

# Fatty Acid Content of Seed at Different Development Stages in Canola on Different Soil Types with Low Organic Matter

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**Abstract:** Physical and chemical properties of vegetable oils and consequently their use, depend on the composition of fatty acids that accumulate in storage lipids during seed development. The objective of this study was to determine the combined effects of seed development stages and organic matter content of soil on oil fatty acid composition of canola. The experiments were carried out under field conditions on four soils with different organic matter contents. To evaluate seed oil content and fatty acid composition of canola, we harvested plants at six growth stages (GS), GS 75, GS 79, GS 83, GS 87, GS 92 and GS 99 including development of seed, ripening and senescence. The synthesis of oil and fatty acids were largely influenced by seed maturity and soil type. Seeds had maximum content of stearic and palmitic acids at GS 75 (50% of pods reach final size). The seed yield, oil content of seeds and oleic acid percentage of seed oil significantly increased with increasing rate of soil organic matter in canola. This study addresses the organic matter content in poor soils should be ameliorated not only to obtain higher crop yields but also quality production.

**Key words:** Canola, Fatty acid, Oil content, Seed development, Soil organic matter.

Canola (*Brassica napus* L.) is one of the most important oilseed crops in the world, and its acreage has increased dramatically over the past decade (Przybylski et al., 2005). It is also a potential alternative cash crop for farmers in Turkey. Canola oil is high in oleic acid which is commonly used for food and industrial purposes. It is considered to be a very healthy edible oil. The functional characteristics, quality and commercial importance of the seed oil, both for food and industrial use, are primarily determined by the proportion of its main constituent fatty acids. Chain length, degree of unsaturation, and functional groups of vegetable oil fatty acids determine mainly the quality of oils (Zhang et al., 2004; Stoll et al., 2005; Baud and Lepiniec, 2010).

The biosynthetic pathways leading to the formation of oil fatty acids have been widely studied in oilseeds, such as sunflower (*Helianthus annuus* L.) (Garcia-Diaz et al., 2002; Rondanini et al., 2003; Rolletschek et al., 2007; Zlatanov et al., 2009), groundnut (*Arachis hypogaea* L.) (Hassan et al., 2005b), rapeseed species (*Brassica* sp.) (Bhardwaj and Hamama, 2003), safflower (*Carthamus tinctorius* L.) (Rahamatalla et al., 2001) and cuphea (*Cuphea* sp.) (Berti and Johnson, 2008).

The synthesis of fatty acids in rapeseed is known to be affected by environmental conditions (Zhang et al., 2004;

Hassan et al., 2005a; Omid et al., 2010). Temperature during seed development is generally considered to account for most of the variations in fatty acid composition (Baux et al., 2008). Other important factors are solar radiation (Izquierdo et al., 2009) and water stress (Pavlista et al., 2011).

Soil organic matter is often chosen as the most important indicator of soil quality and agricultural sustainability (Liu et al., 2006). It has a profound physical, chemical and biological impact on soil. Organic matter has several functions in soil: it increases nutrient holding capacity of soil, is a pool of nutrients for plants, improves water infiltration, decreases evaporation, increases water holding capacity, reduces crusting, improves aggregation, prevents erosion, and prevents compaction. Soil organic matter has to be at least 2% for soil productivity (FAO, 2006). With conventional wheat (*Triticum aestivum* L.) and sunflower rotation, erosion, incorrect tillage and other applications caused negative changes in the fertility of soils in the Thrace Region of Turkey. In this region, while 35.2% of the total arable area had organic matter over 2% in 1970, only 6% of the total arable area currently has more than 2% organic matter. Loss of soil organic matter is gathering concern because it is often associated with reduced crop performance (Onemli, 2004).

Table 1. Main characteristics of four soil types at experimental site.

Parameters	Value				Unit	Method
	Soil 1	Soil 2	Soil 3	Soil 4		
Organic matter	1.30	1.11	0.97	0.85	(%)	Walkley-Black
pH	8.0	8.0	8.1	8.1		Saturated extraction
Phosphorus	15	14	13	13	(ppm)	Olsen-ICP
Potassium	130	126	126	120	(ppm)	Ammonium acetate-ICP
EC	0.07	0.07	0.07	0.07	(dS m <sup>-1</sup> )	Saturated extraction
Texture	Clay loam	Clay loam	Clay loam	Clay loam		Saturated extraction

In recent years, the increasing demand for oil crops has led to a renewed interest on the accumulation of fatty acids during seed development. The main objective of this study was to examine the accumulation of fatty acids during seed development in canola on four soil types containing different amounts of organic matter. The second objective was to determine seed yields and days from planting to harvest on these soils.

## Materials and Methods

### 1. Experimental site

The experiments were carried out on the field of the Faculty of Agriculture at Namik Kemal University in Tekirda, Turkey (40°59' N, 27°33' E, elevation 3 m), on four clay loam soils differing in organic matter content in 2009 and 2010. The main chemical characteristics of four soils are shown in Table 1. The physical and chemical properties of soils were similar, except the organic matter. Phosphorus (P), potassium (K) and pH of soils ranged from 13 to 15 ppm, 120 to 130 ppm and 8.0 to 8.1 respectively. Organic matter content of the soils were 1.30, 1.11, 0.97 and 0.85% in Soil 1, Soil 2, Soil 3 and Soil 4, respectively.

### 2. Seed monitoring

ES Hydromel, a canola hybrid belonging to "Euralis Semences International" with mid early flowering and early maturity, was sown at a rate of 3.5 kg ha<sup>-1</sup> at a 15 mm soil depth in the second week of September. The experimental design was a randomized complete block in a split plot with three replications. Soil type and seed development were placed on the main plot and subplot; respectively. Experimental units consisted of 40 rows 10-m in length with 12.5 cm spacing between rows. For the evaluation of yield and quality components, 16 rows 5 m in length (10 m<sup>2</sup>) from each subplot were harvested at six different growth stages including development of seeds (GS 75 and GS 79), ripening (GS 83 and GS 87) and senescence (GS 92 and GS 99) according to Canola Council of Canada Grower's Manual (CCC, 2012).

Twelve border rows were on the one side of subplots to make interplot competition effects minimal. Soil fertility

Table 2. The six harvest times according to Canola Council of Canada Grower's Manual (CCC, 2012).

Growth stages	Description
Growth stage 7: Development of seed	
1 GS 75	50% pods reach final size
2 GS 79	Nearly all of the pods reach final size
Growth stage 8: Ripening	
3 GS 83	30% pods ripe, seeds black and hard
4 GS 87	70% of pods ripe, seeds black and hard
Growth stage 9: Senescence	
5 GS 92	Plants dead and dry (Fully ripe)
6 GS 99	Harvested product

was adjusted to 180 kg ha<sup>-1</sup> of N and 60 kg ha<sup>-1</sup> of P. The first part of N and the whole of P were applied at seeding time as composed fertilizer (20-20-0 N-P-K). The remaining nitrogen was applied at GS 30 (beginning of stem elongation) as urea fertilizer and at GS 61 (flowering: 10% of flowers on the main raceme open, main raceme elongating) as ammonium nitrate fertilizer. Flowers were tagged with the date of anthesis.

### 3. Seed oil and fatty acids analysis

To evaluate seed development, we harvested plants six times between GS 75 and 99 with three replications for each soil type. The six harvest times are according to Canola Council of Canada Grower's Manual (CCC, 2012) as shown in Table 2.

Seed oil and fatty acid analyses were conducted at the laboratory of Trakya Birlik, a Turkish Agricultural Cooperative. Seed oil content was determined with a pulsed NMR instrument (Bruker Minispec-Bruker Analytische Messtechnik, Karlsruhe, Germany). Oil content was expressed as a percentage of dry seed weight. Gas chromatography of fatty acid methyl esters was performed with an Agilent 6890 N gas chromatography equipped with a flame ionization detector. Analyses were conducted on an Agilent capillary column with 100 m × 0.25 mm i.d., 0.2 μm according to ISO 5508 (Ackman, 2002). The column temperature was programmed from

Table 3. Analyses of variance of the effects of seed development stages, soil types and the interaction effects of seed development and soil type on oil and fatty acid contents of canola.

	DF	Mean squares						
		Oil Content	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)
Seed development	5	74.812**	0.846**	0.594**	19.460**	3.115**	0.675**	0.140**
Soil type	3	28.755**	0.326**	0.062**	17.042**	7.235**	0.236**	0.030**
Seed development × soil type	15	11.699**	0.107**	0.037**	7.396**	2.305**	0.255**	0.030**

\*\* : Significant differences are shown at  $P < 0.01$  based on ANOVA.

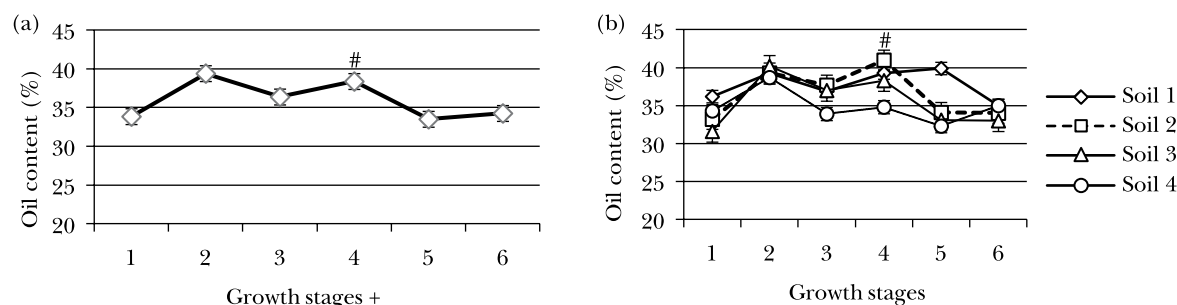


Fig. 1. Oil content of canola at different seed development stages from GS 75 to GS 99, (a) average of values on different soils, (b) values on each soil type. Error bars show SD ( $n = 3$ ).

+: See Table 2.

#: The time of harvesting.

120 to 230°C; the injector and detector temperature was set at 250°C using helium, air and hydrogen. Six major fatty acids (Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linolenic (C18:3), Arachidic (C20:0)) were identified as percentage of total fatty acids.

#### 4. Seed yield and number of days from planting to senescence

Seed yield and number of days from planting to GS 99 (Senescence) were examined on soils with different organic matter contents. Seed yield ( $\text{kg ha}^{-1}$ ) was calculated using subplot yield. For the yield determination, 16 rows 5 m in length ( $10 \text{ m}^2$ ) from each subplot were harvested. Seed yield was expressed at 10% moisture using subplot values. Days from planting to GS 99 was determined as the number of days between sowing date and GS 99.

#### 5. Data analysis

Statistical analysis was conducted according to standard procedures for a randomized complete block design with a split plot design (Steele et al., 1997). The SAS System was used to generate the analysis of variance (ANOVA) for determining treatment effects on the dependent variables (SAS Institute, 1997). Independent variables were seed oil content, fatty acid content, seed yield, and days from planting to GS 99. Treatment mean comparisons were

based on F-Protected Least Significance Differences (LSD) comparisons at  $P \leq 0.05$ . Correlation coefficients among the oil fatty acids were calculated with all the data as given by Kwon and Torrie (1964).

### Results and Discussion

The seed oil content and fatty acid composition of canola grown on four soil types were analyzed at six growth stages GS 75, GS 79, GS 83, GS 87, GS 92 and GS 99 including development of seed, ripening and senescence of GS based on Canola Council of Canada Grower's Manual. According to ANOVA (Table 3); seed development, soil type and the interaction of seed development and soil type significantly affected the oil content and the amount of fatty acids: palmitic, stearic, oleic, linoleic, linolenic and arachidic acids.

#### 1. Seed oil content

Seed oil content changed with the seed development from GS 75 to GS 99. (Fig. 1a). A significant difference in oil content was observed between GS 75 (50% of pods reach final size) and GS 79 (nearly all pods reached the final size). After GS 87 (70% of pods ripe, seeds black and hard), oil content decreased to GS 99 (Senescence, plants dead and dry, harvested product) due to seed enlargement and accumulation of other seed components (Baud and Lepiniec, 2010). Reduced seed oil content in developing

Table 4. Oil and fatty acid contents of canola seed on different soil types with different percentages of organic matter at GS 92 (Fully ripe).

Soils	Organic matter (%)	Oil Content (%)	Fatty acids (%) <sup>†</sup>					
			C16:0	C18:0	C18:1	C18:2	C18:3	C20:0
Soil 1	1.30	39.970 a*	4.729 d	1.134 c	66.245 a	21.286 d	7.762 c	1.021 c
Soil 2	1.11	34.083 b	5.054 b	1.228 a	64.834 b	22.141 b	7.970 b	1.230 a
Soil 3	0.97	33.130 c	5.021 c	1.213 b	62.404 c	21.962 c	8.320 a	1.081 b
Soil 4	0.85	32.297 c	5.454 a	1.227 a	60.575 d	23.753 a	7.743 d	1.230 a
LSD <sub>0.05</sub>		0.8951	0.0130	0.0066	0.0322	0.0311	0.0136	0.0095

\*: Within each column, means followed by same small letters are not significantly different by the LSD test at  $P < 0.05$ .

†: Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linolenic (18:3), Arachidic (C20:0) acids.

*Brassica* seeds before full maturity has been reported by Bhardwaj and Hamama (2003).

Oil content of canola seed was also influenced by the soil type (Table 3). Soil 1 gave the highest oil content, but the oil content was analyzed in seeds of canola on Soil 4 at GS 92 (fully ripe). Although all soil types had less than 2% soil organic matter, soil with a higher organic matter content gave a higher oil content (Table 4).

Similar results in oil content were observed for

Table 5. Seed yields at GS 99 and the number of days from planting to GS 99 of canola on soils with different organic matter contents.

	Organic matter (%)	Seed yields (kg ha <sup>-1</sup> )	Number of days from planting dates to GS 99 <sup>+</sup>
Soil 1	1.30	3183 a*	287.38 a
Soil 2	1.11	2684 b	278.25 b
Soil 3	0.97	2379 c	269.75 c
Soil 4	0.85	2117 d	264.25 d
LSD <sub>0.05</sub>		12.082	10.365
Mean squares of ANOVA		1675735.58**	818.115**

\*: Within each column, means followed by same small letters are not significantly different by the LSD test at  $P < 0.05$ .

+: See Table 2.

\*\* : Significant differences are shown at  $P < 0.01$  based on ANOVA.

interaction between seed development and soil type (Fig. 1b). Seed oil content increased from GS 83 to GS 87 on Soil 1 and Soil 3 with a higher percentage of organic matter while maximum oil content was reached at GS 79 on Soil 3 and Soil 4. Table 5 shows that soil organic matter was advantageous for plant growth and seed development to produce higher oil yields (Onemli, 2004).

The results show that seed development and soil type affects oil accumulation of canola seeds, and oil yield should be ameliorated by agronomic applications depend on environment.

## 2. Oil fatty acid composition

At maturity (GS 92), the oleic acid content was highest on soil 1, and palmitic, linoleic and arachidic acids contents were highest on Soil 4 (Table 4), Linolenic acid content was highest on Soil 3. It was also shown that soil organic matter had a positive effect on oleic acid content but a negative effect on linolenic acid.

Fatty acid composition of canola seed at growth stage is presented in Table 6. The greatest accumulation of palmitic and stearic acids as the primary saturated fatty acids was attained at GS 75. Then, they decreased constantly to GS 99 and GS 87, respectively. Although stearic acid was most amenable to change during seed development stages (GS 75 – 79), it did not show a linear

Table 6. Changes in fatty acid contents of canola seed during development.

Evaluated six growth stages	Fatty acids (%)					
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0
GS 75 <sup>+</sup>	5.620 a*	1.735 a	61.359 e	22.272 c	7.746 f	1.266 b
GS 79	5.262 b	1.227 b	61.000 f	23.007 a	8.410 a	1.092 e
GS 83	5.248 c	1.177 d	61.668 d	22.932 a	8.166 b	1.129 d
GS 87	5.055 d	1.161 e	62.426 c	22.366 b	7.863 e	1.051 f
GS 92	5.064 d	1.201 c	63.514 b	22.286 c	7.949 d	1.141 c
GS 99	4.836 e	1.197 c	64.215 a	21.615 d	7.964 c	1.333 a
LSD	0.013	0.006	0.103	0.081	0.013	0.0041

\*: Within each column, means followed by same small letters are not significantly different by the LSD test at  $P < 0.05$ .

+: See Table 2.

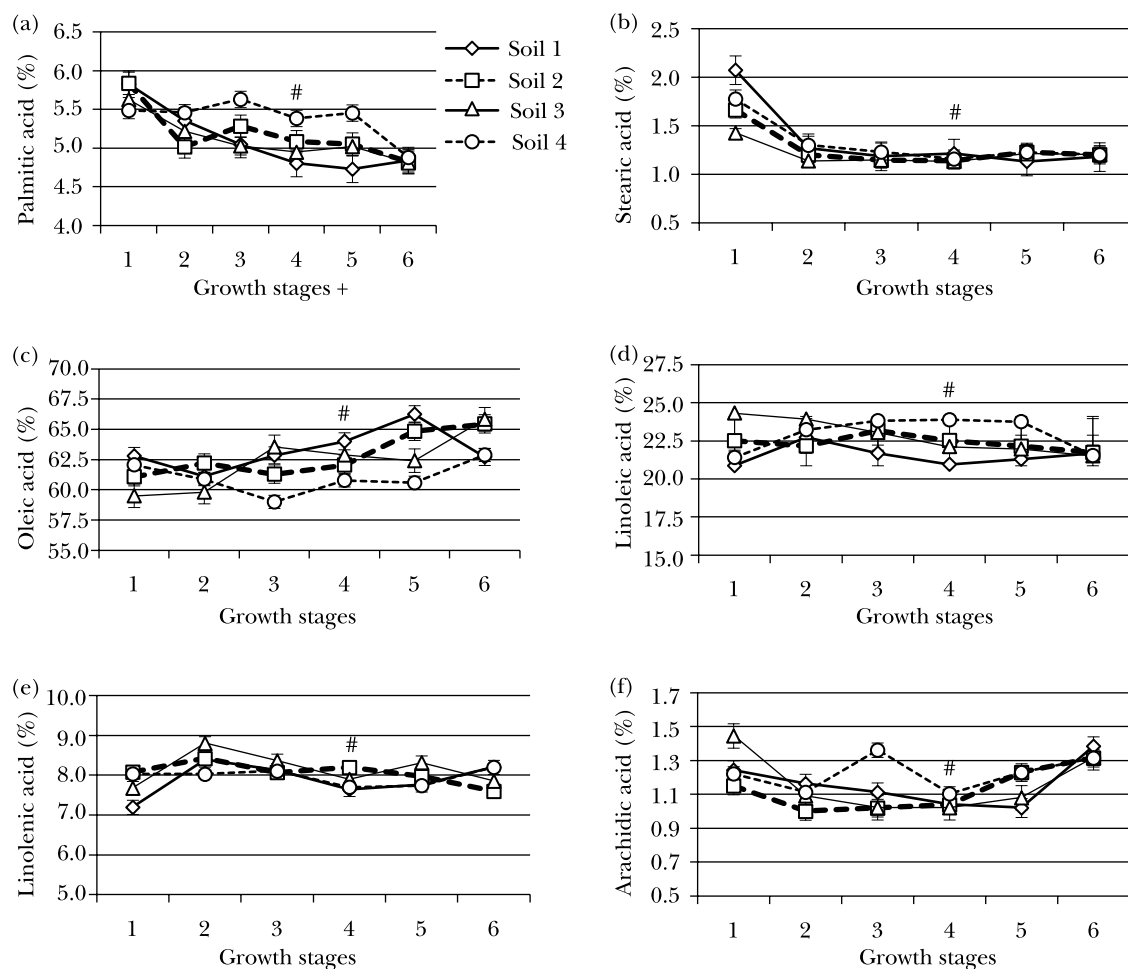


Fig. 2. Content of each oil fatty acid at different seed development stages from GS 75 to GS 99 on four soil types containing organic matter at different percentages. Error bars show SD ( $n = 3$ ).

+: See Table 2.

#: The time of harvesting.

decrease after GS 87.

Oleic acid content increased from GS 79 to GS 99. The highest linoleic acid content was found at GS 79 (nearly all of pods reach final size) and then decreased. The linolenic acid content was also highest at GS 79, and lowest at GS 75. Thus, linolenic acid increased during seed development. Similar unsteady variations were also observed in arachidic acid. Nevertheless, seeds at GS 99 had the highest arachidic acid content. The synthesis of fatty acids during seed development of rapeseed is affected by the environmental conditions (Zhang et al., 2004, Hassan et al., 2005a). DaMatta et al. (2010) also indicated that impacts of climate changes on crop physiology and food quality is significant.

Although the variation curves of some fatty acids during seed development were similar on all four soil types, soil type affected the fatty acid contents (Fig. 2). Organic matter percentages of the soils were 1.30, 1.11, 0.97 and 0.85 in Soil 1, Soil 2, Soil 3 and Soil 4, respectively (Table 1). Higher organic matter content of soil resulted in higher oleic acid content, higher seed yields and larger

number of days from planting to GS 99 (Table 1, 4, 5 and Fig. 2). Maximum oleic acid percentage in canola seed was reached at the fully ripe stage on Soil 1, but on the other soils oleic acid content was higher after the fully ripe stage (GS99). Generally, seeds of canola had maximum contents of stearic and palmitic acids at the beginning of the seed filling stage. The results indicate that a higher soil organic matter provide a higher unsaturated and lower saturated fatty acid contents after growth stage 8 (GS 83, GS 87) in canola (Fig. 2). Organic matter and microbial activity have been reported to have positive effects on fatty acid composition and physiological growth of oil crops (Onemli, 2004; Khosro et al., 2011).

### 3. Correlations among oil fatty acids

Oil content had significant positive correlations with linolenic ( $r = 0.341$ ) and stearic acids ( $r = 0.236$ ), whereas it had a negative correlation with arachidic ( $r = -0.678$ ) and palmitic acids ( $r = -0.233$ ) (Table 7). Positive correlations were observed between palmitic and stearic ( $r = 0.660$ ),

Table 7. Correlation coefficients on overall basis among oil content and fatty acids in canola.

	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (18:3)	Arachidic (C20:0)
Oil content (%)	-0.233*	0.236*	0.014	-0.121	0.341**	-0.678**
Palmitic (C16:0)		0.660**	-0.739**	0.491**	-0.193	0.244*
Stearic (C18:0)			-0.134	-0.263*	-0.434**	0.279*
Oleic (C18:1)				-0.760**	-0.293*	-0.116
Linoleic (C18:2)					0.276*	0.068
Linolenic (18:3)						-0.286*

\* and \*\* means statistical significance at the  $P < 0.05$  and  $P < 0.01$  of probability, respectively.

palmitic and linoleic ( $r = 0.491$ ), palmitic and arachidic ( $r = 0.244$ ), stearic and arachidic ( $r = 0.279$ ), and between linoleic and linolenic acids ( $r = 0.276$ ). Oleic acid showed highly negative correlation ( $r = -0.760$ ) with linoleic acid. The other negative significant correlations were observed between oleic and palmitic ( $r = -0.739$ ), linoleic and stearic ( $r = -0.263$ ), linolenic and stearic ( $r = -0.434$ ), oleic and linolenic ( $r = -0.293$ ), and between linolenic and arachidic acids ( $r = -0.286$ ). These results are essentially similar to published correlations in canola (Bhardwaj and Hamama, 2003; Hassan et al., 2005a; Khan et al., 2008; Omidi et al., 2010).

These correlations have an important role in the seed fatty acids synthesis. For instance, the increase in oleic acid synthesis depending on genetic trait and environmental conditions during seed development causes a reduction of linoleic acid percentage of seed oil.

#### 4. Seed yield and the number of days from planting to GS 99

Seed yield ranged from 2117 to 3183 kg ha<sup>-1</sup> depending on soil type (Table 5). Higher organic matter content of soil resulted in higher seed yields. Organic matter contents of the soils were 1.30, 1.11, 0.97 and 0.85% in Soil 1, Soil 2, Soil 3 and Soil 4, respectively (Table 1). The seed yield was highest on Soil 1 which contained 1.30% organic matter and lowest on Soil 4 (0.85% organic matter).

The number of days from planting to GS 99 (Senescence-harvest product) varied from 264.25 to 287.38 (Table 5) according to organic matter content of soils. Canola plants grown on Soil 4 reached GS 99 about 23 days earlier than those on Soil 1.

Oil content and fatty acid composition are affected by seed development stage and soil type. Oil content increases during GS 75 – 79, and then decreases until GS 83 (ripening) (Fig. 1). Saturated short chain fatty acids (C16:0, C18:0) are synthesized at GS 75 (50% of pods reach final size). Oleic acid showed a significantly high and negative correlation with linoleic acid during seed development, and maximum oleic content at GS 99 (harvest product). Accumulation of fatty acids, especially linoleic, linolenic

and arachidic acids appears to be largely associated with climatic conditions during seed development. Although the highest oil content was found at GS 87, the best harvest time in canola for high oleic content and low saturated fatty acids is GS 99. The results also show that the oil content, oil quality and seed yield of canola significantly increases with increasing organic matter content of soil (Table 4, 5, Fig. 1, 2). Higher soil organic matter gives a higher oleic acid content.

The results of this study will be useful for agronomic and genetic research in determining the optimal harvest period. The significance of the results lies in the demonstration of the feasibility of seed growth stages at which developing canola seed could be modified to change oil and fatty acid contents. The results also show that the seed yield, oil content and oil quality of canola significantly increases with increasing rate of soil organic matter content of soil. This study revealed that the organic matter content of poor soils should be ameliorated not only to obtain higher crop yields but also to improve the quality of production. Soil organic matter is one of our most important resources; unwise exploitation is devastating; and it must be given proper attention in any conservation policy as one of the major factors affecting the levels and value of crop production in the future.

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