Pak. J. Bot., 55(2): 711-717, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-2(33)

EFFECT OF ROW SPACING AND HARVEST METHOD ON SEED YIELD OF ALFALFA (*MEDICAGO SATIVA* L.)

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Abstract

The optimal seed yield achieving from forage legumes production on of the biggest challenge to modern farming system under changing of climate. This research was conducted to evaluate the effect of row spacing and harvest method on the seed yield and yield components in alfalfa. The research was done at East Mediterranean Agricultural Research Institute during 2012 and 2015 arranging in a split-plot design with four replications. Row spacings (25, 50, 75 and 100 cm) were assigned in the main plots and harvest methods (HM1:cutting with mower to form windrows to air dry and then threshing with plot combine,HM2:harvest directly with the plot combine, HM3:harvest with the plot combine afterethephon plus cyclanilide application as defoliant,and HM4:harvest with the plot combine after thidiazuron plus diuron application as defoliant) were in the subplots. Row spacing and harvest method significantly affected on the number of pods per plant, the number of seeds per pod and seed yield, but it was not significant influence on seed germination. The highest seed yield (829 kg ha^{-1}) (three years average) was obtained with 25 cm row spacing with HM3, while the lowest yield (412 kg ha^{-1}) was obtained with 100 cm row spacing with HM1.Generally , The results of the study showed that defoliant application with 25 cm row spacing and narrower row spacing (25-50 cm) with HM2 or HM3 were the most effective combinations of treatments for alfalfa seed production in the ukurova region of Turkey.

Key words: Alfalfa, Defoliant, Harvest method, Row spacing, Seed yield.

Introduction

Crops of legumes are important not only for their nutritional value but also for their contribution to the economy. Additionally, grain legumes are a great source of vitamins and energy (Ahmad *et al*., 2022). .Alfalfa is the oldest crop grown for forage, having been cultivated for about 9000 years (Sheaffer *et al*., 2020). It is a highly productive protein- and mineral-rich legume adapted to a wide range of environmental conditions. Its erect growth habit makes it suitable for hay, silage and artificially dehydrated forage, and it has drought resistance. It has many benefits, such as extensive adaptability, a very high degree of abiotic stressors, including cold resistance, heat, drought, heavy metals, and salt (Hossain *et al*., 2020). It has high voluntary intake characteristics and nutritive value and is a valuable break crop in arable and organic systems on account of its N2 fixation ability and as source of organic matter It is a major source of honey production (Frame, 2018). The alfalfa seed with a high seed qualit and genetic capacity of high yield and quality is needed for an effective alfalfa production. Many genetic and environmental factors affect seed yield. Successful alfalfa seed production is favored in regions that are characterized by clear, sunny, warm summer days in combination with little or no rainfall (Acikgoz, 2021). These climatic conditions promote good flowering of alfalfa and provide an environment conductive to the pollinating activity of bees. In addition to a favorable climate, there are other variables that will influence the yield and quality of alfalfa seed. Stand density is known to be an important factor in seed production because competition between and within plants affects a plant's ability to produce vegetative and reproductive material Thin, uncrowded stands are recognized capable of producing higher seed yield than dense stands in solid planted fields. Low seed production from dense stands can be explained in part by low nectar production, unattractiveness to pollinators, and increased floral abortion. Proper row spacing for alfalfa depends on soil depth and texture, total water availability, length of growing season, cultivar and possibly other factors (Stanisavljevic *et al*., 2012; Kavut, 2016; Avci *et al*., 2017, Abasov *et al*., 2019; Patra & Apul, 2019; Pajcin *et al*., 2020). According to Rincker *et al*. (1988), seed yield is a complex trait and it depends on the number of seeds per unit area and individual seed weight. Seeds are produced in pods and the yield components include number of seeds per pod, number of pods per raceme, number of stems per plant and number of plants per unit area. They also stated that alfalfa seed yields range from 0 (crop failures) to a verified yield of 2110 kg ha-1 of clean seed. Lush vegetative growth of alfalfa is desirable in the production of fodder, but there is a problem in seed production when, due to a large amount of above-ground mass, the alfalfa stem falls, which negatively affects on the yield and seed quality (Pajcin *et al*., 2020). On the other hand, flowering and seed ripening in alfalfa plants do not occur simultaneously. For this reason, the green parts of the plant and the immature pods in the seed harvest reduce the quality of the seeds. Seed harvest in alfalfa is made by different methods such as by windrowing and combining or by chemical curing and direct combining. The seeds are usually direct combined following spraying with a crop desiccant to speed up drying (Frame, 2018; Acikgoz, 2021). Chemical curing eliminates the need for cutting and windrowing, which may increase loss of seeds (40-50 kg ha^{-1}) due to shattering Desiccants, defoliants and growth regulators are chemicals used in agricultural production to accelerate the preparation of crops for mechanical harvest. For example, diquat and paraquat are non-selective contact herbicides that cause desiccation and defoliation (He *et al*., 2015). Ethephon is a plant growth regulator used as a defoliant or desiccant (Ben-Tal, 1987; Pajcin, 2020). Effects of the using these chemicals in pre-harvest desiccation have been investigated in alfalfa (May *et al*., 2003; Pajcin *et al*., 2020). However, the effects of pre-harvest chemical defoliant applications on seed yield and seed quality have not been so thoroughly examined in alfalfa.

Despite high forage yield potential under the lowland conditions of the Cukurova region, drying and harvesting problems occur, and seed quality decreases due to the continuing vegetative growth of the plant in high temperature and humidity during summer months. The study was therefore conducted to ascertain the impact of row spacing and harvest methods on the seed yield and some seed yield components of alfalfa under Cukurova conditions in order to bridge the study and knowledge gaps.

Materials and Methods

Plant material and experimental design: In the research, non-dormant synthetic alfalfa cultivar Nimet which was developed and registered by the East Mediterranean Agricultural Research Institute, was used. Field trial was conducted in the mentioned Institute, located in Adana province (36°51'35" N, 35°20'43" E), under irrigated management conditions during 2012 and 2015.

Adana which is located in the Mediterranean region of Turkey has a long growing season and moderate climatic conditions during winter. Summers are hot and humid with mean temperature of some days reaching above 30°C. The mean long-term annual temperature is 18.8°C, the lowest and highest average temperatures occur in January and August, respectively. Long-term mean annual precipitation of the locality is 668 mm. During the time period (2013 to 2015) in which the study was conducted, annual average temperatures were 19.3, 20.7 and 18.8°C, total annual precipitation was found to be 290, 501 and 661 mm with 63.1, 66.0 and 67.2% relative humidity, respectively. The soil was silty-clay with organic matter content 1.7%, pH 7.88, lime16.3% and phosphorus content 87.0 kg ha-1 and rich in lime.

The seedbed was prepared by ploughing with a field cultivator followed by planking. According to pre-sowing soil analysis, the site was fertilized with 40 kg ha^{-1} Nitrogen and 35 kg ha^{-1} Phosphorus.Sowing was done by hand in November 2012 along the previously opened marker tracks with a seeding rate of 5 kg ha^{-1}.The experiment was arranged in a split-plot design with four replications. The main plot treatments were row spacings (25, 50, 75 and 100 cm) and the subplots were 04 harvest methods (**HM1, HM2, HM3 and HM4**).

HM1: When approximately 60-75% of the pods in the plots reached black- browned color, they were cut with a self-propelled sickle bar mower (Grillo GF1), and dried naturally on the plot and threshed with plot combine to obtain clean seeds.

HM2: (considered as a control method): Plants in these plots were harvested directly by plot combine when they had reached similar development as in HM1.

HM3: Ethephon-cyclanilide mixture(600 ml ha^{-1}) applied with a pressurized sprayer in first week of July in all years when 60-75% of alfalfa pods had turned brown to the plots and harvested directly 10 days later by plot combine.

HM4: Thidiazuron-diuron mixture (1 L ha^{-1}) applied with plots harvested as HM4.

The subplots were 3 × 5 m (15 m^2), 12 rows with 25 cm row spacing, 6 rows with 50 cm row spacing, 4 rows with 75 cm row spacing, and 3 rows with 100 cm row spacing. A 01 m buffer was left between the plots in order to prevent the applications from affecting each other.

Weeds were controlled by hand hoeing as and when needed throughout the growing seasons of alfalfaa. Pollination was facilitated by honey bees (*Apis mellifera*) and other pollinators, such as leaf cutter bees (*Megachile rotundata*), naturally occuring in the region (Avci *et al*., 2010). In the first growing season, to determine the number of pods per plant and the number of seeds per pod, 10 plants were chosen randomly in each plot. After counting the pods on 01 plant, 30 randomly selected pods were threshed by hand and the average number of seeds per pod was calculated.

Data collection: Seed was harvested after ripening with harvesting dates between 12 to 25 July depending on the treatment. Germination tests were conducted on seeds harvested and cleaned from each treatment by placing 100 seeds (four replicates) on moist filter paper and allowing the seeds to imbibe water at 25°C for 7 days in a dark incubator.

Statistical analysis

Data obtained from the experiment was subjected to analysis of variance (ANOVA) technique using the MSTAT-C statistical analysis program and differences were compared using Duncan's multiple range at 5% level of probability.

Results and Discussion

Number of pods per plant: Row spacing, harvest technique, and their interplay all had a substantial impact on the number of pods per plant (Table 1). Row spacing made a significant difference in the number of pods per plant, and the values varied from 59.6 to 78.9 per plant depending on the row spacing. However, the highest mean number of pods was recorded with 100 cm row spacing, followed by 25 row spacing, but these values were least significant. The number of pods per plant was significantly higher with 25 cm row spacing than with 50 cm row spacing, but not significantly different compared to 75 and 100 cm row spacing. Under 50 cm of row spacing, plants might produce greater vegetative growth and branching but less generative growth, which could account for this. As opposed to narrow row spacing, increased pod counts with an increase in row spacing from 50 to 100 cm may be the result of the plants' balanced vegetative growth, which resulted in the creation of more pods.

Harvest methods significantly differed the number of pods per plant, varying from 65.8 to 75.1 pods per plant.The number of pods per plant was found significantly higher with HM2 and HM1 compared to HM3. This finding indicates that under HM3, fewer pods were produced per plant because the pods had not yet dried and fully matured. The interaction between row spacing and harvest method showed that the quantity of pods per plant was considerably affected differently depending on the row spacing by the harvest method. The number of pods per plant tended to increase as row spacing increased from 50 to 100 cm with HM1 and HM2, however, no consistent trend was detected for HM3and HM4.

Although the number of pods per plant was not significantly affected by harvest methods with a 25 cm row spacing, HM3 with 50 and 100 cm row spacing significantly reduced the number of pods per plant when compared to HM1 and HM2. Similarly, with 75 cm row spacing, treatment HM4 caused a significantly lower number of pods per plant compared to other harvest methods. Alfalfa has a highly specialized flower with a unique tripping mechanism that limits the types of insects providing pollination. The percentage of flowers setting pods in a highly self-sterile plant may vary considerably, depending partly upon the environment. Kavut (2016) reported that number of pods per plant at 20 cm row spacing was significantly higher than at 40 cm row spacing. This result is in harmony with our result. Zhang *et al*. (2008), Rashidi *et al*. (2009) and Abadouz *et al*., (2010) found that row spacing had not significant effect on number of pods per plant or raceme.

Number of seeds per pod: Depending on the row spacing, the typical number of seeds per pod ranged from 4.95 to 5.76 (Table 2). The number of seeds per pod with 50 cm row spacing was significantly lower than other row spacings. Ahmad *et al*., (2020) found that icreaseing the row spacing from 30 cm to 60 cm decreased the number of seeds per pod. This result is in harmony with our result. In contrast to this, Kavut (2016) found that number of seeds per pod at 40 cm row spacing was significantly higher than that at 20 cm row spacing. Row spacing and harvest method have a strong interaction effect on the quantity of seeds per pod. However, the number of seeds per pod in plants grown with 100 cm row spacing with HM1 was lower than the plants grown with 75 cm row spacing, while it was not significantly affected by row spacing with HM2. With HM3, plants grown with 50 cm row spacing had lower numbers of seeds per pod than those grown with 75 and 100 cm row spacing. With HM4, 50 cm row spacing had significantly lower number of seeds per pod than the plants grown with 25 and 100 cm row spacing. Seeds per pod varied according to the row spacing and harvest method, and generally no consistent pattern was evident. This may be due to an alfalfa plant structural characteristic, effective pollination, competition between plants for nutrition, or other environmental variables. Wang *et al*., (2011) reported that an alfalfa flower has 7-13 ovules, but only a small proportion of them are capable of producing the mature seed. They also inferred that the numbers of ovules per ovary, fertile ovules per floret and the number of seeds per pod vary between the alfalfa cultivars. Few-to-many ovules can be sterile and unavailable for fertilization (Rosellini *et al*., 1998). A low seed/ovule ratio is a general characteristic of outcrossing species. In general, no more than five ovules per pod develop mature seeds (Lorenzetti, 1993). It has been reported in the previous research findings that the number of seed per pod varied from 1.85-9.16 (Dordas, 2006), 4.1 to 4.8 (Abdouz *et al*., 2010), 5.5 to 5.7 (Rashidi *et al*., 2009) and 1.63 to 2.41 (Abasov *et al*., 2019).

Table 1. Effects of row spacing and harvest methods onnumber of pod per plant in alfalfa.

Row spacing (cm)	Harvest method				
	HM1	HM2	HM3	HM4	Mean
25	72.1 b-e[1]	84.7 ab	71.1 b-e	77.0 a-d	76.2 ab*
50	64.8 d-f	64.0 d-f	50.3 g	59.3 e-g	59.6 c
75	70.5 c-e	71.2 c-e	80.6 a-c	54.8 fg	69.3 b
100	86.7 a	80.4 a-c	61.3 e-g	87.1 a	78.9 a
Mean	73.5 a+	75.1 a	65.8 b	69.5 ab	
CV (%)			11.1		
F Row spacing (RS)			12.0 **		
F Harvest method (HM)			3.96 *		
F RSx HM			5.23 **		

*) Means indicated with the same letter in the same column are not significantly different according to the Duncan test at p≤0.05
+) Means indicated with the same letter in the same row are not significantly different according to the Duncan test at p≤0.05 Means of the different combinations of row spacing and harvest method indicated with the same letter are not significantly different according to the Duncan test at p≤0.05

Table 2. Effects of row spacing and harvest methods onseeds number per pod in alfalfa.

Row spacing (cm)	Harvest method				
	HM1	HM2	HM3	HM4	Mean
25	5.26 a-d 1	6.02 ab	5.28 a-d	6.02 ab	5.64 a*
50	5.07 b-d	5.21 a-d	4.53 d	5.01 cd	4.95 b
75	5.97 ab	5.46 a-c	6.00 ab	5.52 a-c	5.76 a
100	4.70 cd	5.37 a-d	5.51 a-c	6.17 a	5.44 a
Mean	5.25	5.54	5.33	5.68	
CV (%)	10.4				
F Row spacing (RS)	8.50 **				
F Harvest method (HM)	N.S.				
F RS xHM	2.29 *				

*) Means indicated with the same letter in the same column are not significantly different according to the Duncan test at p≤0.05

1) Means of the different combinations of row spacing and harvest method indicated with the same letter are not significantly different according to the Duncan test at p≤0.05

Table 3. Effect of row spacing and harvest methods on seed yield (kg ha^{-1})of alfalfa.

Year	Row spacing (cm)	Harvest method					Year mean
		HM1	HM2	HM3	HM4	Mean	
2013	25	474.3	711.8	713.3	492.9	598.1 d[1]	537.8 b*
	50	473.9	608.5	546.4	506.4	533.8 ef	
	75	509.4	526.2	681.5	597.1	578.6 de	
	100	322.0	493.9	489.3	457.9	440.8 g	
	Mean	444.9 f[2]	585.1 c	607.6 bc	513.6 de		
2014	25	621.6	980.7	1158.5	867.1	907.0 a	831.0 a
	50	666.2	1119.2	845.9	1135.1	941.5 a	
	75	545.3	845.8	921.7	746.9	764.9 b	
	100	562.5	578.0	768.8	933.7	710.8 c	
	Mean	598.9 bc	880.9 a	923.7 a	920.7 a		
2015	25	360.7	753.8	613.6	624.2	588.1 de	509.1 c
	50	378.8	715.9	479.4	629.3	550.8 d-f	
	75	350.4	668.6	474.9	497.5	497.8 f	
	100	352.1	447.6	308.1	490.0	399.5 g	
	Mean	360.5 g	646.5 b	469.0 ef	560.2 cd		

Row spacing (cm)	Harvest method				
	HM1	HM2	HM3	HM4	Mean
25	485.5 f[4]	815.4 ab	828.5 a	661.4 c-e	697.7 a[3]
50	506.3 f	814.5 ab	623.8 de	756.9 b	675.4 a
75	468.4 fg	680.2 cd	692.7 c	613.8 e	613.8 b
100	412.2 g	506.5 f	522.1 f	627.2 de	517.0 c
Mean	468.1 c+	704.2 a	666.8 b	664.8 b	
CV (%)	11.94				
F Year (Y)	418.19**				
F Row spacing (RS)	30.99**				
F Harvest method (HM)	105.51**				
F Y × RS	7.04**.				
F Y × HM	15.21**				
F RS × HM	4.15**				

*) Means indicated with the same letter in the same column are not significantly different according to the Duncan test at p≤.05

+) Means indicated with the same letter in the same row are not significantly different according to the Duncan test at p≤0.05

1) Means of year by row spacing indicated with the same letter are not significantly different according to the Duncan test at p≤0.05

2) Means of year by harvest method indicated with the same letter are not significantly different according to the Duncan test at p≤0.05

3) Means indicated with the same letter in the same column are not significantly different according to the Duncan test at p≤0.05

4) Means of row spacing by harvest method indicated with the same letter are not significantly different according to the Duncan test at p≤0.05

Table 4.Effect of row spacing and harvest methods on seed germination rate (%) in alfalfa.

Year	Row spacing (cm)	Harvest method					Year mean
		HM1	HM2	HM3	HM4	Mean	
2013	25	98.3	98.0	98.8	98.8	98.4	98.6 a*
	50	99.8	98.5	99.0	99.0	99.1	
	75	99.0	99.5	98.5	98.0	98.8	
	100	98.5	98.3	98.5	97.8	98.3	
	Mean	98.9	98.6	98.7	98.4		
2014	25	99.5	99.3	99.3	97.5	98.9	98.8 a
	50	100.0	99.0	98.0	98.5	98.9	
	75	99.5	98.5	99.0	99.0	99.0	
	100	99.5	98.5	97.5	98.3	98.4	
	Mean	99.6	98.8	98.4	98.3		
2015	25	97.5	97.5	98.3	97.3	97.6	97.9 b
	50	98.3	97.8	96.8	97.3	97.5	
	75	99.8	96.5	97.5	98.0	97.9	
	100	99.0	98.0	98.3	98.5	98.4	
	Mean	98.6	97.4	97.7	97.8		

Row spacing (cm)	Harvest method				
	HM1	HM2	HM3	HM4	Mean
25	98.4	98.3	98.8	97.8	98.3
50	99.3	98.4	97.9	98.3	98.5
75	99.4	98.2	98.3	98.3	98.6
100	99.0	98.3	98.1	98.2	98.4
Mean	99.0 a+	98.3b	98.3b	98.2 b	
CV (%)			1.53		
F Year (Y)			10.54**		
F Row spacing (RS)			N.S.		
F Harvest method (HM)			3.50*		
F Y × RS			N.S.		
F Y × HM			N.S.		
F RS × HM			N.S.		

*) Means with the same letter in the same column are not significantly different according to the Duncan test at p≤0.05
+) Means indicated with the same letter in the same row are not significantly different according to the Duncan test at p≤0.05

Seed yield: When the row spacing was increased from 25 to 100 cm, the seed yield was noticeably decreased (Table 3). However, this decrease was only statistically significant at 75 and 100 cm row spacing. Higher number of pods per plant and seeds per pod (Tables 1 and 2), as well as the higher number of plants per unit arearesulted in higher seed yield with 25 cm row spacing compared to other spacings. Although the number of pods per plant and the number of seeds per pod were lower with 50 cm row spacing, and the seed yield was higher due to the higher number of plants per unit area, than with 75 and 100 cm row spacing. Luo (2021) reported that lower plant density decreased seeed yield of alfalfa. This result supports our result. Compared to other harvest methods, significantly higher seed yield was obtained with HM2 whereas less seed yield was obtained with HM1. There was no considerable difference in seed yield between pre-harvest defoliant applications. Row spacing and harvest methods had different effects on seed yields depending on the year and each other, according to the statistical significance of the interactions between year, row spacing, and harvest method. In three-year averages, the highest seed yield was obtained in the plots with 25 cm row spacing and HM3. In addition, the seed yield obtained from the plots grown with 25 or 50 cm row spacings with HM2 were not statistically different to those obtained in the plots with 25 cm row spacing withHM3.

In the previous researches, the seed yield (kg ha^{-1}) of alfalfa was found 606-805(Rashidi *et al.*, 2009), 537-634 (Avci *et al.*, 2007), 265-311 (Bekovic *et al.*, 2008), 189-610.9 (Pajcin *et al.*, 2020). The mean values related to seed yield recorded in the present study (412.2 -828.5 kg ha^{-1}) were lower than those reported by Rashidi *et al.*, (2009), but similar to Kavut (2016), Avci *et al.*, (2017) and Jabessa & Bekele (2021). Variation among the yields may be due to differences in location, climatic conditions and management techniques among the researches.

Seed germination rates: The third year's average seed germination rate was much lower than it was in the first and second years (Table 4). The alfalfa plants grew physiologically enough in their second year to increase the germination rates, which were correlated with seed yield. Average germination rates obtained from different row spacings in 2013, 2014 and 2015 varied between 98.3 and 98.6%, but this was not statistically significant. The highest germination rate was obtained with HM1, and the lowest value with HM3 whereHM3 with the defoliant application slightly reduced the germination rate. Dawson (1992) also reported that seed quality of alfalfa was not affected by the pre-harvest application of glyphosate. Moyer *et al*., (1996) found that type of desiccant (diquat and glufosinate) had little and similar effects on seed germination, and the proportion of viable seed (93% or greater) was similar to the control. The current findings are consistent with previous reports that desiccants do not have a significant effect on germination (Rincker *et al*., 1988). From a detailed study conducted by May *et al*., (2003) pertaining to the effect of pre-harvest glyphosate, glufosinate and diquat application on seed yield, seed quality, seed germination and regrowth of alfalfa, it was concluded that desiccant applications do not affect negativelyon seed germination.

Conclusions

According to the study's findings, HM3 was combined with a 25 cm row spacing to produce the best seed output, while HM1 produced the lowest seed yield across all row spacings due to greater pod breakage during plant drying. Both pre-harvest defoliant applications slightly decreased the seed yield compared to the control treatment (HM2) in the third year, possibly due to failure of immature seeds to develop after defoliant spraying. Both defoliants effectively desiccated alfalfa for combine harvesting. It could also be opined that narrower row spacing (25-50 cm) with the direct harvesting (HM2) optionally with defoliant application (HM3) and with 25 cm row spacing) hold potential for boosting alfalfa seed production in the Cukurova region in abiologically viable manner.

Acknowledgments

The authors thank Ayman ELSABAGH for his contributions and improvement of paper.

Conflicts of Interest: The authors declare no conflict of interest

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(Received for publication 18 November 2021)