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Effect of different levels of nitrogen fertilizer and cultivars on quantitative and qualitative characteristics in canola forage (*Brassica napus* L.)

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ABSTRACT

In order to investigate the effect of Nitrogen on quantitative and qualitative characteristics of spring type of forage canola in summer cultivation, this experiment was conducted in Ghazvin at 2012. This experiment was done in split plot form and with a randomized complete block design with three replications. The main factor included five levels of Nitrogen (N) :(0, 75, 150, 225 and 300 kg\h). The sub factor included two varieties of spring type of Canola including RGS003 and SARIGOL. The impact of Nitrogen (p≤0/05) on final fresh forage and final dry forage was significant. The highest fresh forage yield was obtained from applying 300(kg/h) Nitrogen and RGS003 variety with the average of 46382.42(kg/h). The highest forage Glucosinolate content was obtained from using 300(kg/h) and SARIGOL variety with the average of 29.80(mg/g) where as the lowest forage Glucosinolate content was approached from non using of Nitrogen fertilizer and RGS003 variety with average of 12.20(mg/g). The highest protein percentage was obtained from using 300(kg/h) with average of 13.17(%). The RGS003 variety with average of 12.76(%) compared to SARIGOL variety with the average of 12.30(%) produced more protein .The highest fat percentage was obtained from using 150(kg/h) of Nitrogen with average of 2.56(%). Increasing or decreasing in Nitrogen application resulted to decreasing in forage oil percentage.

Key words: Canola, Nitrogen (N), Glucosinolate, protein

INTRODUCTION

Canola (*Brassica napus* L.) belonged to *Crucifer* a family has received remarkable attentions for forage production potential as well as oil and meal source. To the best of our knowledge, there are rare researches in literatureson forage canola in Iran, however, in recent years; it has been central focused research area. Canola forage has been widely cultivated and used since 600 years ago for feeding livestock (Fitzerald and Black, 1991), although its water demand is exorbitant as summer forage [1]. Average Canola forage yield in three harvesting dates ranged from 4350 to 5690 (kg/h). Harvesting at September gave 5540 (kg/h) forage yield, while at end of October, it was amounted to 7900 (kg/h) (Morison, 1960)[5]. In case of applying too much nitrogen fertilizer, it causes some health problems in livestock. U.Biljelli et al. (2007) while studying effect of two different harvesting dates in canola as forage found that the more close to ripening time, the more dry weight and the less total protein concentration will be [6]. Thanks to its cold hardness and higher tolerance during winter, canola can meets part of livestock forage demands during fall and winter when there is forage limitation. In addition to forage production during cold seasons, it can provide favorable forage in short term as the second rotation following rice and wheat harvested. Considering canola nutrients status has a great deal of importance to achieve high quality and quantity forage yield. The main objective for the present research is to shed light on the best nitrogen application treatment and subsequently to determine suitable cultivar for cultivation.

Canola contains 40-48% oil, 38-45% protein in the meal with 5% grain moisture. Linoleic to linolenic acids ratio in canola oil is known to be 2:1 which is normal for human diets purposes. Canola meals contain 13% fiber. Much fiber concentration present in meal serves as a limiting factor for feeding livestock, because it loses potential to release energy in ration.

Based on some estimation, sixty percent of arable lands encounter to mineral nutrients limitation (Gran et al., 2000). Application of wide verities of chemical fertilizers plays vital role in modern agriculture and fertilization is found to be one of the main determinant criteria for sustainable agriculture production[3]. Fertilization accounting for 30-50% total crop yields. Minerals have substantial role in plants resistance to cope with environmental biotic and abiotic stress (Marschener, 1995, Yangi and Smijed, 2005)[4]. Sanches (1997) stated that investigation of oil and protein percent in eight canola varieties in Brazil showed that oil and protein percent are 41.3, 36.8, 24.7 and 20.9 respectively and varieties difference significantly in terms of oil and protein percent. Adding nitrogen fertilizers, improves grain yield and protein percent, however, too much nitrogen levels may loses canola's oil percent, lowering oil percent, due to high nitrogen rate application, oil yield is enhanced in unit area to increase total yield. As results, it is worthy to note that lack of nitrogen (nitrogen deficiency) might impose adverse effect on plant quality (percent of protein) and crop yield.

MATERIALS AND METHODS

In order to evaluate effect of different nitrogen fertilizer applications on quantity and quality forage of two spring canola cultivars in summer cultivation, an experiment was conducted in Ghazvin province in agronomical year of 2012. Study area is located at 1285 m above sea level with annual average rainfall 310-320(mm), annual average temperature 13.9(C), minimum and maximum absolute annual temperatures of 17.4 and 37.8(C) respectively. Soil texture in study area is loam and silt loamy with pH 7.5-8.2 and its electrical conductivity found to be 0.8-1.5(ds/m) (table1). This experiment was arranged as split plot in completely randomized block designs in the 3 replication. Nitrogen was considered as the main factor involving five levels of 0, 75, 150, 225 and 300 (kg/h) from 46% urea. Fertilizer was applied in three phenology stages of 4-8 leaves emergence, early sheath growing and budding and the secondary factor involving two spring canola cultivars RGS003 and SARIGOL. Two spring canola cultivars RGS003 and SARIGOL were used in the present research. Seeds provided from department of oil seed researches, research center of seed and seedling breeding and preparation in Karaj (RGS003: German and spring type, SARIGOL: Iranian and spring type).

Table1. Analysis results of soil experiment.

Depth	0-30	30-60
EC(ds/m)	1	1.39
PH	8	7.9
SAR	3.75	4
T.N.V%	7.5	7.8
O.C%	0.74	0.5
Total N %	0.07	0.05
Texture	Loam	Loam

RESULTS AND DISCUSSION

Final yield (fresh forage): Variance analysis showed that nitrogen and nitrogen*cultivar interaction are significant on final fresh forage yield in probability levels of 1% and 5% respectively, however this was not for cultivar individually. Mean comparison of cultivar effect showed that RGS003 had the highest final fresh forage yield (with average 32520.27 kg/h) followed by SARIGOL (29850.03 kg/h).given that, nitrogen levels fell into different statistical classes. The highest yield (with average 45472.46 kg/ha) was obtained when 300 (kg/h) nitrogen treatment was applied, showing no significant difference to yield (with average 42880.29kg/h), obtained by adding 225 (kg) nitrogen in h^{-1}.The lowest yield (13697.92 kg/h) was obtained when no nitrogen application (control) was applied. Mean comparison analysis on nitrogen*cultivar interaction indicated the least final fresh forage yield (9020.83 kg/h) in cultivar SARIGOL and control treatments. Accordingly, RGS003 and 300 (kg/h) N showed the highest final fresh forage yield about 46382.42 (kg/h). RGS003 genotypes provided the highest final fresh forage yield (45104.17 and 46382.42 kg/h) when 225 and 300 (kg/h) nitrogen were applied, whereas, the highest final fresh forage yield (44562.50 kg/h) in SARIGOL once 300 (kg/h) nitrogen was considered showing no significance difference to 225 (kg/h) nitrogen application which gave 40656.42 (kg/h)(Tables 2, 3 4).

Final yield (dry forage): Variance analysis showed that nitrogen and nitrogen*cultivar interaction are significant on Final dry forage yieldin probability levels of 1% and 5% respectively, however this was not case for cultivar individually. Mean comparison analysis of cultivar effect showed that RGS003 had the highest final dry forage yield

(with average 5423.23kg/h) followed by SARIGOL with average 4747.27(kg/h). Given that, nitrogen levels fell into different statistical classes. The highest yields (7606.59 and 6460.23 kg/ha) were obtained when 225 and 300 (kg/h) nitrogen treatments were applied. The lowest yield (2618.66 kg/h) was obtained when no nitrogen application (control) was applied. Mean comparison analysis on nitrogen*cultivar interaction indicated, the highest final dry forage yield in cultivar RGS003 and with use 225(kg/h) nitrogen. Accordingly, SARIGOL and no nitrogen application (control) showed the least final dry forage yield about 1633.80(kg/h). RGS003 exhibited provided the highest final dry forage yield with average 8132.86(kg/h) when use 225(kg/h) nitrogen, showing no significant difference to 300 (kg/h) nitrogen (69008.54 kg/h) whereas, the highest final dry forage yield (7082.32) in SARIGOL once 225 (kg/h) nitrogen was considered showing no significance difference to 300 (kg/h) nitrogen application which yielded 40656.42 (kg/h) (Tables 2, 3 4).

Table2. Variance analysis of dry weight and fresh weight

SOV	df	Final Dry weight in25% flowering(kg/h)	Final Fresh weight in25% flowering(kg/h)
Replication	2	47869861.106 ^{ns}	1523956.694 ^{ns}
Nitrogen(N)	4	1025691832.420 **	23173388.283 **
error	8	47995088.249	1328796.687
Cultivars (V)	1	53476095.408 ^{ns}	3366266.819 ^{ns}
N* V	4	94551257.539 *	3672825.732 *
error	10	26527761.050	790934.315
Total	29		
CV%		16.52	17.50

**, ** and ^{ns}: significantat5%, 1% probability levels, and Non-significant.*

Table 3. Mean comparison of effects nitrogen and cultivars on dry and fresh weight

	Final Dry weight	Final Fresh weight
0	13697.92 c	2618.66 c
75	27645.83 b	4472.66 b
150	26229.25 b	4268.11 b
225	42880.29 a	7606.59 a
300	45472.46 a	6460.23 a
RGS003	32520.27 a	5423.23 a
SARIGOL	29850.03 b	4747.27 b

Means in each column having similar letter (s), are not significantly at the 5% level.

Table4. Mean comparison of nitrogen * cultivars interaction on dry and fresh weight

Nitrogen	Cultivar	Dry weight	Fresh weight
0	RGS003	18375.00 e	3603.53 d
0	SARIGOL	9020.83 f	1633.80 e
75	**RGS003**	22406.25 de	3456.20 d
75	**SARIGOL**	32885.42 bc	5489.11 bc
150	RGS003	30333.50 cd	5014.99 cd
150	SARIGOL	22125.00 de	3521.23 d
225	**RGS003**	45104.17 a	8132.86 a
225	**SARIGOL**	40656.42 ab	7080.32 ab
300	RGS003	46382.42 a	6908.54 ab
300	SARIGOL	44562.50 a	6011.91 bc

Means in each column having similar letter (s), are not significantly at the 5% level.

Glucosinolate content: Results of variance analysis revealed that Glucosinolate content in forage was significantly, nitrogen and nitrogen*cultivar interaction was significant in probability levels of 1% and 5% respectively. Analysis of mean comparison on cultivar effect showed that SARIGOL had the highest forages Glucosinolate (22.76 mg/g DW) followed by RGS003 (19.92 mg/g DW).Different nitrogen levels were classified in various statistical classes. The highest Glucosinolate content (28.95 mg/g DW) was obtained when 300 (kg/h) N was applied, while the lowest Glucosinolate content (13.50 mg/g DW) was related to no nitrogen application (control treatment). Mean comparison of nitrogen*cultivar interaction indicated nitrogen and cultivars treatments into separate statistical classes so that SARIGOL showed the highest Glucosinolate content (29.8 mg/g DW) when 300(kg) nitrogen was applied. The least Glucosinolate content (12.20mg/g) was obtained in RGS003 once no nitrogen application (control) was used. Taken together, the highest Glucosinolate content in RGS003 and SARIGOL were obtained (28.10 and 29.8 mg/g DW), when 300 (kg/h) nitrogen were used (Table 5, 6, 7).

Fiber percentage: Results of variance analysis revealed that fiber in forage was significantly by nitrogen and cultivar individually in probability levels of 1% and but it was not true for nitrogen*cultivar interaction though. Analysis of mean comparison on cultivar effect showed that RGS003 had the much more forage fiber (35.61%) and then SARIGOL (32.79%). Different nitrogen levels were classified in various statistical classes. The lowest forage

fiber were related to control and 300 (kg/h)a nitrogen treatments (31.86 and 32.05% respectively), while the highest fiber with average 38.04(%) was obtained when 150 (kg/h) nitrogen was applied. mean comparison of nitrogen*cultivar interaction revealed the least fiber in SARIGOL when no nitrogen application and 300 (kg/h) N were applied so that 30.97 and 30.98% fiber were obtained respectively. The highest forage fiber was achieved in RGS003 once nitrogen150 (kg/h) was used with 39.97(%). Under the same 150 (kg/h) nitrogen treatment, RGS003 and SARIGOL showed the highest fiber percent with 39.97 and 36.12 respectively (Tables 5, 6, 7).

Protein percentage: Variance analysis indicated that nitrogen and cultivar individually in probability levels of 1% and but it was not significant for nitrogen*cultivar interaction although. Analysis of mean comparison on cultivar effect showed that RGS003 had the much more protein percent with 12.76 and then SARIGOL (12.30%). Different nitrogen levels were classified in various statistical classes. The lowest protein percent was related to control (11.14%). Applying nitrogen 150, 225 and 300 (kg/h) resulted in 13.02, 13.11 and 13.15% protein, which all fell into the same statistical class with no significant difference. Mean comparison of nitrogen*cultivar interaction revealed the least protein with average 11.05(%) in SARIGOL when no nitrogen application was applied. The highest proteinpercent wereachieved in RGS003 once 225 and 300(kg/h) nitrogen were used respectively with averages 13.42 and 13.43(%) (Tables5, 6, 7).

Table5. Variance analysis of Glucosinolate Content, Forage fiber and Forage protein

SOV	df	Glucosinolate content(mg/g)	Forage fiber (%)	Forage protein (%)
Replication	2	8.829 ^{ns}	0.807 ^{ns}	0.022 ^{ns}
Nitrogen(N)	4	248.163 **	38.025 **	4.544 **
error	8	1.930	0.815 **	0.053
Cultivars (V)	1	60.461 **	62.699 **	1.582 **
N* V	4	1.497 *	1.187 ^{ns}	0.040 **
error	10	2.142	0.533	0.046
Total	29			
CV%		6.86	2.14	1.71

**, ** and ^{ns}: significantat5%, 1% probability levels, and Non-significant.*

Table6. Mean comparison of nitrogen and cultivars on Glucosinolate Content, Forage fiber and Forage protein

	Glucosinolate	Forage fiber	Forage protein
0	13.50 e	31.86 c	11.14 c
75	17.45 d	34.86 b	12.21 b
150	20.05 c	38.04 a	13.02 a
225	26.75 b	34.00 b	13.11 a
300	28.95 a	32.05 c	13.17 a
RGS003	19.92 b	35.61 a	12.76 a
SARIGOL	22.76 a	32.72 b	12.30 b

Means in each column having similar letter (s), are not significantly at the 5% level.

Table7. Mean comparison of nitrogen × cultivars interaction on Glucosinolate Content, Forage fiber and Forage protein

Nitrogen	Cultivar	Glucosinolate	Forage fiber	Forage protein
0	**RGS003**	12.20 f	32.75 c	11.23 f
0	**SARIGOL**	14.80 ef	30.97 d	11.05 f
75	RGS003	15.30 e	36.48 b	12.46 d
75	SARIGOL	19.60 cd	33.25 c	11.96 e
150	**RGS003**	18.40 d	39.97 a	13.27 ab
150	**SARIGOL**	21.70 c	36.12 b	12.78 cd
225	RGS003	25.60 b	35.73 b	13.42 a
225	SARIGOL	27.90 ab	32.27 cd	12.81 cd
300	**RGS003**	28.10 ab	33.12 c	13.43 a
300	**SARIGOL**	29.80 a	30.98 d	12.91 bc

Means in each column having similar letter (s), are not significantly at the 5% level.

Fat percent: Results of variance analysis revealed that fat percent in forage was affected by nitrogen and cultivar individually in probability levels of 1% and but it was not significantly for nitrogen*cultivar interaction although. Analysis of mean comparison on cultivar effect showed that RGS003 had the less fat percent (1.71) in Comparison to SARIGOL (2.05%). mean comparison of nitrogen*cultivar interaction revealed the highest fat percent (2.98%) in RGS003 when 150(kg/h) nitrogen was applied. The lowest fat percent was achieved in SARIGOL once no nitrogen (control) was applied with average1.42(%). under the same 150 (kg/h) nitrogen treatment, RGS003 and SARIGOL showed the highest fat percent 2.89 and 2.15% respectively(Table 8, 9, 10).

Protein yield: Protein yield is calculated through multiplying forage yield by proteins percent, since forages raw protein serves as one of the most important criteria widely used to evaluate forage quality. Variance analysis showed

that nitrogen, cultivar and nitrogen*cultivar interaction were significant at protein yield in probability levels of 1 and 5% respectively. Mean comparison on cultivar effect showed that RGS003 had much protein yield (703.76kg/h) than SARIGOL (593.89 kg/h). Different nitrogen levels were classified in various statistical classes. The lowest protein yield was related to control treatment (292.5 kg/h). Applying 225 and 300(kg) nitrogen in h^{-1}, resulted in 999.8 and 851.1 (kg/h) protein yieldrespectively, categorized into the same statistical class. Mean comparison of nitrogen*cultivar interaction revealed the leastprotein yield (180.5 kg/h) in SARIGOL when control treatment was applied. The highest protein yield was achieved in RGS003 once 225 (kg/h) nitrogen was applied with average 1092.00(kg/h), there are no significant differences to SARIGOL and RGS003 cultivars when 225 and 300(kg/h) N were applied respectively. RGS003 showed the highest protein yield (1092.00 kg/h) when 225 (kg/h) nitrogen was applied. Under the 225 (kg/h) nitrogen treatment, SARIGOL showed the highest fat percent (907.5) showing no significant difference to when 300 (kg/h) which produced 776.5 (kg/h) (Table 8, 9, 10).

Fat yield: Analysis of variance clearly showed that fat yield was affected by nitrogen and cultivar at probability level of 1% but this was not case for nitrogen*cultivar interaction. Mean comparison analysis of cultivar effect showed that RGS003 had higher fat yield (110.27 kg/h) than SARIGOL (76.91 kg/h). Different nitrogen levels were classified in different statistical classes such that the highest fat yield (151.8 kg/h) was obtained when 225 kg/h nitrogen was applied. Similarly, the least fat yield (39.02 kg/h) obtained when no nitrogen (control) was applied. Mean comparison analysis conducted on nitrogen*cultivar interaction showed that SARIGOL genotypes gave the least fat yield with average 22.94(kg/h). The highest yield was in RGS003 (175.4 kg/h) when 225 (kg/h) nitrogen was applied. Both RGS003 and SARIGOL showed the highest fat yields (175.4 and 128.1 kg/h) when the same treatments of 225 (kg/h) nitrogen were applied respectively (Table 8, 9, 10).

Table8. Variance analysis of Forage fat, Protein yield and Oil yield

SOV	df	Forage fat (%)	Protein yield(kg/h)	Fat yield(kg/h)
Replication	2	0.004 ns	22012.698 ns	649.036 ns
Nitrogen(N)	4	1.079 **	465717.639 **	11074.760 **
error	8	0.025	21144.432	565.004
Cultivars (V)	1	0.850 **	90544.420 *	8345.338 **
N* V	4	0.060 ns	53027.694 *	645.807 *
error	10	0.035	12543.745	400.944
Total	29			
CV%		9.90	17.26	21.40

**, ** and ^{ns}: significantat5%, 1% probability levels, and Non-significant.*

Table9. Mean comparison of nitrogen and cultivars on Forage fat, Protein yield and fat yield

	Forage fat	Protein yield	Fat yield
0	1.48 d	292.5 c	39.02 c
75	1.76 c	543.4 b	79.19 b
150	2.56 a	557.3 b	105.80 b
225	1.99 b	999.8 a	151.80 a
300	1.62 cd	851.1 a	104.90 b
RGS003	2.05 a	703.8 a	110.27 a
SARIGOL	1.71 b	593.9 b	76.91 b

Means in each column having similar letter (s), are not significantly at the 5% level.

Table10. Mean comparison of nitrogen × cultivars interaction on Forage oil, Protein yield and Oil yield

Nitrogen	Cultivar	Forage fat	Protein yield	Fat yield
0	RGS003	1.53 ef	404.6 d	55.1 ef
0	SARIGOL	1.42 f	180.5 e	22.9 f
75	**RGS003**	1.92 bcd	432.3 d	66.4 e
75	**SARIGOL**	1.63 def	654.6 c	91.9 cde
150	RGS003	2.89 a	664.3 c	132.8 b
150	SARIGOL	2.23 b	450.3 d	78.9 e
225	**RGS003**	2.15 bc	1092.0 a	175.4 a
225	**SARIGOL**	1.82 cde	907.5 ab	128.1 bc
300	RGS003	1.76 def	925.6 ab	21.5 bcd
300	SARIGOL	1.47 ef	776.5 bc	88.4 de

Means in each column having similar letter (s), are not significantly at the 5% level.

It can be concluded that the highest dry and fresh forage yields were obtained when 225 (kg/h) was applied and at the same time, adding nitrogen up to 300 (kg/h), made no significant difference in yield. Adding nitrogen about 150 (kg/h) made no significant difference in protein percent. The highest fiber percentage in forage obtained when 150 (kg/h) nitrogen was applied. The highest fat percent was achieved when 150 (kg/h) nitrogen was applied followed by 225 (kg/h) nitrogen. Applying 225 (kg/h) nitrogen resulted in the highest fat yield and forage yield. Affected by

increased nitrogen application to large extent, Glucosinolate content increased substantially so that the highest Glucosinolate content was recorded when 300 (kg/h) nitrogen treatment was applied. Cultivar RGS003 was proved superior to SARIGOL in terms of dry forage yield, fresh forage yield, fiber percent, protein percent, fat percent and Glucosinolate content.

Effect of nitrogen and nitrogen*cultivar interaction found to be significant on final dry and fresh forage yields.Increased level of nitrogen led to higher dry and fresh forage yields, while, adding nitrogen up to 300 (kg/h) made no significant difference on yield. Mean while, RGS003 found to be superior to SARIGOL. Glucosinolate content in forage was affected by nitrogen and cultivar as well as nitrogen*cultivar interaction. Glucosinolate content was improved under nitrogen application so that the highest Glucosinolate content was observed in SARIGOL when, 300 (kg/h) nitrogen was applied. Nitrogen and cultivar showed significant effect on fiber percent individually. Adding nitrogen about 150 (kg/h), increased fiber percent remarkably, but adding it above this threshold resulted in low fiber percent. Comparison of SARIGOL with RGS003 produced higher percent of fiber. Forage protein percent was affected by nitrogen and cultivar significantly. However, there was no significant difference in protein percent when 150,225 and 300 (kg/h) nitrogen were applied.RGS003 had superiority to SARIGOL in protein percent. Fat yield was affected by nitrogen and cultivar significantly. Adding nitrogen up to 150 (kg/h), led to increased fat percent, RGS003 was superior to SARIGOL in this respect. Results obtained in the present research showed that under Ghazvin provinces climate condition, clearly demonstrated that cultivar RGS003 is superior than SARIGOL in respect to qualitative and quantitative traits. So it is recommended that RGS003 be exploited in farming programs. The best and most promising nitrogen rate to achieve maximum fresh and dry forage yield, dry forage, fat and protein yield is 225 (kg/h).

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