Research Article

A Comparison of Weeds Interference and Non-Interference at Different Planting Densities, on Yield, Nutritional Value and Some Morphological Traits of Alfalfa (*Medicago sativa* L.)

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Abstract | In this study, an experiment was conducted to evaluate the effect of weed interference and non-interference in different densities of alfalfa on yield, nutritional value and some morphological traits of alfalfa, forage crop, as factorial and based on a randomized complete block design with three replications in two cuttings during 2013-14 crop years. The experimental treatments were weed interference and non-interference in two levels (hand weeding and non-hand weeding) and plant density in four levels (20, 40, 60 and 80 stems per square meter). The results obtained in two cuttings in the third year of established alfalfa showed that the weeds caused quantitative and qualitative reduction in alfalfa. Alfalfa in the second cutting had better growth compared to the first cutting, so that the plant produced the highest fresh and dry yield in the second cutting. However, weed non-interference increased the levels of superior morphological traits of alfalfa, such as fresh and dry yield, plant height, and number of leaves per plant, number of main-stem nodes, leaf area and percentage of vegetation cover as well as increased the nutritional value of alfalfa in terms of nutrient elements, proteins and factors such as ADF, Ash, CF, NDF.

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Introduction

Alfalfa as a forage crop constitutes the largest area of planting (Lanini et al., 1991; 1999), and as a crude material plays an important role in feeding livestock due to its high protein content (Khanjani and SoleimaniPari, 2005). More than 25% of the dry weight of alfalfa consists of fiber (Meighan et al., 2011). Unfortunately, particular attention has not been paid to the production of this plant in Iran (Raoofi and Giti, 2015). One of the challenges of alfalfa production is the presence of weeds (Meighani et al., 2011; Raoofi et al., 2013). The weeds directly compete with the main plant for light, nutrients and soil moisture and reduce plant yield (Wilson 1981, 1997).

Weed interference can suppress alfalfa yield (Wilson, 1981) and impact stand densities (Becker et al., 1998). In addition to competition with the alfalfa, weed cause a reduction in the quality and quantity of alfalfa and decrease the price by 33% to 60%

(Khanjani and SoleimaniPari, 2005; Khanjani, 2000). Similar reduction of alfalfa density due to weeds has been shown elsewhere; Wilson and Burgener (2009) and Bradley et al. (2010) found that weed interference can reduce alfalfa density 20–30%. Temme et al. (1979) found that lower quality weeds primarily were responsible for decreasing the quality of alfalfa. Frequently, weeds compare in quality with alfalfa; however, nutritive quality rapidly declines as weeds mature (Doll, 1986).

Weeds also alter the composition of the forage, increasing drying time (Doll, 1984) and reducing palatability of the alfalfa (Marten et al., 1987). Generally, weeds will cause severe competition with the crop. Alfalfa seedlings are particularly susceptible to weed competition because they are not vigorous competitors and weeds emerging shortly after seeding can reduce alfalfa success (Fischer et al., 1988; Zimdahl, 2004). As with any crop, weed competition can reduce yields. Higher alfalfa seeding rate often resulted in greater alfalfa and total forage yield, decreased weed biomass, and increased alfalfa density (Calvin et al., 2011).

Weeds interfere with alfalfa during establishment, reducing dry matter yields and plant persistence by competing for light, water, and nutrients (Fischer et al., 1988; Wolfe and Southwood, 1980). The most damage by weeds in an alfalfa field occurs in the first harvest (Zand et al., 2010), however, weeds in many areas, including Hamadan, damage all harvests (Raoofi et al., 2014).

The alfalfa growth stage is considered as the most important factor influencing the composition and nutritional value of forage. The need for structural tissues will rise by ageing the plant, resulting in increased contains of main structural carbohydrates (such as cellulose and hemicellulose) and lignin (AOAC, 1990). Protein content decreases by ageing the plant; therefore, there is an inverse relationship between the protein content and fiber in a plant species (Janmohammadi et al., 2013).

Moreover, the levels of inorganic elements, potassium, calcium, phosphorous and other micronutrients will be reduced by raising plant age, parallel to reduced total forage ash (Sufi Siavash and Janmohammadi, 2011; Ghorbani and Khosravinia, 2012) and (Kellems and Church, 2009). Generally, the weeds in alfalfa farms are often controlled by herbicides (Myhre et al., 1991); in this regard, six herbicides are recommended for treatment of alfalfa (Zand et al., 2007).

Unfortunately, obsolescence and lack of sufficient attention to the weeds in forage crops will lead to serious damages in the alfalfa crop. In addition to the quantitative damages (Raoofi and Giti, 2015), the nutritional value of forage for feeding livestock will also decrease, which unfortunately has not been the subject of attention. In this study, the effects of weed interference on alfalfa and traits such as fresh yield and dry yield, ash content, plant height, number of leaves per plant, number of main-stem nodes, leaf area and percentage of vegetation cover were studied, as well as the elements contained in the alfalfa were also evaluated to assess the nutritional value of alfalfa.

Materials and methods

The alfalfa seeds were planted on 25 September 2010. On 14 April 2011, the first year of alfalfa began to grow. In the spring of 2013, the treatments were performed at the alfalfa that was the third year of growth. This season crop year is 2013-14. To investigate and comparison the interference and Non-interference weeds on Alfalfa, this experiment, was conducted using a randomized complete block design in a three year field of alfalfa in two cuttings. Farm under investigation, located at Km 7 Hamadan-Tehran road at latitude of 34° 51' N and longitude 48° 32' E.

The study was conducted during two growing seasons of the spring and summer of season crop year is 2013-14 respectively. The experimental treatments were weed interference and non-interference (hand weeding, when the weeds Does not exist and non-hand weeding, when the weeds inhabitancy there.) and plant density in four levels (20, 40, 60 and 80 stems per square meter). We note that there were natural pollution of weeds and their distribution were uniform, so due to the wide range and high density weeds, weed interference and non-interference with plant density of alfalfa, were criteria evaluated. The desired alfalfa was planted in Sept. 25, 2010 with the required densities. Considering that all sampling and research design were conducted in the third growth year of alfalfa, so required stem density per unit area was imposed to specified number by the clipper after alfalfa growing at the beginning of 2013-14 crop year.

Each plot consisted of ten rows with a distance of 25 cm and a length of 4 meters. The distance between the two plots was 60 cm and the distance between the two blocks was 130 cm. For proper evaluation, before performing treatments and concurrent with identifying plots and blocks, three fixed quadrants were installed, each with an area of one square meter per plot. The traits during the study were as follows: fresh and dry yield, plant height, number of leaves per plant, number of main-stem nodes, leaf area and percentage of vegetation cover (which was measured by a framework divided into 100 equal parts), minerals (calcium, phosphorus, sodium, potassium and magnesium), protein, ADF, NDF, Ash and CF.

Chart 1: *Environmental conditions of the test site.*

Operative Measurement	appraisal
Absolute maximum air temperature	36.8 °C
Absolute minimum air temperature	-29.6 °C
Average air temperature	9.6 °C
The hottest months of the year	July and August
Average air temperature of hottest months of the year	35°C
The Coldest months of the year	December and junuary
Average air temperature of coldest months of the year	-25.4 °C
The annual amount of precipitation	300 ml.
The number of frost days	143 Days
Wind direction	multifarious

All samples were performed in two cuttings in the third year of established alfalfa. In the non-interference treatment, the weeds were weeding by hand until the last sampling. In the treatments without weed control, all weeds stayed with alfalfa until the end of sampling without any control. To increase the accuracy in weed control, hand weeding was done on average every three days, as well as alfalfa density was examined and with further growth of the density of lateral branches, alfalfa density was determined. On average, irrigation was put on the agenda to avoid farm wilting about every 3 to 6 days and almost after every hand weeding by sprinkler system. Environmental conditions of the test site, expressed in Chart 1.

Samples were taken to determine the traits expressed in the first and second cutting. In any cutting, with 40 percent of farm flowering, plant sampling from the soil surface was carried out by fixed quadrants (considering 50 cm from both sides of each plot as margin). The first cutting harvest was done in the second week of June and the second cutting was harvested in the third week of July. The fresh samples taken from each plot were weighted and their areas were determined by leaf area meter. The samples were dried in the oven at 74°C for 48 hours to calculate the dry weight.

To prepare laboratory samples for each of the treatments, the dry forage samples were split into smaller pieces and then grinded with a coarse grind. The samples were crushed into smaller pieces to chemical analysis at baseline and again were grinded using a mill with a sieves No. 4. Then 300 g samples were grinded again with the help of laboratory mills with a millimeter mesh, and mixed in a blender for 7 to 10 minutes. The crude ash, ether extract and crude fiber were determined in accordance with the relevant protocol (AOAC, 1990), the crude protein by Micro-Kjeldahl devices and acid detergent fiber (ADF) using a Fibertek device (Goering and Van soest, 1970). NDF were measured according to Van soest et al. (1991). Minerals (calcium, phosphorus, sodium, potassium and magnesium) were measured according to standard methods of mineral analysis in plants (Waking et al., 1989). Data obtained from sampling of both cuttings were analyzed as factorial based on randomized complete block design, and data obtained from one year were analyzed as split plot in time by SAS ver. 9.1 software. The means were compared by LSD test.

Chart 2: *weeds were observed.*

Scientific name	common name
Centaurea spp	Cornflower
Convolvalus arvensis L.	field bindweed
Cuscuta spp	Small seed dodder
Cynodon doctylon	Bermuda grass
Descarainia Sophia L.	Hedge mustard
Euphorbia spp	Sun spurge
Hordeum murinam L.	Mouse barley
Lactuca spp	Prickleylettuce
Rumex crispus L.	_
Sismbrium irio L	London rocket
Sorghum halepense L.	Johnson grass
Taraxacum officinale	dandelion
Tragopogon spp	goatsbeard

Chart 3: *Alfalfa weeds with their distribution levels and importance.*

Scientific name	Family	English common name	Importance value
Carthamus spp	Astraceae	Safflower	*
Centaurea spp	Astraceae	Cornflower	*
Ceratocephalus falcatus	Ranunculaceae	_	*
Convolvolus arvensis L.	Convolvulaceae	field bindweed	*
Cuscuta spp	cuscutacae	Small seed dodder	****
Cynodon doctylon	Poaceae	Bermuda grass	**
Descarainia sophia L.	Cruciferae	Hedge mustard	****
Euphorbia spp	Euphorbiaceae	Sun spurge	*
Hordeum bulbosum L.	Poaceae	_	*
Hordeum murinam L.	Poaceae	Mouse barley	*
Lactuca spp	Astraceae	Prickleylettuce	*
Rumex crispus L.	Rosaceae	_	****
Salvia nemorosa	Lamiaceae	Violet sage	**
Sismbrium irio L.	Cruciferae	London rocket	****
Sorghum halepense L.	Poaceae	Johnson grass	*
Taraxacum officinale	Astraceae	dandelion	**
Tragopogon spp	Astraceae	goatsbeard	**
Vaccaria pyramidata Medic	Caryophyllaceae	vaccaria	*

Chart 4: *Chemical analyzes of farm's soil.*

Soil contexture	Sand (%)	Silt (%)	Clay (%)	potassium absorbable (ppm)	Phosphor Absorbable (ppm)	Organic Carbon (%)	Epicene material (%)	PH	EC ds/m	Depth of sampling (cm)
Silty Loamy	33	40	27	332.9	26.4	0.58	9.5	7.6	0.298	30

Results and discussion

According to previous studies on this farm, the weeds in established alfalfa aged over three years can cause more damages and thus the third growth year was considered into research agenda. In the study of two cuttings of three-year established alfalfa, the following weeds (Chart 2) were observed and identified that their distribution levels and importance are as follows, corresponded with the findings of Khanjani and Soleymani pery (2005) and Raoofi and Giti (2015). Chart 3. Also, Chemical analyzes of farm's soil under investigation as well as the Chart 4.

Fresh weight

The results showed that the effects of weed non-interference and plant density on fresh yield (fresh weight) of plant in both cuttings were significant at 1% level (Tables 1 and 3). According to the Table 7, it was observed that weed non-interference (hand weeding) had significant impact on the fresh weight of alfalfa. With increasing plant density, fresh yield level showed upward trend so that the highest alfalfa fresh weight was observed in density of 80 plants per square meter (Table 9). Combined analysis of weed non-interference and plant density in both cuttings on fresh weight of alfalfa (Table 5) showed that the cutting had significant effect on the level of 1%. In general, increasing the number of plants per unit area was directly related to fresh weight. Given the proper density of crop will act more successfully in competition with weeds (Koochaki et al., 2006).

In this regard, Giti et al. (2013) stated that the plant fresh weight was improved by increasing the alfalfa density per unit area. The weed non-interference provided more space to expand and grow alfalfa. As a result, the plant was able to achieve higher weight, because the presence of weeds led to competition on resources and consequently a reduction in the growth of alfalfa. Raoofi et al. (2013) and Raoofi et al. (2014) said

Table 1: *Analysis of variance of weed non-interference and plant density for some morphological traits in the first cutting of alfalfa.*

				Mean	Squares			
Sources variations	df	Fresh weight	Dry weight	Stem height	number of main-stem nodes	Number of main-stem leaves	Leaf area	Percentage of green vegetation cover
Replication	2	4955784**	517455 ns	29.7 ns	2.1 ns	219 ns	66626 ns	383.5*
Hand weeding	1	7924091**	5089428**	3.7 ns	4.4 ns	396 ns	1243725*	192.7*
Plant density	3	2666795**	289 992**	10.1 ns	1.8 ns	1119*	15485.2 ns	179.6**
Hand weeding × plant density	3	155408 ns	19351 ns	1.95 ns	5.2 ns	35 ns	182.1 ns	1.15 ns
Error	14	354 928	42955	2.1	1.8	74	227 966	3.2
CV %		25.3	26.3	8.3	7.5	9.6	21.9	7.7

ns,* and * *: *No significant and significant at* the levels of 5% and 1%, *respectively*

Table 2: *Analysis of variance of weed non-interference and plant density for nutritional value in the first cutting of alfalfa*

Sources			Mean	Squares		
variations	df	Crude protein	ADF	Ash	CF	NDF
Replication	2	147.2*	87.2*	38.6 ns	61.7*	77.9*
Hand weeding	1	74.7*	43.3*	577.2*	31.1*	38.6*
Plant density	3	68.3**	41.9**	114.1*	28.6**	36.8**
Hand weeding × plant density	3	0.44 ns	0.3 ns	7.3 ns	0.31 ns	0.32 ns
Error	14	2.1	2.6	32.6	1.9	2.7
CV %		6.3	6.8	33.1	5.7	7.2

ns,* and * *: *No significant and significant at* the levels of 5% and 1%, *respectively*

Table 3: *Analysis of variance of weed non-interference and plant density for some morphological traits in the second cutting of alfalfa.*

Sources				Mean	Squares			
variations	df	Fresh weight	Dry weight	Stem height	number of main-stem nodes	Number of main-stem leaves	Leaf area	Percentage of green vegetation cover
Replication	2	11832882**	1379303.7**	59.8**	0.65 ns	373 ns	362975.8 ns	347.3**
Hand weeding	1	18519563**	731,932.2**	45.44 ns	16.5*	3.8*	460,948.3**	577.3**
Plant density	3	16486402**	686,625.3**	16.2*	10.11 ns	* 807	43961.5 ns	342*
Hand weeding × plant density	3	267656.2 ns	12199.2 ns	6.8 ns	* 5.4	297 ns	88111.3 ns	31 ns
Error	14	1112333.7	103,532.6	28.9	13.2	320	17353	408
CV %		22.8	23.2	24.4	17.4	14.7	24.7	6.5

ns,* and * *: *No significant and significant at the levels of 5% and 1%, respectively*

that the weeds cause competition with alfalfa and thus reduce their fresh weight. There was also a wide range of weeds on the farm that inhibited the proper growth of alfalfa due to adsorption of resources and by shadowing in some cases. This fact is evident that yield loss enhances with increasing number of weeds (Rashed Mohassel et al., 2008). It was very clear relationship between the weighted yield and density of alfalfa. At higher densities, alfalfa was very successful in competition with weeds. The weed non-interference provided space for growing alfalfa that had accompanied its optimum growth. Generally, in terms of weed non-interference, alfalfa was able to better use of resources and conditions and caused fresh weight gain by higher canopy.

Dry weight

The results of the dry weight yield were consistent with the fresh weight yield. In the first and second cuttings, the dry weight was affected by the weed non-interference and plant density (Tables 1 and 3). The weed non-interference significantly increased dry weight of alfalfa and it could have a direct impact on dry yield. In general, we saw an increase in dry weight of forage by raising the density, but there was no significant difference between 40 and 60 stems per square meter (Table 9) though the trend was on the rise. Elevated density per unit area was directly related to the dry weight, and the highest dry weight yield was obtained at density of 80 stems per square meter; hence, the appropriate planting density is very noteworthy. The researchers have considered repeatedly the importance of determining the appropriate density of alfalfa planting and other crops (Raoofi and Giti, 2015). The results of combined analysis showed that the fresh weight and dry weight were under the

Table 4: *Analysis of variance of weed non-interference and plant density for nutritional value in the second cutting of alfalfa*

Sources			Mean	Squares		
variations	Degrees of freedom	Crude protein	ADF	Ash	CF	NDF
Replication	2	153.44**	91.3**	78.3*	64.5**	82.6**
Hand weeding	1	116.3 ns	151.9**	373.3**	98.6**	129.77**
Plant density	3	166.8*	87.6*	12.3*	61.4*	77.9*
Hand weeding × plant density	3	1.1 ns	6.1 ns	11.8 ns	5.6 ns	5.9 ns
Error	14	2.9	3.4	22.4	2.1	3.4
CV %		5.4	6.7	36.8	6.9	6.9

ns,* and * *: *No significant and significant at the levels of 5% and 1%, respectively*

Table 5: *Combined analysis of hand weeding and plant density on some morphological traits in both cuttings* of alfalfa.

Sources				Mean	Squares			
variations	df	Fresh weight	Dry weight	Stem height	number of main-stem nodes	Number of main-stem leaves	Leaf area	Percentage of green vegetation cover
Replication	2	20045595**	10051475**	112.6 ns	2.8 ns	110.9 ns	431966 ns	781.9*
(non-interference) Hand weeding	1	36670825**	230 672**	52.5 ns	1.21 ns	249.1 ns	7345982*	898.5*
Plant density	3	23979996**	1679911**	34.2 ns	9.34 ns	1908.2*	8562 ns	749.1*
(non-interference) Hand weeding × plant density	3	2577129 ns	48411 ns	6.3 ns	8.6 ns	93.5 ns	320755 ns	3.8 ns
Experimental error	14	1358242	157 497	27.3	6.4	266.9	480 542	16.9
Time (cutting)	1	1145111**	6039699*	8485*	859.1*	888179.2 ns	404 193*	2666**
Cutting × replication	2	3442917 ns	87377 ns	5.6 ns	1.3 ns	5761.1 ns	887349 ns	69.2 ns
Cutting ×(non-interference) Hand weeding	1	3079425 ns	285318 ns	15.4 ns	17.4 ns	173.5 ns	902791 ns	52.11 ns
Cutting × Plant density	3	611011 ns	117998 ns	2.7 ns	2.6 ns	161.8 ns	26944 ns	88.6*
Cutting ×(non-interference) Hand weeding× Plant density	3	376517 ns	6529 ns	4.1 ns	4.1 ns	293.3 ns	195811 ns	7.1 ns
Error	14	901 588	122037	26.6	9.3	202.6	4737	24.9
CV %		24.8	26.7	16.4	14.7	8.9	12.8	9.5

ns,* and * *: *No significant and significant at the levels of 5% and 1%, respectively*

Table 7: *Mean comparison of hand weeding on some traits in both cuttings of alfalfa.*

Weed control	Fresh weight	Dry weight	Leaf area	Green cover	Crude protein	Crude ash	ADF	NDF	Crude fiber
Interference	3566 b	1027 b	1941b	106b	21.3b	18.75b	22.7b	32.9b	27.9b
Non-Interference	5364 a	1572 a	3344a	131a	23.7a	32.36a	28.6a	50.2a	31.8a

Means with same letters in each column in each treatment have no significantly difference according to LSD test (5%)

Table 6: *Combined analysis of hand weeding and plant density on nutritional value in both cuttings of alfalfa.*

Sources			Mean	Squares		
variations	df	Crude protein	ADF	Ash	CF	NDF
Replication	2	228072.08 ns	3479353**	7345982*	2144252**	2783482**
(non-interference) Hand weeding	1	3,878,677.18*	798 495**	1105**	492 866**	638 788**
Plant density	3	3099 ns	581 506**	162.22 ns	358 911**	466 115**
(non-interference) Hand weeding × plant density	3	169311 ns	16754.7 ns	8.36 ns	10335.11 ns	13411.2 ns
Experimental error	14	253 726	54518.5	28.77	33588.36	43616.5
Time (cutting)	1	213 545*	2112553**	9011.36**	1303188.2*	1691144.5*
Cutting × replication	2	468520.33 ns	30246.5 ns	19.32 ns	18633.4 ns	24198.2 ns
Cutting ×(non-interference) Hand weeding	1	476672.11 ns	98765.3 ns	33.49 ns	61915.2 ns	79113.4 ns
Cutting × Plant density	3	14225.7 ns	40846.2 ns	41.08 ns	25166.3 ns	32685.2 ns
Cutting ×(non-interference) Hand weeding× Plant density	3	103388.22 ns	2262 ns	4.09 ns	1492.4 ns	1811 ns
Experimental error	14	2411.3	42244	30.21	27113	33799
CV %		12.3	9.7	28.77	8.9	9.4

ns,* and * *: *No significant and significant at the levels of 5% and 1%, respectively*

influence of cutting, hand weeding and plant density (Table 5). In general, the space was provided for the success of alfalfa forage crop through the weed control, by more favorable taking advantage of resources and conditions and by overcome the weeds.

Height and number of main-stem nodes

Analysis of variance for the effects of hand weeding and plant density on alfalfa plant height showed that apart from the plant density in the first cutting that had significant effect on this trait at the level of 5%, the rest had no significant effect on any of the two cuttings (Tables 1 and 3). Alfalfa height will be of paramount importance at the time of the presence of weeds in the farm, as if the weed growth rate is higher than the main plant, so they could adversely affect the main plant and reduce the growth in the main plant by shading and absorption of nutrient elements from the soil. The combined analysis of hand weeding and plant density in both cuttings on the height of alfalfa indicated that the cutting had significant effect on plant height at 5% level and the interactions between weed non-interference and plant density on this trait did not affect significantly (Table 5). Overall, a wide range of weeds with different growth habits led to reduced space for successful alfalfa. We observed a reduction in the growth of alfalfa by excelling weed growth, creating shadow and competing with alfalfa. The weed interference with alfalfa can significantly reduce its height. In the first cutting, the effect of plant density on number of main-stem nodes was non-significant. The effects of weed non-interference and its interaction with density were significant at 5% level (Table 1). The effect of weed non-interference, plant density and their interaction on the number of main-stem nodes in the second cutting were non-significant (Table 3). Generally, number of main-stem nodes was greater in the first cutting than in the second cutting. In this experiment, alfalfa growth in the second cutting was better than in the first cutting due to the significant decline in the second cutting weeds. These results were consistent with the findings of Raoofi et al. (2016) based on the damage caused by weeds in the first cutting.

Number of leaves and leaf area

The effect of hand weeding and plant density in the first cutting on the number of leaves per plant was significant at the levels of 5% and 1%, respectively. However, their interaction was not significant (Table 1). The hand weeding effect on the number of leaves per plant was non-significant in the second cutting but the effect of plant density on this trait was significant at the 5% level. Their interaction on the number of leaves per plant was non-significant (Table 3). The hand weeding effect on leaf area was significant in the first and second cuttings at 1% and 5%, respectively, but the plant density had no significant effect on leaf area in both cuttings. Interaction of hand weeding and density in both cuttings on the leaf area was non-significant (Tables 1 and 3). The number of leaves per plant decreased with increasing plant density (Table 9). The most (270) and the least (235) numbers of leaves were related to densities of 20 and 80 stems per square meter, respectively. Low plant density reduced competition among plants and increased the number of leaves. These results were in line with the findings of Delaluz et al. (2002) based on a decrease in the number of leaves in high plant densities. Although the number of leaves in density of 80 stems per square meter was lower compared to low densities, but the leaf area was greater, as well as the percentage of green vegetation cover was higher that had direct relationship with it. The high growth rate as well as the rapid increase in weed density will cause sooner competition with the crop (Akey et al., 1990). In this experiment, with increasing alfalfa density, although the number of leaves dropped, but since the plant could use resources and conditions more favorably compared to the weeds, so increased their leaf area and thereby compensated the lower number of leaves. Mean comparison of data showed that weed non-interference increased significantly alfalfa leaf area (Table 7). Combined analysis of hand weeding and plant density in both cuttings revealed that cutting and hand weeding had significant effect on leaf area at the level of 5% (Table 5). The whole range of weeds (narrow leaf, broadleaf and parasites) absorbed nutrient elements from the soil and reduced alfalfa access to nutrient elements. Alfalfa growth was limited by reducing access to these elements. This is consistent with studies of Bazdirev et al. (2004). Because of strong and fast initial velocity of roots, the weeds were more successful compared to alfalfa. Thus, in this experiment, the weeds caused a reduction in alfalfa leaf area.

Percentage of green vegetation cover

The effects of weed non-interference and density on the percentage of green vegetation cover were significant at 1% and 5% levels in the first cutting, as well as at 5% and 1% levels in the second cutting. Interaction between weed non-interference and density was non-significant in both cuttings (Tables 1 and 3). The mean comparisons showed that the percentage of green vegetation cover in the treatment of weed non-interference was higher than in non-weeding or non-interference (Table 7), and the percentage of green vegetation cover intensified by increasing plant density (Table 9). Liebman et al. (2001) and Bazdirev et al. (2004) reported that in the presence of a chance in favor of crops (such as non-interference and weeding), the yield and development of the crop would be much more than competition with weeds and green cover will increase. In this experiment, the weed non-interference in accordance with the instruction of hand weeding led to better growth and development of alfalfa and to the formation of alfalfa canopy more effectively, and caused a greater extent. Tulikov (1974) suggested that the green cover could be improved by increasing the density per unit area.

Crude protein content

Crude protein content is one of the main factors examined in alfalfa. In the first cutting, hand weeding and plant density effect on crude protein was significant at the levels of 5% and 1%, respectively, but their interaction was not significant (Table 2). In the second cutting, the effect of hand weeding on crude protein was non-significant, but the plant density showed a significant effect on this trait at the level of 5% and their interaction on the crude protein was not significant (Table 4). The crude protein content was intensified by increasing plant density (Table 9). The highest crude protein content was related to the density of 80 stems per square meter. With the increasing density of alfalfa, the weeds were more influenced by density, and alfalfa showed better use of resources and conditions in the farm. This was an important factor in increasing the crude protein content so that the weed non-interference led to a significant increase in crude protein content of alfalfa (Table 7). Majidi Dizaji et al. (2014) also emphasized that the pure alfalfa accounted for the most crude protein content. Combined analysis of hand weeding and plant density in both cuttings showed that cutting and hand weeding had significant effect on the crude protein at the level of 5% (Table 6). Reduced access of alfalfa to nutrient elements because of competition with weeds led to decrease in the alfalfa growth and crude protein. Reducing the alfalfa crude protein can be directly related to the presence of weeds because of the uniformity of the farm in every respect. With regard to the wide range of weeds on the farm, we observed very high competition between weeds and alfalfa, reducing crude protein content. Alfalfa interact with other crops might also reduce the protein content in Alfalfa (Majidi Dizaji et al., 2014). Certainly, it will be much more intense on interaction with weeds; even in cases where mixed cultivation is concerned with alfalfa, the emphasis is on the lack of competition among the plants. If there is no severe competition among species, such cultures have been recommended. However, the study farm had a variety of narrow-leaf, broadleaf and parasitic weeds. It is clear that high competition will be established between alfalfa and weeds. These results are consistent with investigation of VanderMeer (1989).

Acid detergent insoluble fiber (acid detergent fiber percentage)

The effects of hand weeding and plant density on acid detergent insoluble fiber (ADF) were significant in the first cutting at 5% level. In the second cutting, hand weeding and planting density had significant effects on these traits at the levels of 1% and 5%, respectively (Tables 2 and 4). Table 7 shows that hand weeding caused a significant increase in alfalfa ADF. In addition, ADF content improved with increasing plant density (Table 9). Combined analysis of hand weeding and plant density in both cuttings on ADF content (Table 6) showed a significant effect of cutting and hand weeding at 1% level; the interaction betweenhand weeding and plant density was non-significant.

Crude ash content (Ash %)

The results indicated that the effect of hand weeding and plant density on crude ash content in the first cutting was significant at the 5% level. Also in the second cutting, hand weeding and plant density effect on crude ash content was significant at 1% and 5%, respectively (Table 2 and 4). According to the Table 7, we see that the hand weeding led to significant increase in crude ash content in alfalfa. A direct relationship was found between density and crude ash content. The crude ash content had upward trend with increasing plant density. The highest crude ash content was observed at a density of 80 stems per square meter (Table 9). Combined analysis of hand weeding and plant density in both cuttings on the crude ash content (Table 6) indicated that cutting and hand weeding had significant effect at 1% level; the interaction between hand weeding and plant density was non-significant. While, the crude ash content was enhanced with increasing plant density in the interference between weeds and alfalfa, because generally adhere to proper plant density of crops will lead to success in competition with weeds (Koochaki et al., 2006).

Crude fiber percentage

The effect of hand weeding and plant density on crude fiber (CF) in the first cutting was significant at 5% level. In the second cutting, hand weeding and plant density had significant effect on the crude fiber at 1% and 5% levels, respectively (Tables 2 and 4). Hand weeding resulted in high crude fiber (Table 7). By increasing stem density, the crude fiber content had upward trend and we found the highest crude fiber content at a density of 80 stems per square meter (Table 9). Combined analysis of hand weeding and plant density in both cuttings revealed significant effect of cutting and hand weeding on the crude fiber at 1% level; the hand weeding and plant density interaction was not significant (Table 6).

Neutral Detergent Fiber

Hand weeding and plant density had significant effect on the neutral detergent insoluble fiber (NDF) in the first cutting at 5% level. In the second cutting, the effect of hand weeding and plant density on this trait was significant at 1% and 5% levels, respectively (Tables 2 and 4). Table 7 shows that hand weeding caused a significant increase in alfalfa NDF content and the content of this trait also went up with increasing plant density (Table 9). Combined analysis of hand weeding and plant density in both cuttings on the NDF (Table 6) indicated that cutting and hand

Table 8: *Mean comparison of hand weeding on some elements in both cuttings of alfalfa.*

Weed control	Calcium	Phosphorus	Sodium	Potassium	Magnesium
Interference	3566 b	0.4 b	0.5b	12.2b	7.5b
Non-Interference	24.3a	1.3 a	1.8a	35.2a	18.2a

Means with same letters in each column in each treatment have no significantly difference according to LSD test (5%)

Table 9: *Mean comparison of alfalfa density on some morphological traits and nutritional value in both cuttings of alfalfa.*

Plant density (Stems/m²)	Fresh weight (g/m²)	Dry weight (g/m²)	Leaf area	Green cover (%)	Crude protein (%)	Crude ash (%)	Crude fiber (%)	ADF (%)	NDF (%)
20	2985.3 d	1056.33 d	270 b	84.3 d	21.6 d	16.9 d	27.9 d	22.6 d	52.2 d
40	3972.7 c	1210.7 bc	266 ab	91.4 c	22.4 c	23.4 c	28.3 c	24.7 c	43.5 c
60	5908.4 b	1545.8 b	240 a	96.8 b	22.9 b	26.6 b	30.4 b	26.4 b	38.1 b
80	7136.4 a	1961.1 a	235 a	103.6 a	23.7 a	23.1 a	31.5 a	28.3 a	31.4 a

Means with same letters in each column in each treatment have no significantly difference according to LSD test (5%)

weeding had significant effect at 1% level; the interaction between hand weeding and plant density was non-significant.

Elements (calcium, phosphorus, sodium, potassium and magnesium)

According to Table 8, it was observed that weed non-interference (hand weeding) had a significant impact on the alfalfa element contents. With increasing plant density, study element contents revealed upward trend, and the maximum content in all the elements was observed at the density of 80 plants per square meter (Table 9). The weed non-interference provide more space for alfalfa growth and development; while in the interference status, the weeds present in the farm consume nutrient elements (Mohammad Doost, 2011) and thus are more successful in competing with the main plant, resulting in reduced nutrient elements. In this regard, Nasiri (2013) also pointed out the important role and effectiveness of nutrient elements on yield and yield components of plants. In general, we observed a decrease in level of alfalfa growth with the growth and spread of weeds (without hand weeding). The presence of weeds can cause the depletion of soil nutrient elements, reducing the contents of elements. Luxury absorption of elements by weeds (Mohammad Doost, 2011) several times higher than the crop causes severe deficiency of these elements in the alfalfa.

Conclusion

The test results indicated contamination of alfalfa farms with a wide range of weeds. The research findings in the study area showed significant effects of plant density and weed control on the alfalfa yield. This plant in suitable sowing density, in addition to producing high yield and ash content, could better use the resources and conditions in comparison with weeds and could significantly outreach superior morphological traits. Thus, according to the importance of forage plants, it is scientifically recommended that suitable cultivation density to be on the agenda of alfalfa farmers in the study and other regions in the country to achieve maximum yield as well as to minimize herbicide use.

Author's Contribution

Both authors contributed equally.

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