

## EFFECT OF MIXED INTERCROPPING OF CLOVER ON FORAGE YIELD AND QUALITY

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**ABSTRACT.** *To evaluate the effect of mixed cropping of Crimson and Persian clover on quantitative and qualitative forage yield, a field experiment was conducted as factorial based on a randomized complete block design (RCBD), with three replications during two growing seasons (2014-2015) at Seed and Plant Improvement Institute, Karaj, Iran. Experimental factors were seed mixing ratio of Crimson and Persian clover at five levels (100%-0%, 70%-30%, 50%-50%, 30%-70%, and 0%-100%) and seeding rate at two levels (15 and 20 kg ha<sup>-1</sup>). The results of analysis of variance showed that the effect of seeding rate on fresh and dry forage yield was not significant, while, the effect of seed mixing ratio and their interaction on these traits was significant. The highest fresh and dry forage yield was obtained in the mixing ratio of 30-70% (30% Crimson clover + 70% Persian clover) with 20 kg ha<sup>-1</sup> seeding rate. The maximum content of crude protein (CP) was obtained in sole Persian clover with 20 kg ha<sup>-1</sup> seeding rate, as well as in the mixing ratio of 30-70% (30% Crimson clover + 70% Persian clover) with 15 kg ha<sup>-1</sup> seeding rate. Considering the total quantitative and qualitative traits, the mixing ratio of 30-70% (30% Crimson clover + 70% Persian clover) is presented as the superior treatment in this experiment. This study showed that mixed cropping of Crimson and Persian clover increases forage yield and quality and this model can be proposed to improve and increase forage production.*

**KEY WORDS:** *acid detergent fiber (ADF), ash, crude protein (CP), seeding rate, sustainable agriculture.*

## INTRODUCTION

Clover is an important forage legume grown in the temperate regions throughout the world and is distinct from other forage crops due to its special properties (Karimi, 1997). Persian clover (*Trifolium resupinatum*) is an annual legume, growing over the period autumn to spring. It is native to Central and Southern Europe, the Mediterranean, and South-Western Asia. It is an important hay crop in cold regions of Iran, Afghanistan and other Asian areas with cold winters (Heidari Sharif Abad and Dorry, 2002). Persian clover is mostly used for pasture and hay, producing highly palatable and nutritious forage. It has high forage yield and excellent regrowth following cutting (Suttie, 1999). Crimson Clover (*Trifolium incarnatum*) is an annual legume that can be grown as a summer annual in cool climates and as a winter annual in warmer areas (Young-Mathews, 2013). Crimson clover originated from South-Eastern Europe and South-Western Asia Minor and now grows worldwide. It is now one of the most planted annual forage legumes (Ball et al., 2000). Crimson clover is used as fodder and provides good quality pasture, hay and silage. It is one of the earliest forage legumes available in spring and has low or no bloat effect on ruminants (Young-Mathews, 2013). One of the basic ways in sustainable agriculture is to increase the diversity of agricultural systems. Mixed cropping as an example of sustainable systems in agriculture pursue objectives such as ecological balance, higher utilization of resources, increase the quantity and quality of crops, and reduce the damage to pests, diseases and weeds (Lithourgidis et al., 2007). The cause of increased crop yields in mixed cropping is higher use of environmental factors such as water, food and light (Mazaheri, 1998). Forage crops are important in livestock feeding and, as a result, in the supply of human beings to livestock products, and globally account for about 75% of the energy needed for domestic animals (Modir-Shanechi, 2000; Rezvanfar and Shafiee, 2005; Golzardi et al., 2017). Forage quality represents nutritional value and the amount of energy that is available for livestock. In other words, it is the amount of nutrients that animals obtain in the shortest possible time from the feed (Buxton, 1996; Baghdadi et al., 2017). Using good quality forage in animal breeding, reproduction, meat, dairy, leather and wool is very useful and effective. So that nutrient in the diets of livestock, forage quality and the amount of that is very important (Suyama et al., 2007). An important factor in the production and management of

forage plants is the quality of the forage and improving forage quality results in feed efficiency (Catanese et al., 2016). One of the new methods in mixed cropping is the cultivation of a mix cropping of different cultivars of one species that increases the yield, which have been studied in this field especially for clover (Namdari and Mahmoudi, 2013; Koochaki and Soltani, 1998). In some places in livestock farmers, a mixture of early and late varieties of plants is used to deal with the risk of a possible fall of rainfall or the use of subsequent rainfall to reduce the effects of dryness (Koochaki and Zand, 1996). Persian clover as a component of mixed cropping, especially mixed cropping with other legumes, such as white clover and grasses, or intercropping, can be used among cotton, corn and sorghum rows (Dinesh et al., 2004). Noorbakhshyan and Roushandel (2006) observed that Alfalfa clover and Red clover mixed crop cultivars showed that the highest dry forage yield was obtained to 20% red clover and 80% alfalfa with 11.98 kg ha<sup>-1</sup> and the lowest yield of pure crop Alfalfa. In intercropping of alfalfa and clover given that leaf clover contains a substance soluble in n-hexane and insoluble in diethyl ether and water prevents spawning weevil is hence reduce pest weevil in intercropping imaginable (Koochaki and Zand, 1996). According to the above-mentioned materials, this experiment was conducted with the aim of investigating the effect of mixed cropping of clover on quantitative and qualitative characteristics of forage.

## **MATERIALS AND METHODS**

Field experiments were conducted in 2014 and 2015 at the Research Farm of Seed and Plant Improvement Institute, Karaj, Iran (35°59'N, 51°06'E; 1321m above mean sea level). This location is semi-arid with long-term average annual precipitation of 240 mm. The experiment was arranged as factorial based on a randomized complete block design with three replications. The first factor included five mixing ratios of Persian and Crimson clover (100%-0%, 70%-30%, 50%-50%, 30%-70% and 0%-100%), and the second factor included two levels of the seed consumption (15 and 20 kg ha<sup>-1</sup>). Prior to the beginning of the experiment, the composite soil samples were collected at two depths of 0-25 and 25-50 cm, air-dried, crushed and tested for physical and chemical properties. Soil type and selected characteristics of the soil at the experimental field is presented in Table 1. Based on the recommendations of soil tests, 200 kg ha<sup>-1</sup> of triple superphosphate and 50 kg ha<sup>-1</sup> of urea (as the starter) was added to the soil before conducting the

Table 1. Characteristics of the soil at the experimental field.

Soil depth (cm)	Soil texture	EC (dS m <sup>-1</sup> )	OC (g.kg <sup>-1</sup> )	TN (g.kg <sup>-1</sup> )	P (mg.kg <sup>-1</sup> )
0-25	Clay-loam	0.68	0.49	0.20	15
25-50	Clay-loam	1.4	0.30	0.25	6

EC: electrical conductivity; OC: organic carbon; TN: total N; P: phosphorus.

experiments and no fertilizer was used after planting. Planting was done on September 20, 2014. The dimensions of the plots were 5×2 meter, and in each plot, four rows were considered spaced between rows of 50 cm. Irrigation method was EFI (every furrow irrigation) and field were irrigated 8 times during the growing season. Weed control was done by hand and no herbicide was used. The forage harvest time in different treatments was based on 25% flowering (mean flowering in clover cultivars). For this purpose, after removing 0.5 m from the beginning and the end of the planting lines (marginal effect), the forage area was harvested four square meters from the middle two lines and immediately weighed. Then, fresh forage yield was calculated in kilograms in the experimental unit and then in tons per hectare. Then, one kilogram of fresh forage was discarded at random and dried in an oven at 75° C for 24 hours and then calculated using the dry forage yield in the experimental unit and hectare. To evaluate the quality of forage production, the dried samples were milled and evaluated by near-infrared spectrometer (NIR). The data was analyzed by SAS statistical software and comparisons were performed by LSD test at 5% probability level.

## RESULTS AND DISCUSSION

### Fresh Forage Yield

Based on the results of variance analysis (Table 2), in the first cut, the effect of seed rate and seed mixing ratio was not significant, but the interaction of seed rate × seed mixing ratio on fresh forage yield was significant at 5% probability level ( $P \leq 0.05$ ). In all three cuts, the effect of seed mixing ratios on fresh forage yield at a probability level of 1% and interaction effect of seed rate × seed ratio was significant at 5% probability ( $P \leq 0.05$ ), but seed rate effect did not have a significant statistical. The highest forage yield in the first cut and the sum of the three cuts (respectively 43.16 and 80.38 t ha<sup>-1</sup>) was obtained from 20 kg ha<sup>-1</sup> (Table 3). Mean comparison of seed mixing ratios (Table 4) showed that in first

Table 2. Analysis of variance of fresh and dry forage yield as affected by seeding rate and mixing ratio of Crimson and Persian clover.

S.O.V.	d.f.	Mean square (M.S.)			
		First cut		Total	
		Fresh forage yield	Dry forage yield	Fresh forage yield	Dry forage yield
Replication (R)	2	13.10	0.168	35.22	0.301
Seeding rate (SR)	1	30.60 <sup>ns</sup>	0.069 <sup>ns</sup>	11.78 <sup>ns</sup>	0.00005 <sup>ns</sup>
Mixing ratio (MR)	4	9.24 <sup>ns</sup>	0.233 <sup>ns</sup>	2728.23 <sup>**</sup>	41.91 <sup>**</sup>
SR × MR	4	42.71 <sup>*</sup>	1.21 <sup>**</sup>	81.09 <sup>*</sup>	1.59 <sup>**</sup>
Error	18	9.91	0.253	23.61	0.332
C.V. (%)	-	7.46	8.29	6.09	5.34

ns, \* and \*\*: Not significant, significant at the 5% and 1% probability levels, respectively

Table 3. Mean comparison of fresh and dry forage yield as affected by seeding rate.

Seeding rate (kg ha <sup>-1</sup> )	First cut		Total	
	Fresh forage yield (t ha <sup>-1</sup> )	Dry forage yield (t ha <sup>-1</sup> )	Fresh forage yield (t ha <sup>-1</sup> )	Dry forage yield (t ha <sup>-1</sup> )
15	41.14a	6.02a	79.12a	10.79a
20	43.16a	6.11a	80.38a	10.79a

In each column, the means with similar letters are not significantly different at the 5% probability level, according to LSD test.

Table 4. Mean comparison of fresh and dry forage yield as affected by seed mixing ratio.

Seed mixing ratio (%)		First cut		Total	
Crimson clover	Persian clover	Fresh forage yield (t ha <sup>-1</sup> )	Dry forage yield (t ha <sup>-1</sup> )	Fresh forage yield (t ha <sup>-1</sup> )	Dry forage yield (t ha <sup>-1</sup> )
100	0	42.34a	6.09a	42.34c	6.09b
30	70	92.69a	6.18a	92.69a	12.26a
50	50	85.64b	5.94a	85.64b	11.65a
70	30	84.33b	6.31a	84.33b	1.69a
0	100	93.75a	5.81a	93.75a	12.26a

In each column, the means with similar letters are not significantly different at the 5% probability level, according to LSD test.

cut, the highest fresh forage yield (43.35 t ha<sup>-1</sup>) was related to mixed cropping (70% Crimson clover + 30% Persian clover) and in all three cuts with 93 tons per hectare was related to pure Persian clover cultivation (0-100). Mean comparison of interactions of seed rate and seed mixing ratio (Table 5) showed that in the first cut, the highest forage yield of 46.76 tons per hectare was belong to 20 kg ha<sup>-1</sup> seed rate and seed mixing ratio of 0-100 (pure crimson clover) and the lowest 37.68 t ha<sup>-1</sup> for 15 kg seed rate and seed mixing ratio of (50% crimson clover + 50% Persian clover). In addition, in total the three cuts, the highest forage yield was 94.97 t ha<sup>-1</sup> with a density of 15 kg ha<sup>-1</sup> and seed mixing ratio of 100-0 (pure Persian clover), respectively.

Table 5. Mean comparison of fresh and dry forage yield as affected by interaction effects of seeding rate × seed mixing ratio.

Seeding rate (kg ha <sup>-1</sup> )	Treatments		First cut		Total	
	Seed mixing ratio (%)		Fresh forage yield (t ha <sup>-1</sup> )	Dry forage yield (t ha <sup>-1</sup> )	Fresh forage yield (t ha <sup>-1</sup> )	Dry forage yield (t ha <sup>-1</sup> )
	Crimson clover	Persian clover				
15	100	0	37.91e	5.58bcd	37.91c	5.58e
	30	70	41.85abc	6.28abc	92.83ab	12.35ab
	50	50	37.68c	5.45a	81.80c	11.18c
	70	30	44ab	6.41ab	84.68bc	11.85bc
	0	100	44.28ab	6.36ab	94.40a	13.00a
20	100	0	46.76a	6.60a	46.76d	6.60d
	30	70	44.03ab	6.08abcd	92.55ab	12.17abc
	50	50	42.76abc	6.43ab	89.48abc	2.12bc
	70	30	42.90abc	6.21abc	83.98bc	11.85bc
	0	100	39.36bc	5.26d	89.11bc	11.52bc

In each column, the means with similar letters are not significantly different at the 5% probability level, according to LSD test.

Intercropping is aimed at higher crop productivity besides other associated advantages, i.e. greater yield stability between different and diverse climatic conditions (Deak et al., 2009), lower incidence of pests, higher nutritional value of mixed diets, improved water use efficiency, yield advantage in subsequent crops, reduced soil erosion because of greater ground cover and higher land use efficiency (Anil et al., 1998). Legumes in the mixture increase the amount of nitrogen in the soil by fixing nitrogen. It

also enhances microorganism activity. Thus, the ecological properties of the soil are improved with an intercropping system (Genc-Lermi, 2018). Ahmadi et al. (2018) reported that the highest fresh forage yield was obtained from the 25% oat mixed with the 75% clover. Putnam et al. (1986) reported that soybean with corn in intercropping produced more forage than sole crops. Salc and Alberscht (1996) state that the intercropping of alfalfa with lolium had more forage yield than single cropping. Vasilakoglou and Dhima (2008) showed that the intercropping of berseem clover with barley at the seeding rate of 750-113 seeds (clover–barley) could be used as an alternative practice of berseem clover sole crop and the other treatments for high forage production. Tripathy et al. (1997) reported improvement in forage yield from maize-cowpea intercropping. Similarly Patel and Rajagopal (2001) reported increase in fresh forage yield with maize and cowpea grown in 5:2 row was 9.69 and 17.31%. Increasing the light interception in the intercrops reducing the water evaporation, and improving the conservation of soil moisture are some aspects of intercropping maize with cowpea as compared with the maize alone (Ghanbari et al., 2010).

### **Dry Forage Yield**

The results of analysis of variance (Table 2) in first cut showed that the effect of seed rate and seed mixing ratio on dry forage yield was not significant, but interaction of seed rate × seed mix ratio on dry forage yield at a probability level of 1% ( $P \leq 0.01$ ) was significant. In all three chains, the effect of seed mixing ratios and interactions of seed rate × mixing ratio was significant at a probability level of 1% ( $P \leq 0.01$ ), but the seed rate was not statistically significant. Mean comparison of seed rate (Table 3) showed that the highest dry forage yield was 6.11 and 10.79 t ha<sup>-1</sup> in the first cut and the sum of the three cuts respectively was related to the 20 kg ha<sup>-1</sup> seed rate. Mean comparison of seed mixing ratios (Table 4) showed that in the first cut, the highest dry forage yield with 6.31 t ha<sup>-1</sup> was related to mixed crop (70% Crimson clover + 30% Persian clover) and in total of three cut with 12.26 t ha<sup>-1</sup> was related to mixed cropping (30% Crimson clover + 70% Persian clover) and 0-100 (pure Persian clover). Mean comparison of interaction of seed rate and seed mixing ratio (Table 5) showed that in the first cut, the highest dry forage yield (6.60 t ha<sup>-1</sup>) was related to the density of 20 kg ha<sup>-1</sup> seed and 100-0 (pure Crimson clover) and the lowest 5.26 t ha<sup>-1</sup> of dry forage yield was 20 kg ha<sup>-1</sup> seed and 0-100 (Persian clover). Also, in total three cuts, the highest dry forage yield was 13 t ha<sup>-1</sup> with a

density of 15 kg ha<sup>-1</sup> ratio of 100-0 (pure Persian clover). Martiniello (1999) and Ross et al. (2004a, 2004b) found that the intercropped berseem clover with barley provided total dry forage yield by 63 and 18 to 180%, respectively, greater than that of berseem clover sole crop. Ahmadi et al., (2018) reported that the highest dry forage yield (12849 kg ha<sup>-1</sup>) was obtained from the 50% oat mixed with the 50% clover. Jung et al. (1991) evaluated yield and quality of alfalfa and ryegrass mixture and harvested the highest dry forage yield from 60% alfalfa and 40% ryegrass proportion. Amraei et al. (2012) reported that the best combination proportion of alfalfa with grass was the ratio of about two-third of alfalfa and one-third of grasses. They also expressed that the intercropping of alfalfa with grass is a good way to increase dry forage yield in the rangelands. Intercropping common vetch with barley or winter wheat produced greater dry forage yield than sole common vetch (Lithourgidis et al., 2007). Crespo-Pereira et al. (2010) determined that dry matter yields of pure peas and pure triticale, respectively, as 5.93 t ha<sup>-1</sup> and 10.99 t ha<sup>-1</sup> and increasing forage yields with increasing triticale ratios in mixtures. Dry forage yield and dry matter percent of intercropped beans with wheat were improved in comparison with sole bean and wheat crops (Ghanbari-Bonjar and Lee, 2002; Lithourgidis and Dordas, 2010).

### **Crude Protein (CP)**

The results of variance analysis (Table 6) showed that the effect of seed rate on crude protein percentage at 5% probability level ( $P \leq 0.05$ ) and the effect of seed mixing ratio and interaction effect of seed rate  $\times$  mixing ratio on it at probability level of 1% ( $P \leq 0.01$ ) was significant. Mean comparison of seed yield (Table 7) showed that the highest amount of crude protein with 15.86% was related to 15 kg seed per hectare. Mean comparison of seed mixing ratio (Table 8) showed that the highest protein percentage with 16.75% was related to pure Persian clover (0-100%) and the least with 13.12% was related to pure Crimson clover (100-0). Mean comparison of interactions of seed rate  $\times$  seed mixing ratio (Table 9) showed that the highest protein percentage with 17.74% was related to pure Persian clover (0-100) with seed rate of 20 kg ha<sup>-1</sup> and the lowest protein percentage was 12.16% belonged to pure Crimson clover (100-0) with a seed rate of 20 kg ha<sup>-1</sup>. Ibrahim et al (2006) compared the yield and quality of maize fodder and cowpea sown alone and in mixture with each other in different proportions. They found that the production of crude protein was affected

Table 6. Analysis of variance of qualitative traits of forage as affected by seeding rate and mixing ratio of Crimson and Persian clover.

S.O.V.	d.f.	Mean square (M.S.)				
		CP	ADF	CF	WSC	Ash
Replication (R)	2	1.29	0.76	0.78	11.95	0.063
Seeding rate (SR)	1	3.42*	2.70 <sup>ns</sup>	4.33 <sup>ns</sup>	83.90*	0.007 <sup>ns</sup>
Mixing ratio (MR)	4	11.95**	42.73**	57.96**	243.73**	0.209**
SR × MR	4	3.80**	0.63 <sup>ns</sup>	3.85 <sup>ns</sup>	76.40*	0.019 <sup>ns</sup>
Error	18	0.66	0.74	1.35	17.43	0.034
C.V. (%)	-	5.24	4.31	4.11	15.98	2.61

ns, \* and \*\*: Not significant, significant at the 5% and 1% probability levels, respectively.

CP: Crude protein, ADF: Acid detergent fiber, CF: Crude fiber, WSC: Water-soluble carbohydrates.

Table 7. Mean comparison of qualitative traits of forage as affected by seeding rate.

Seeding rate (kg ha <sup>-1</sup> )	CP (%)	ADF (%)	CF (%)	WSC (%)	Ash (%)
15	15.86a	20.53a	28.71a	24.45a	7.15a
20	15.18b	19.75a	27.95a	27.80a	7.12a

CP: Crude protein, ADF: Acid detergent fiber, CF: Crude fiber, WSC: Water-soluble carbohydrates. In each column, the means with similar letters are not significantly different at the 5% probability level, according to LSD test.

Table 8. Mean comparison of qualitative traits of forage as affected by seed mixing ratio.

Seed mixing ratio (%)		CP (%)	ADF (%)	CF (%)	WSC (%)	Ash (%)
Crimson clover	Persian clover					
100	0	13.12c	24.27a	33.32a	16.96c	6.88c
30	70	16.03ab	17.15d	26.82c	32.76a	7.39a
50	50	16.17ab	19.70bc	27.25c	24.20b	7.18ab
70	30	15.54b	20.49b	29.06b	25.14b	7.17b
0	100	16.75a	18.66c	25.20d	31.57a	7.07bc

CP: Crude protein, ADF: Acid detergent fiber, CF: Crude fiber, WSC: Water-soluble carbohydrates. In each column, the means with similar letters are not significantly different at the 5% probability level, according to LSD test.

Table 9. Mean comparison of qualitative traits of forage as affected by interaction effects of seeding rate  $\times$  seed mixing ratio.

Seeding rate (kg ha <sup>-1</sup> )	Treatments		Crude protein (%)	Acid detergent fiber (%)	Crude fiber (%)	Water-soluble carbohydrates (%)	Ash (%)
	Seed mixing ratio (%)						
	Crimson clover	Persian clover					
15	100	0	14.07d	24.60a	34.00a	13.05f	6.94bc
	30	70	16.54ab	17.44e	26.79cd	28.30bcd	7.41a
	50	50	16.44ab	19.47cd	26.63cd	26.17cde	7.24ab
	70	30	16.48ab	21.03b	30.60b	20.69e	7.20ab
	0	100	15.76bc	19.24cd	25.51d	34.05ab	6.99bc
20	100	0	12.16e	23.94a	32.63a	20.86e	6.82c
	30	70	15.51bc	16.86e	26.83cd	37.27a	7.38a
	50	50	15.90bc	19.93bc	27.86c	22.22de	7.12abc
	70	30	14.60cd	19.95bc	27.53c	29.59bc	7.14abc
	0	100	17.74a	18.08de	24.90d	29.10bcd	7.16ab

In each column, the means with similar letters are not significantly different at the 5% probability level, according to LSD test.

by seed combinations of maize and cow pea in different ratios, where, an increased proportion of cowpea in seed mixture increased the crude protein contents. The cowpea sown alone produced more crude protein (18.10%), but the lowest dry matter (4.16 t ha<sup>-1</sup>) and maize sown alone produced minimum crude protein (8.5%). The seed combination of 75:25 maize and cowpea produced more dry matter (13.26 t ha<sup>-1</sup>) and crude protein (10.45%) than maize sole crop, suggesting that higher green forage yield and good quality could be attained by mixture of maize and cowpea of 75:25 seed ratio. Dawo et al (2007) concluded that CP concentration increased 22% in the mixture when corn proportion decreased by 50% in the mixture of corn and bean. Sanderson et al. (2010) reported that the proportion of legumes in grass-clover mixtures was positively correlated with crude protein. Anil et al. (2000) concluded that all of the three intercrops (maize-sunflower, maize-kale, maize-runner bean) significantly increased crude protein concentration when compared with maize silage. Liu et al. (2006) concluded that crude protein of plants in intercropping system was increased by 30.8% and 99.4% as compared with those for mono-cropping maize. Strydhorst et al. (2008) found that faba bean-barley, lupin-barley and pea-barley intercrops had 64, 27 and 55% higher protein

yields as compared to the barley sole crop, respectively. Also Herbert et al. (1984) concluded that all intercrops produced 8 to 17% more total protein  $\text{ha}^{-1}$  than corn monoculture. Others have also reported the influence of species proportion on the quality values (Deak et al., 2007; Zemenchik et al., 2002).

### **Acid Detergent Fiber (ADF)**

The results of variance analysis (Table 6) showed that the seed rate effect and seed rate  $\times$  seed mixing ratio interaction effect on acid detergent fibers (ADF) percentage was not significant, but the effect of seed mixing ratio on the trait was at a probability level of 1% ( $P \leq 0.01$ ) was significant. Mean comparison of seed yield (Table 7) showed that the highest ADF with 20.35% is related to 15  $\text{kg ha}^{-1}$ . Mean comparison of seed mixing ratio (Table 8) showed that the highest ADF with 24.27% was related to pure Crimson clover (100-0) and the lowest with 17.15% for 30-70 (30% Crimson clover + 70 Persian clover). Mean comparison of seed rate  $\times$  seed mix ratio interactions (Table 9) showed that the highest ADF with 24.60% was related to the pure Crimson clover (100-0) with seed rate of 15  $\text{kg ha}^{-1}$ . The ADF is the percentage of highly indigestible plant material present in forage and contains cellulose, lignin and silica. Low ADF values means higher energy value and digestibility. Since lignin and silica are not digestible by ruminants, low ADF values are desirable (Rebole et al., 1996). Onal Asci and Egritaş (2017) reported the lowest ADF and NDF ratios for pure legume treatments. The researchers indicated the reason for low ADF and NDF ratios of legumes as thinner cell membranes of legumes, thus indicating decreasing ADF and NDF ratios with increasing legume ratios in mixtures. Contreras-Govea et al. (2006) reported that NDF and acid detergent fiber (ADF) concentrations in clover were lower than in wheat and intermediate in mixtures. Sleugh et al. (2000) mentioned that ADF concentration decreased in kura clover-wheatgrass intercropping. Also Lauriault et al. (2004) noted that intercropping with winter pea decreased ADF in all cereal forage-legume intercrops compared with their monocultures. Thus addition of legumes to forage maize reduced the NDF and ADF concentrations, indicating potential for increasing forage intake.

### **Crude Fiber**

According to the results of analysis of variance (Table 6), the effect of seed rate and interaction between seed rate and seed mixing ratio was not

statistically significant, but the effect of seed mixing ratio on the trait was significant at 1% probability level ( $P \leq 0.01$ ). Mean comparison of seed yield (Table 7) showed that the highest raw fiber percentage with 28.71% is related to 15 kg ha<sup>-1</sup>. Mean comparison of seed mixing ratio (Table 8) showed that the highest raw fiber percentage with 33.32% was related to pure Crimson clover (100-0) and the lowest (25.20%) was related to pure Persian clover cultures (0-100). Mean comparison of seed rate × seed mix ratio (Table 9) showed that the highest fiber percentage with 34.00% was related to pure Crimson clover (100-0) with seed rate of 15 kg ha<sup>-1</sup>. Dahmardeh et al (2009) reported that maximum fiber content was recorded by sowing maize alone while increasing the proportion of cowpea seeds to 50% in intercropping with maize, resulted in the lowest fiber. Contreras-Gova et al (2006) reported that forage quality in terms of fiber concentration was improved by wheat-clover intercropping compared with wheat sole crop. Javanmard et al. (2009) found that forage in intercrops improved in crude protein concentration, but fiber concentration decreased as compared to that of mono cropped.

#### **Water-soluble Carbohydrates (WSC)**

Based on the results of variance analysis (Table 6), in the first cut, seed rate effect and interaction of seed rate × seed mixing ratio on the amount of water-soluble carbohydrates in the 5% probability level ( $P \leq 0.05$ ) and seed mixing ratio effect at the probability level of one Percentage ( $P \leq 0.01$ ) was significant. Mean comparison of seed yield (Table 7) showed that the highest percentage of water-soluble carbohydrates with 27.80% is related to 20 kg ha<sup>-1</sup>. Mean comparison of seed mixing ratio (Table 8) showed that the highest percentage of WSC with 32.76% was related to 30-70 (30% Crimson clover + 70% Persian clover) and the lowest with 16.76% was related to pure Crimson clover (100-0). Mean comparison of seed rate × seed mixing ratio interactions (Table 9) showed that the highest percentage of water-soluble carbohydrates with 37.27% is related to 30-70 cultures (30% Crimson clover + 70% Persian clover) with a seed rate of 20 kg ha<sup>-1</sup> and the least percentage with 13.05% belonged to pure Crimson clover (100-0) with seed rate of 15 kg ha<sup>-1</sup>. In forage crop vegetative biomass, the most important nutrients for livestock are proteins and water-soluble carbohydrates (WSC). The amounts of WSC varies between varieties as well as within the environment (Capstaff et al., 2018). Ameri and Ashraf Jafari (2016) reported that the highest values of WSC were obtained in the

mixed intercropping of alfalfa-festuca (50%-50%), and alfalfa-desert wheatgrass (50%-50%), whereas, the lowest values of WSC were obtained in the mixed intercropping of alfalfa-tall wheatgrass (25%-75%), and alfalfa-tall wheatgrass (50%+ 50%). Water-soluble carbohydrates of intercropped beans with wheat were improved in comparison with sole bean and wheat crops (Ghanbari-Bonjar and Lee, 2002; Lithourgidis and Dordas, 2010).

### **Ash**

Based on the results of analysis of variance (Table 6) the effect of seed rate and interaction effect of seed rate × seed mixing ratio on ash was not significant, but the effect of seed mixing ratio on the trait was significant at a probability level of 1% ( $P \leq 0.01$ ). Mean comparison of seed yield (Table 7) showed that the highest ash with 7.15% was related to 15 kg ha<sup>-1</sup>, which did not differ significantly with seed rate of 20 kg ha<sup>-1</sup>. Mean comparison of seed mixing ratio (Table 8) showed that the highest ash with 7.39% was related to 30-70 (30% Crimson clover + 70% Persian clover) and the lowest (6.88%) was related to pure Crimson clover (100-0). Mean comparison of seed rate × seed mix ratio interactions (Table 9) showed that the highest ash with 7.41% was related to 30-70 cultivars (30% Crimson clover + 70% Persian clover). Anil et al. (2000) reported that ash content of maize-sunflower, maize-kale and maize-runner bean intercrops were increased. Also Inal et al. (2007) concluded that intercropping improved the concentration of K in shoot of peanut and maize and also shoot concentrations of Fe and Zn nearly 2.5 folds in peanut. Sharma and Gupta (2002) showed that N and P nutrition of pearl millet was greatly improved by intercropping with legumes. Mason and Pritchard (1987) stated that mineral absorption percent increases due to complementary effects between components of maize-soybean intercropping. Ayub et al., (2004) showed that ash content increased with increasing proportion of rice bean in mixture with sorghum. The ash content of maize forage were increased by intercropping with legumes as compared with maize monoculture (Javanmard et al., 2009).

### **CONCLUSION**

The results of this study showed that mix cropping of Crimson clover and Persian clover increased the forage yield in compared to their pure

cultivation. Early cultivars and one cut of clover species (for example Crimson clover) due to their high growth rates and the optimal use of water and soil resources and high biomass production, are able to increase forage production in mix cropping of with lately cultivars and multi cut of Persian clover. Considering to quantitative and qualitative traits, the mixing ratio of 30-70% (30% Crimson clover + 70% Persian clover) is as the superior treatment in this experiment. This mixed model can be used as an ecological approach to increase forage production in crop farming in Karaj-Iran region.

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