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Seed Yield and Oil Compositions of Flax (*Linum usitatissimum* L.) Plant as Affected by Sowing Date and Nitrogen

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Abstract: To study the effect of sowing date and nitrogen application on seed yield and fatty acids of flaxseed, an experiment was conducted using split plot on the basis of randomized complete block design with three replications in Experimental Field of Islamic Azad University of Yasooj during 2006-2008. Main plots were consisted of five sowing dates (Mar. 5, Mar. 20, Apr. 3, Apr. 18 and May 4) and sub plots including nitrogen fertilizer at rates of 0, 50, 100 and 150 kg ha^{-1}. The results of two-year analysis of data indicated that the highest kernel weight obtained by Mar. 5 and 20 and 150 kg N ha $^{-1}$ and the lowest value were belonged to May 4 and 0 kg N ha $^{-1}$. Mar. 5 and 100 kg N ha $^{-1}$ was the best combination to produce the highest harvest index. Among the different sowing dates, Mar. 5 produced the highest grain yield at either N level. Grain yield after Mar.5 was reduced by 8 to 130 %. Sowing date × N interaction was also significant in this trait. Oil content ranged from 24.4 to 32. 6% and was average 30%. Delay in sowing date after Mar. 5 decreased oil seed about 33% at May.4. Increasing in nitrogen fertilizer decreased oil seed content. Apr. 18 + 150 kg N ha^{-1} was the best combinations for grain protein percent. The unsaturated fatty acid contents varied from 14.6 to 52.6 %. The best combination in order to obtain the highest linolenic acid and oleic acid was Mar. 5+150 kg N ha^{-1} and Mar. 5+0 kg N ha^{-1}, respectively. In general, results of this study demonstrate that Mar.5 sowing date was the best date for increasing all traits except grain protein percent. 100 or 150 kg N ha^{-1} also increased all traits except oil percent and oleic acid of linseed.

Key words: Flax · Seed yield · Oil yield · Fatty acids · Grain protein

INTRODUCTION

Linseed (*Linum usitatissimum L*), also called flaxseed, has got significance due to its nutrients and pharmaceutical uses. It is used for edible and lightening purposes in some Asian countries and can also be used as a substitute part of animal fat in poultry diets [1].

False flax is a member of the plant family *Brassicaceae*,which it relates to rape and mustard, This plant is one of the oldest oil crops in the temperate regions. It is native in south and south-west Asian countries. In the late 1930 some varieties of the genus *Camelina* had been grown on poor sandy soil in most parts of European countries, namely: Russia, Poland, Netherlands, Belgium and etc. mainly to replace winter-killed rape [2]

Flax is now unknown in the wild but originally it may have been a native of Asia. It has been cultivated since at least 5000 BC, probably first by the ancient Mesopotamians and later by the Egyptians who rapped their mummies in linen cloth. The Romans spread flax cultivation to Northern Europe and now the plant is grown all over the world for the oil extracted from the seeds and for its fibers, which are made into linen and other cloths. Various parts of the plant have been used to make fabric, dye, paper, medicines, fishing nets, hair gels and soap. It is also grown as an ornamental plant in gardens [2].

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Seeds are widely used medicinally. Their contains about 36-48% oil content which is high in unsaturated fatty acids, with esters of linoleic acid, linolenic acid, stearic acid and oleic acid; also mucilage, proteins, a cyanogenic glycoside (linamarin) and enzymes [3, 4 and 5]. Crushed seeds mixed to a paste with water are used to make hot poultices to relieve pain and to heal septic wounds, skin rashes and ulcers. The extracted oil is used in the pharmaceutical industry to make liniments for burns and rheumatic pain. The oil is also important in the manufacture of paints, soap and printer's ink. Inclusion of linseed in the diet results in increased polyunsaturated fatty acids [6, 7], which protect the body against cancer [8], reduce the chances of cardiovascular diseases and certain other health related problems [9]. So, linseed may play a vital role in reducing these health risk problems due to its nutritive value which depends upon different factors like cultivar, locality, planting date, fertilizers and year of production [10].

In Iran, flax is cultivated as a dual purpose (seeds for oil and stems for fiber). The cultivated area through the last 20 years was decreased due to the great competition of other economic winter crops resulting in a gap between production and consumption. Therefore, it is necessary to increase flax productivity per unit area which could be achieved by using high yielding cultivars and improving the agricultural treatments [11,12]. One of these treatments is planting date which is important factor for vigorous growth and consequently higher yield of different plant species [11,13,14].

Sow flax early enough to allow the plants to become well established before freezing temperatures occur. After plants have branched at the crown, cold-hardy varieties can withstand much lower temperatures without serious injury. Avoid planting flax too early in the fall or late spring frosts may damage the crop at the bloom stage. In South Texas, when moisture conditions permit, plant between November 10 and December 10. In this area, flax seeded after January 1 usually produced lower yields than December seeding. If moisture is available, October 15 to October 30 is an ideal time for planting flax in the temperate area.

In western Canada, flax (*Linum usitatissimum* L.) is often sown in early May when soils are usually cool and moist [15]. This practice has been adopted so that the crop flowers early to escape the hot, dry conditions generally experienced in mid to late July. Cool temperatures combined with a long photoperiod during and after flowering increases both seed yield and the production of linolenic acid. Unfortunately, seeding flax into cool wet soils can delay germination and result in a poor stand due to the invasion of seeds by soil fungi. Plants emerging under these conditions have reduced vigour and yield potential. Also, if emergence is delayed due to low soil temperatures and/or deep seeding, soil incorporated herbicide residues such as trifluralin or triasulfuron from a previous crop can injure and reduce the emerging seedlings [15]. The importance of early stand establishment has been documented in several studies. Ford [16] demonstrated that the number of seeds per capsule as well as the number of capsules per plant was greatly reduced if seeding was delayed. A delay in seeding also resulted in a reduction in oil content, iodine value and linolenic acid [17]. Sosulski and Gore [18] reported that oil content, iodine number and linolenic acid increased under long photoperiods whereas short photoperiods resulted in delayed flowering and reduction in both oil and fatty acid content.

Responses to fertilizer applications depend upon moisture conditions, previous crop and fertility status of the soil. Experimental data generally show fertilizer applications to be profitable under good management and favorable moisture. When large amounts of low nitrogen residue, such as grain sorghum stubble, are returned to the soil before planting flax, apply 3 pounds of nitrogen per expected bushel of yield. A strong interaction of S and N for seed yield was found in rapeseed and mustard [19-25], sunflower [26] linseed [27] Groundnut [28,29] and Soybean [30,31].

Due to the environmental characteristics of Iran, oil flax crop is more adaptable to fiber one. High temperatures and intense radiation in fact favor seed production and oil accumulation. Moreover, at high temperatures the oil is richer in saturated glycerides while at low temperatures and less intense radiation the plants tend to remain in the vegetative phase yielding more fiber of better quality. The average crop yields about 1 t ha-1, but experimental trials proved that the best varieties can produce up to 3 t ha^{-1} of seed with 40% of oil content and 20% of proteins. The objectives of this study were to determine the optimum nitrogen fertilizer and planting date on various plant and seed characteristics for a new flax cultivar.

MATERIALS AND METHODS

The studies were conducted at Agricultural Researches Station of Islamic Azad University of Yaouj, 51°, 41′ E, 30°, 50′N, 1832 m above sea level) during 2006-2008. The experiment was complete randomized block, split plot design with three replications. Main plots, sowing date, were Mar. 5, Mar. 20, Apr. 3, Apr. 18 and May. 4. Sub plots, fertilizer treatment, were N at rates of 0, 50,100 and

Table 1: Soil physical and chemical characteristics of the experimental site

	Year	
Variable	2006-2007	2007-2008
Texture	Si-C	S-C-L
PH	7.5	7.85
EC dS m^{-1}	0.9	1.0
Organic matter (%)	0.92	0.69
N(%)	0.088	0.076
P mg Kg^{-1}	13.4	16
K mg Kg^{-1}	340	206
Fe	3.7	5.4
Zn	0.64	0.1
Mn	5.8	9.9
Cu	0.48	0.98

150 kg ha^{-1}. The cultivar used in this experiment was Olay ozon. To determine the soil characteristics 15 samples from 30 cm depth were collected and analyzed by Shiraz Soil Testing Laboratory for basic soil physical and chemical properties (Table 1). P and K fertilizer were applied according to recommendations of Soil Testing Laboratory of Shiraz Agricultural Researches Station in forms of superphosphates and potassium sulfate, respectively. Plots were sown with a cone seeder and were 6 m long and 2.4 m wide, with 12 rows 0.2 m apart. Plant density was 570000 plants ha^{-1}. Plots were plowed and disked before planting in July. The plots were disked again before seeding in February. Irrigation of each main plot was measured volumetrically by field calibrated gypsum block. The Ψsoil at 30-cm depth was kept -0.025 MPa to maturity. The irrigation system was operated so that runoff did not occur. At maturity, 10 plants were taken randomly from each subplot for recording the following morphological, yield components and yield.

The various parameters within the linseed plant that are discussed in this paper were evaluated as fallows:

- Specific seed weight (average weight of 1000 seeds in grams); recorded on 10 random samples from each sub-plot.
- Seed yield. Center eight rows (of 12 rows) of each plot were harvested for grain yield and converted to grain yield per hectares.
- The oil concentration of a sample of whole seeds from each plot was determined by Near-Infrared Reflectance Spectroscopy as described by Bhatty [32].
- Oil yield (kg/ha); calculated by multiplying seed oil percentage ×seed yield per ha.
- Grain protein percent was estimated by Kjeldhal method and crude protein (CP) was calculated by multiplying nitrogen (%) with factor 6.25 [33].

Samples were dried in a forced-air oven at 70° C for 48 h.

After determination of the oil yield (in the dry matter), fatty acids were esterified as methyl esters [33] and analyzed by Agilent 6890 N GC equipped with a DB-23 capillary column (60 m x 0.25 µm) and a FID (Flame Ionization Detector) detector. The carrier gas was helium, at a flow rate of 1.2 mL/min. Both injector and detector temperatures were kept at 250°C. Column temperature was initially kept at 165°C for 15 min and then increased to 200°C at a rate of 5°C/min, where it was maintained for 15 min. Samples of 1 µl were injected by auto-sampler, in the split mode (1:50). The fatty acid identification was performed by retention time comparisons with corresponding fatty acid methyl ester standards. The standards were purchased from Sigma-Aldrich Ltd.

Data were analyzed by analysis of variance [34].When significant differences were found ($P=0.05$) among means, Duncan's multiple range test (DMRT) were applied.

RESULTS AND DISCUSSION

Thousand-kernel Weight: Year and sowing dates had significant effect on kernel weight in 1 % probability level (Table 2). The highest value of kernel weight was obtained in Mar. 5 and 20 and the lowest value were belonged to May.4. At later sowing date, because of confronting the grain filling period to high temperature at the end of growing season kernel weight severely decreased (Table 3). Significant sowing date × nitrogen fertilizer interactions were detected at $P \geq 0.01$ in this trait (Table 4). Dubey [36] concluded that delay in planting date caused the decrease in 1000-kernel weight, oil rate and grain yield.

Harvest Index: There was significant difference between year and sowing dates treatments in this trait (Table 2). Mar.5 sowing date was more successful than other treatments to transport of assimilate from sources to plant sinks (Table 3). Significant sowing date × year and sowing date × nitrogen fertilizer interactions were detected at $P \geq 0.05$ on harvest index. Mar. 5 and 100 kg N ha^{-1} produced the highest value.

Table 2: Results of analysis of variance combined across years, sowing date and N fertilizer to both years.

SOV	df	1000-Kernel weight (g)	Grain yield Kg ha^{-1}	Harvest index (%)	Oil percent	Oil yield Kg ha^{-1}	Grain protein (%)	Linolenic acid (%)	Linoleic acid (%)	Oleic acid (%)
Year (Y)	1	**	**	*	NS	**	NS	NS	NS	**
Sowing Date (SD)	4	**	**	**	**	**	**	**	NS	NS
Y×S	4	NS	NS	*	NS	NS	NS	NS	NS	NS
Nitrogen(N)	3	NS	**	NS	**	**	**	**	**	**
Y×N	3	NS	NS	NS	**	NS	NS	NS	**	*
S×N	12	*	**	*	*	**	**	**	NS	*
Y×SD×N	12	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV, %		7.5	10.7	12.5	9.35	13.5	9.3	13.1	14.8	11.5

* , ** Significant at 0.05 and 0.01 probability levels, respectively. NS=nonsignificant at P> 0.05.

Table 3: Mean values of traits of linseed cultivar at five sowing date and four N fertilizers to both years.

Treatments	1000-Kernel weight(g)	Grain yieldKg ha^{-1}	Harvest index(%)	Oil percent	OilyieldKg ha^{-1}	Grain protein (%)
Sowing date						
Mar. 5	6.1 A	1371 A	41 A	32.6 A	431 A	22.2 C
Mar. 20	5.9 A	1277 B	36 B	32.2 A	403 B	22.1 C
Apr. 3	4.7 B	1134 C	27 C	29.8 B	317 C	23.7 B
Apr.18	3.9 C	706 D	23 D	26.7 BC	187 D	25.6 A
May. 4	3.0 D	603 E	19 E	24.4 C	141 E	25.4 A
Nitrogen(N) Kg ha^{-1}						
0	4.7 A	459 C	27 A	30.5 A	151 C	19.9 D
50	4.7 A	584 B	28 A	30.5 A	181 B	22.7 C
100	4.7 A	1518 A	29 A	27.7 B	426 A	24.7 B
150	4.8 A	1511 A	29 A	26.8 B	425 A	28.1 A

Table 4: Interaction between sowing date and N fertilizers on mean values traits in both years.

Treatments		1000-Kernel weight (g)	Grain yield Kg ha^{-1}	Harvest Index (%)	Oil percent	Oil yield Kg ha^{-1}	Grain protein (%)
Sowing date	Nitrogen Kg ha^{-1}						
Mar. 5	0	6.2 A	625 EFG	40 A	35 A	625 EFG	113 GHI
	50	6.1 A	736 E	41 A	35 A	736 E	156 G
	100	6.1 A	2087 A	41 A	32 ABCD	2087 A	460 BC
	150	6.1 A	2039 A	41 A	29 DEFG	2039 A	551 A
Mar. 20	0	6.0 A	605 FG	36 B	34 AB	605 FG	108 GHI
	50	6.0 A	734 E	36 B	34 ABC	734 E	158 G
	100	6.0 A	1901 B	36 B	31 BCDE	1901 B	435 C
	150	6.0 A	1867 B	37 B	29 DEFG	1867 B	507 AB
Apr. 3	0	4.8 B	520 GH	27 C	30 CDEF	520 GH	104 HI
	50	4.7 B	675 EF	27 C	31 BCDE	675 EF	150 GH
	100	4.7 B	1646 C	27 C	28 EFGD	1646 C	388 D
	150	4.7 B	1696 C	27 C	27 FGHI	1696 C	492 B
Apr. 18	0	4.0 C	198 I	23 D	27 EFGHF	198 I	75 IJ
	50	4.0 C	464 H	23 D	28 DEFG	464 H	111 GHI
	100	3.9 C	1001 D	23 D	26 GHID	1001 D	256 F
	150	4.1 C	1008 D	23 D	26 GHI	1008 D	311 E
May. 4	0	2.9 D	198 J	19 E	26 GHI	198 J	43 J
	50	3.0 D	314 I	19 E	25 GHIF	314 I	76 IJ
	100	3.1 D	954 D	19 E	23 I	954 D	237 F
	150	3.2 D	946 D	19 E	24 HI	946 D	274 EF

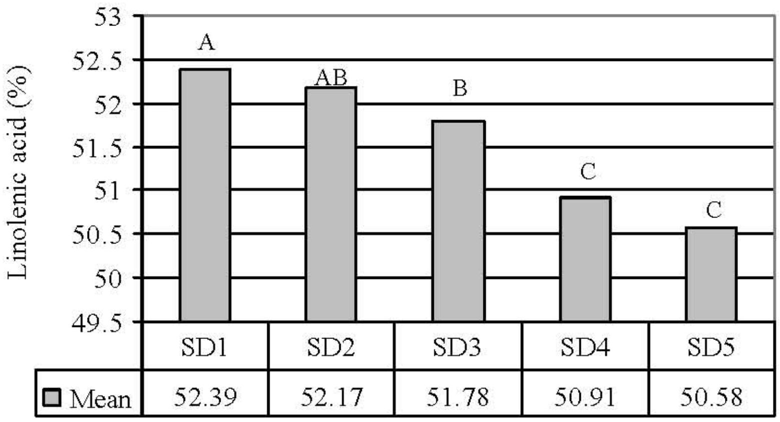

Fig. 1: Effect of sowing date on linolenic acid

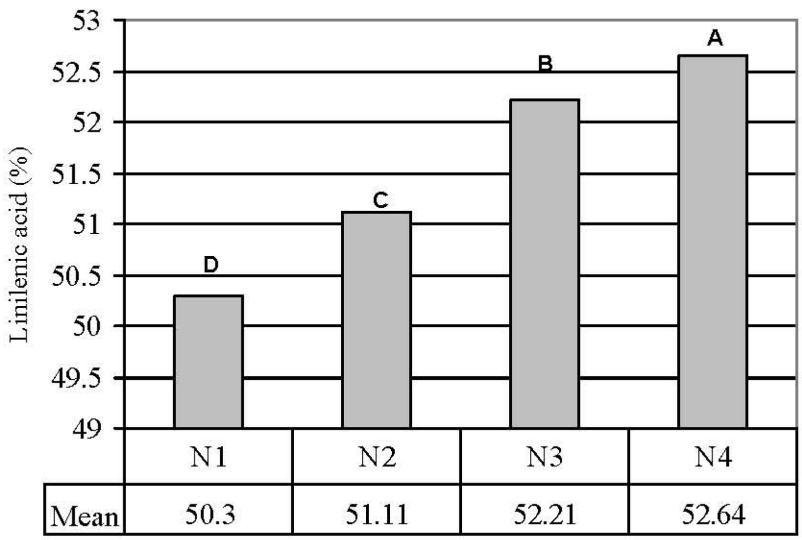

Fig. 2: Effect of nitrogen fertilizer on linolenic acid

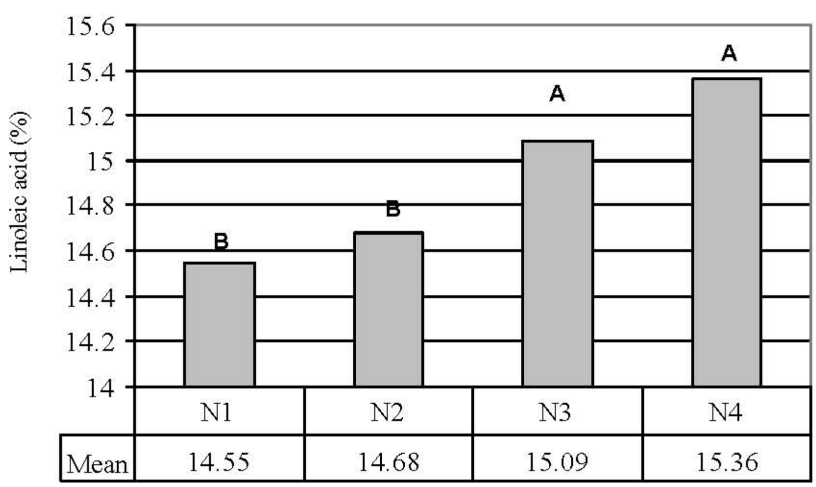

Fig. 3: Effect of nitrogen fertilizer on linoleic acid

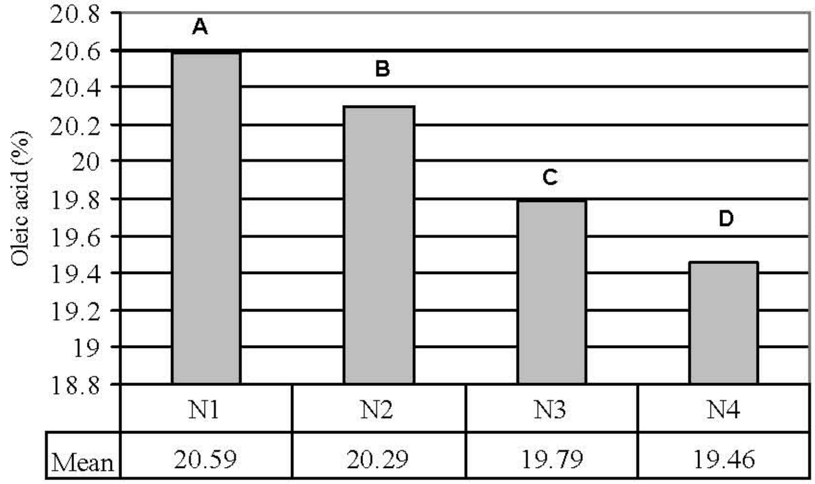

Fig. 4: Effect of nitrogen fertilizer on oleic acid

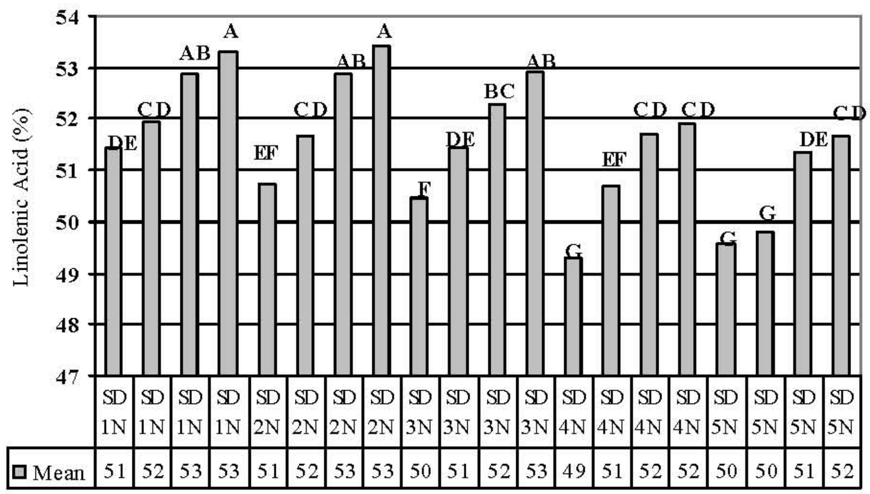

Fig. 5: Interaction effects between sowing date and nitrogen fertilizer on linolenic acid

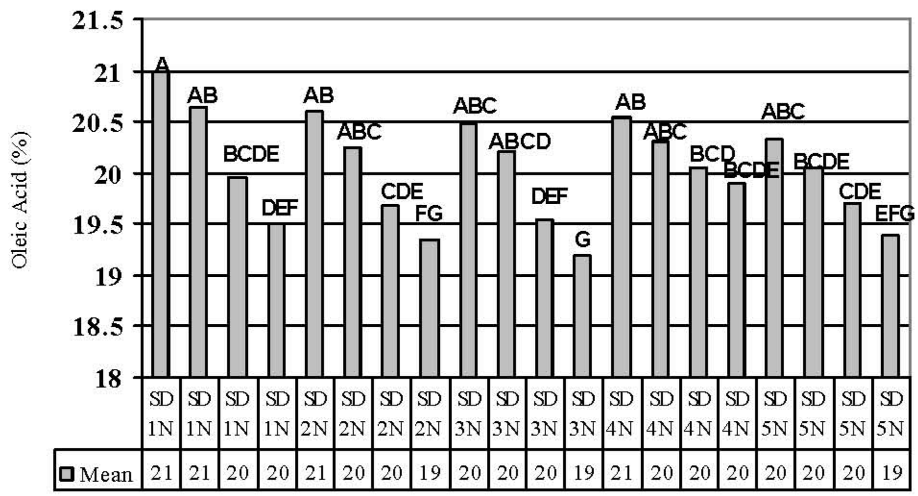

Fig. 6: Interaction effects between sowing date and nitrogen fertilizer on oleic acid

Grain Yield: Grain yield after Mar.5 was reduced by 8 to 130 % (Table 3). Grain yield increased in response to applied N, with the grain yield of the crop that no received N being three fold than for that receiving 150 kg N ha^{-1} (Table 3). However, increasing in grain yield beyond 100 kg N ha^{-1} was marginal. This dose not implies that there was no response to the application of higher rates of N at different sowing dates. Among the five sowing dates, Mar. 5 produced the highest grain yield at either N level (Table 4) and the doubling of N fertilization increased grain yield by 1351 kg ha^{-1}. A hundred kilograms of N ha^{-1} was adequate for maximum grain yields with Mar.5 sowing date. The N response data may be used in determining N fertilizer rates for specific yield goals. Sowing date × N interaction was only significant in this trait. High temperatures during the period of reproductive growth lead to decline of reproductive growth period, failure in the number of crop and finally decrease the number of seed and then the decline comes to grain yield. Therefore, in most studies the grain yield decreases with the delay in planting [38].

Oil Percent and Oil Yield: Sowing date, nitrogen fertilizer and interaction between them were significant in oil percent and oil yield (Table 2). Mar. 5 produced the highest oil seed and oil seed yield. Delay in sowing date after Mar. 5 decreased oil seed about 33% at May. 4 (Table 3). Increasing in nitrogen fertilizer decreased oil seed content (Table 3). However, oil seed yield increased with applying in nitrogen fertilizer, due to increase in grain yield. Oil content ranged 24.4 to 32. 6% and was average 30% in the approximately 50% of the material used. Generally, linseed contains about 40% oil in the seed [39]. Oil content of linseed recorded in this study was lower compared to those reported by Hume [40] and Kouba [4].

Grain Protein Percent: Both sowing date and N fertilizer had significant effect of grain protein percent (Table 2). Delay in sowing date resulted in increase grain protein percent up to 15 % at May.4 than Mar. 5 sowing date (Table 3). Because of shorter growth period at late sowing dates, starch accumulation was low. Therefore, grain protein content increased. The crop received 150 kg N ha^{-1} produced 41 % more protein than the crop received 0-N (Table 3). Sowing date × N interaction was only significant in this trait. Apr. 18 + 150 kg N ha^{-1} was the best combinations for grain protein percent.

Fatty Acids: Figures of 1 to 6 show summary of the fatty acid composition and percentage of unsaturated fatty acids. The unsaturated fatty acid contents varied from 14.6 to 52.6 %. Generally, linseed oil contains approximately 9-11% saturated (5-6% palmitic acid and 4-5% stearic acid) and 75-90% unsaturated fatty acids (50-55% linolenic acid, 15-20% oleic acid) [41]. The highest linolenic acid obtained in Mar. 5 with average 52.4 % (Fig. 1). However, there were no differences in linoleic acid and oleic acid with change in sowing date (Table 2). linolenic acid and linoleic acid increased with increasing in nitrogen fertilizer (Figs 2 and 3). Conversely, oleic acid content decreased with increasing in nitrogen fertilizer (Fig. 4). The best combination in order to obtain the highest linolenic acid and oleic acid was Mar.5 +150 kg N ha^{-1} and Mar.5 +0 kg N ha^{-1}, respectively (Figs 5 and 6). Temperature is generally considered to be the most critical climatic factor affecting oil yields and quality [42]. Carder [43] has shown that day length (photoperiod) is also an important factor responsible for the differential growth behavior of grain crops when grown at two diverse latitudes.

CONCLUSION

The results of this study demonstrate that Mar.5 sowing date was the best date for increasing all traits except grain protein percent. 100 or 150 kg N ha^{-1} also increased all traits except oil percent and oleic acid of linseed. It recommends carry out the experiment under irrigation levels, soil types and other conditions.

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