dust Chamanabad (2011) reported that weeds in the field using nutrients were more successful in competing with the main plant, thereby reducing the EO yield. Raoufi and Giti (2015) reported that treatments of weeding and density increased fresh and dry weight, green cover percentage and EO yield of peppermint. Hence, the maximum yield of the mentioned items was obtained in densities of 18 plants m² with hand weeding.

In sweet basil (*Ocimum basilicum* L.), the highest yield of linalool (63.88%) and consequently of the oxygenated monoterpenes (75.05%), was achieved in the weeding treatments (Sarrou *et al.,* 2016). Vouzounis *et al.* (2003) reported that weed competition decreased the dry weight of lavender flowers by 52.4%, while weeds control significantly increased the oil content from 5.82% (weedy check) to 6.65%. They added that the dry weight of leaves and flowers of transplanted oregano significantly decreased by 11.9% and oil content by 8.8% under

VI: *The effects of S. hortensis densities and number of weeding times on density and dry weight of Amaranthus retroflexus and Solanum nigrum density*

Density (plants per m²)	Number of hand weeding	Density of *A. retroflexus* (plants per m²)	Dry weight of *A. retroflexus* (g/m²)	Density of *S. nigrum* (plants per m²)	Total density (plants per m²)
50	Weedy check	113.00 ± 4.00 [a]	100.86 ± 3.91 [a]	67.67 ± 3.51 [a]	188.33 ± 3.21 [a]
	2 times	13.33 ± 2.08 [d]	5.18 ± 0.62 [c]	3.00 ± 00.00 [e]	17.67 ± 2.52 [e]
	3 times	9.67 ± 2.08 [defg]	3.76 ± 0.39 [cd]	3.00 ± 00.00 [e]	14.00 ± 2.00 [ef]
	4 times	6.33 ± 1.15 [gh]	2.60 ± 0.48 [cd]	1.67 ± 0.58 [e]	9.00 ± 1.73 [gh]
75	Weedy check	110.00 ± 2.64 [a]	100.39 ± 1.20 [a]	63.33 ± 3.06 [b]	180.33 ± 3.06 [b]
	2 times	11.33 ± 0.58 [def]	3.89 ± 0.18 [cd]	3.00 ± 1.00 [e]	16.00 ± 2.64 [e]
	3 times	5.33h	7.67 ± 1.15 [fgh]	3.13 ± 0.08 [cd]	14.23 ± 0.29 [efg]
	4 times	7.33 ± 1.15 [gh]	3.20 ± 0.31 [cd]	2.33 ± 0.58 [e]	10.67 ± 1.53 [fgh]
100	Weedy check	96.67 ± 4.04 [b]	83.53 ± 2.15 [b]	59.67 ± 2.31 [c]	162.00 ± 5.57 [c]
	2 times	9.00 ± 1.00 [efgh]	3.27 ± 0.26 [cd]	3.33 ± 0.58 [e]	13.33 ± 1.15 [efg]
	3 times	7.00 ± 1.00 [gh]	3.31 ± 0.26 [cd]	2.00 ± 1.00 [e]	11.00 ± 1.73 [fgh]
	4 times	8.00 ± 1.00 [fgh]	2.76 ± 0.58 [cd]	1.67 ± 1.15 [e]	9.67 ± 0.58 [fgh]
125	Weedy check	91.33 ± 5.51 [c]	80.87 ± 5.01 [b]	54.67 ± 2.08 [d]	152.33 ± 6.11 [d]
	2 times	12.33 ± 1.53 [dxc]	4.04 ± 0.46 [cd]	2.67 ± 0.58 [e]	16.00 ± 1.73 [e]
	3 times	7.00 ± 1.00 [gh]	1.50 ± 0.22 [d]	2.00 ± 1.00 [e]	10.00 ± 1.73 [fgh]
	4 times	5.33 ± 1.53 [h]	2.78 ± 0.27 [cd]	1.33 ± 0.58 [e]	7.67 ± 1.53 [h]
LSD5%		3.97	2.88	2.58	4.70

Different letters in each column represent significance at 5% probability level based on least significant difference (LSD) test

weed competition, whereas the two characters were reduced by 43.0 and 28.0% in the transplanted sage.

The medicinal plant of *S. hortensis* is very sensitive due to low growth rate, height and canopy in the early stages of growth. Therefore, weeding weeds in the early stages of *S. hortensis* growth significantly prevents possible weeds damage, which the results are its evidence. Poor competition of medicinal plants with weeds depends on various factors such as lack of good canopies and root system, high needs for nutrients, weak resistance to severe environmental conditions, a prolonged flowering period, a tendency to scatter seeds; and a low harvest index (Mirshekari, 2013; Abaas, 2014; Carrubba and Militello, 2013; Ibrahim and Qasem, 2001).

The weeds of *A. retroflex*, *S. nigrum* and *C. album* were dominant weeds in the present study. The density and dry weight of the dominant weeds thus decreased with any increase in the density of *S. hortensis*. These findings are in agreement with previous study conducted by Ghahari *et al.,* (2013), who reported that the effects of weeding time and plant density of fenugreek (*Trigonella foenum-graecum*) were highly significant on dry matter of weeds. Also any increase in plant density caused a significant decrease in weed dry weight. Barkhi *et al.* (2009) reported that dry weight of *A. retroflexus* decreased at the higher plant density of maize. Fayzbakhsh *et al.* (2007) also stated that dry weight of stem and leaf of maize decreased with any increasing density.

The lack of any significant effect of *S. hortensis* density on the dry weight of *C. album* and *S. nigrum* indicates that the increase in competition between plants at high densities has not been so enough as to reduce the weight of both dominant weeds. Also, the lack of a significant effect of weeding times on the dry weight of *C. album* compared to the weedy check probably indicates a delay in the emergence of this weed as indicated by field observations.

Basically, the changes in weed population density during the growing season can be attributed to the competition of adjacent plants such as weeds with weeds and weeds with *S. nigrum*, which causes a thinning, especially at higher densities. This finding is consistent with the results of Carrubba (2017), Gholamalipour Alamdari (2011), Gharanjic *et al.* (2013) and Dastorani *et al.* (2018).

Koocheki *et al.* (2005) reported that the decrease in the number of weeds over time is probably due to any increase in the weed inter and intraspecific competition over nutrition sources, light, etc. This competition will lead to the elimination of weaker plants. Therefore, more weeds in the weedy check treatment can be due to the longer period of weed interference and greater use of environmental potential.

CONCLUSION

The presence of weeds reduced the morphological and quality of active metabolites of *S. hortensis*. Also, the adequate density of *S. hortensis* is required due to the increase in the morphological, active metabolites and weeds control. As a result, weeds control in *S. hortensis* with weeding four times, i.e. 2, 4, 6 and 8 weeks after planting- WAP leads to a significant increase in dry weight, content and EO yield. However, delays in weed removal, such as twice weeding (4 and 8 WAP), significantly reduced measured traits. This indicates that weeding in *S. hortensis* due to low competitiveness should be done at the beginning of the growing period. Also, increasing the density of *S. hortensis* from 50 plants upwards provides proper relative control of weeds which in turn intensifies competition in plants and weeds, and hence increases the EO content due to stress conditions. Overall, due to the favorable effect of weed control under three or four weeding times on dry weight and content and EO yield of *S. hortensis*, weeding at the mentioned times is recommended in Gonbad Kavous area and areas with similar conditions to Gonbad Kavous.

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