



Improving of Canola (*Brassica napus* L.) Yield and Oil Quality by Foliar Application of Micro-nutrients Under High-Temperature Stress

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Abstract

High-temperature stress is a major limiting factor to decrease seed yield and oil content. Using micronutrients such as zinc (Zn), manganese (Mn), and boron (B) are considered agricultural management practices for improving the quantity and quality of oil under high temperature at the seed filling stage. The experiment was carried out as a split-plot based on a randomized complete block design with three replications over 2 years. Main plots consisted of three sowing date levels (Nov. 5, Nov. 25 and Dec. 15), and subplots included ten levels of foliar application of micro-nutrients (2, 4, 6 g l⁻¹ Zn; 2, 4, 6 g l⁻¹ Mn; 2, 4, 6 g l⁻¹ B, and the foliar application of distilled water as control treatment). Foliar applications were at rosette (BBCH scale code-30) and the opening of the first flower (BBCH scale code-61). Any delay in planting resulted in higher mean air temperature during the seed-filling period by 4 °C and 0.9 °C in the first and second year, respectively. The reduction of seed yield due to the delay in sowing date on Nov. 25 and Dec. 15 were respectively 7% and 35%. The highest seed yield (4411 kg ha⁻¹) belonged to foliar application of B (4 g l⁻¹) at the first sowing date (Nov. 5) and the lowest ones (1624 kg ha⁻¹) were associated with the last sowing date and using distilled water. High temperature during the seed filling period led to more palmitic, stearic, linolenic, and oleic acids, but generated less linoleic acid. Foliar application of Zn (4 g l⁻¹), Mn (2 g l⁻¹) and B (4 g l⁻¹) led to an increase in the seed yield by 12%, 22% and 29%, respectively. According to the results of this experiment, more than 340 kg ha⁻¹ reduction in the seed yield was observed as the temperature increased by one degree from 28 °C. Based on the results of the experiment, the application of micro-nutrients (Zn, Mn, and B) elevated the seed yield and oil content on all sowing dates.

Keywords B · Delayed sowing date · Fatty acids · Heat stress · Mn · Oil · Zn

1 Introduction

Climate change which is caused by global warming is a global concern (Yerlikaya et al. 2020) and the rise in temperature reduces the yield of many crops (Malhi et al. 2021),

such as canola (Ghani et al. 2021). The global mean temperature has increased by 0.5 °C during the past century and it is predicted to experience a further rise by 0.3–4.8 °C by 2100 (IPCC 2014).

Canola is a major oilseed crop around the world and increasing temperature impacts floral morphology, seed yield, oil content, and fatty acids (Pokharel et al. 2020). Canola oil has a lower content of saturated acids (Shiranirad et al. 2021), high content of monounsaturated fatty acids, and an adequate amount of polyunsaturated fatty acids (Beyzi et al. 2019). The qualitative and quantitative characteristics of canola oil are influenced by environmental factors and nutrition conditions (Shiranirad et al. 2021; Mokhtassi-Bidgoli et al. 2021). Canola grows well if it is sown on the optimum sowing date (Aghdam et al. 2019) when flowering, silique, and seed filling periods are not influenced by terminal heat stress (Kalantar Ahmadi and Daneshian 2021). Heat stress can have adverse effects on reproductive stages as it decreases the seed

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yield and yield components (Zinn et al. 2010; Ahmad et al. 2021b), photosynthetic rate, and water potential (Ahmad et al. 2021a). Exposing flowering plants to high temperatures (30 °C day and 15 °C night) lowers the seed yield due to an increase in the flowers' abortion rate and a consequent decrease in the number of siliques per plant (Gan et al. 2004). Canola oil content is negatively influenced by late-season high temperature (Kutcher et al. 2010), and fatty acids composition has been changed by temperature and sowing date (Izquierdo et al. 2006). High temperatures (30 °C day and 25 °C night) over an extended period up to 40 days after flowering or short-term heat stress (38 °C day and 23 °C night temperatures over 5 days) commenced in 25–29 days post-flowering changed fatty acids amounts and oil content (Aksouh-Harradj et al. 2006; Pritchard et al. 2000).

Heat stress causes a reduction in the uptake of nutrients from the soil and exogenous application of nutrients is proved as a useful way to alleviate the injurious effects of heat stress (Waraich et al. 2012). Plant nutrition improves quantitative and qualitative yields under environmental stresses (Movahhedy-Dehnavy et al. 2009; Ibrahim and Ramadan 2015). Foliar application of nutrient elements is used as a quick and sustainable method to ensure adequate nutrient supply for crops (Fageria et al. 2009) and could be a useful strategy for reducing the harmful effects of heat stress (Waraich et al. 2012). Zn mediates the regulation of water relations, maintaining cell water and osmotic potential under high-temperature conditions (Venugopalan et al. 2022). B increases stomatal opening, and gaseous exchange regulation (Waraich et al. 2011; Imtiaz et al. 2010) and Mn accelerates photosynthesis and nitrogen metabolism under high-temperature conditions (Waraich et al. 2012).

Some studies have revealed that foliar application of a small amount of micro-nutrients like Zn, B, and Mn increased the yield (Sarkar et al. 2007; Wissuwa et al. 2008). Zn is an essential element for the activity of different types of enzymes (Cakmak 2008) and foliar Zn application leads to greater seed yield, oil, and protein content (Bybordi and Mamedov 2010). Zn deficiency influenced carbohydrate metabolism, mitigated pollen germination, and reduced the yield (Mei et al. 2009). Also, there is a relationship between Zn deficiency and susceptibility to high temperatures (Graham and McDonald 2001). Sufficient Zn can soften the negative effects of the short periods of high temperature on seed growth and chloroplast function (Razzaq et al. 2013; Peck and McDonald 2010). The application of Zn improves the quality and quantity of canola oil. The use of Zn at the pollen grain formation stage in canola increases the linolenic acid (Agha Mohammad Reza et al. 2021) and changes other fatty acids (Movahhedy-Dehnavy et al. 2009).

B is absorbed by plants in small amounts, which is involved in many physiological and biochemical processes (Badawy et al. 2016). The application of B can enhance the

seed weight, oil yield, and the oil quality of canola (Mei et al. 2009), and alleviate the harmful effects of the high-temperature stress (Waraich et al. 2011), which can eventually lead to the growth of canola (Badawy et al. 2016). Moreover, B can enhance the antioxidant activities of plants and thereby mitigate ROS damage induced by heat stress. Also, nutrition by B plays an important role in the metabolism of oil (Manaf et al. 2019), carbohydrates, protein, phenol (Bellaloui et al. 2013), and sugar transportation which can improve seed formation (Waraich et al., 2012), enhance photosynthesis, and reduce cell damage (Waraich et al. 2011).

Mn is a vital element for plants and participates in various processes such as photosynthesis, respiration, and enzyme activity (Pourjafar et al. 2016). Furthermore, foliar spraying of Mn enhances the seed yield of canola (Bahrani and Pourreza 2014). Mn can reduce the adverse effects of high temperature by accelerating photosynthesis and nitrogen metabolism (Waraich et al. 2012). The direct influence of Mn as a component of the biotic enzyme could be a contributing factor in the biosynthesis of the fatty acids (Jabeen et al. 2013).

Canola's importance in oil supply and food security is known in many parts of the world and the implementation of proper agricultural management practices has great importance in achieving this goal. Scanty information is available on the effect of micronutrients to decrease the undesirable influences of high temperature on quantitative and qualitative characteristics of canola. Studying the important role of micronutrients (Zn, Mn, and B) in protecting crops under high-temperature conditions might alleviate the negative impact of heat stress. Moreover, it is important to find a suitable sowing date and appropriate management for a successful agricultural practice. Therefore, this experiment was carried out to arrive at the optimum dose and probe the role of Zn, Mn, and B to ameliorate the adverse effect of heat stress on seed yield, oil content, and fatty acids, which can improve both the quantitative and qualitative traits of canola oil in areas with similar climatic conditions.

2 Materials and Methods

2.1 Experimental Site

The study was carried out within 2 years (2015–2017) at Safiabad Agricultural and Natural Resources Research and Education Center which is located in Khuzestan province in the southwest of Iran (82.9 m a.s.l., 48° 26' E, 32° 16' N). The characteristics of the soil are presented in Table 1. The temperature during flowering, siliquing and seed filling periods are shown in Tables 2 and 3, respectively. Climatic

Table 1 Characteristics of physical and chemical properties of soil used in the experiment

| Year | Soil texture | OC (%) | P (mg kg ⁻¹) | K (mg kg ⁻¹) | pH | EC (ds m ⁻¹) | Zn (mg kg ⁻¹) | Mn (mg kg ⁻¹) | B (mg kg ⁻¹) |
|------|--------------|--------|--------------------------|--------------------------|------|--------------------------|---------------------------|---------------------------|--------------------------|
| 2015 | Clay-loam | 0.62 | 8.5 | 178 | 7.64 | 0.57 | 0.71 | 4.5 | 0.6 |
| 2016 | Clay-loam | 0.65 | 10.1 | 185 | 7.61 | 0.56 | 0.73 | 4.2 | 0.62 |

OC organic carbon, P phosphorus, K potassium, pH potential of hydrogen, EC electrical conductivity, Zn zinc, Mn manganese, B boron

data including temperature, rainfall, and relative humidity was provided in Supplementary Figs. 1 and 2.

2.2 Soil Properties

Before conducting the experiment, composite farm soil samples were taken (0–30-cm depth) and then dried out and sieved with a 2-mm sieve. Physical and chemical characteristics of soil were measured. The properties consisted of K and P, electrical conductivity (EC) (Page et al. 1982), organic carbon (OC) (Walkley and Black 1934), pH (Metrohm pH meter, Metrohm, Herisau, Switzerland), and soil texture (Gee and Bauder 1986). Available Zn, Mn, and B were extracted by diethylenetriaminepentaacetic acid (DTPA) (Baker and Amchar 1983) and analyzed by atomic absorption spectrometry (Lindsay and Norvell 1978).

2.3 Experimental Design

A split plot was used based on a completely randomized block design with three replications over 2 years. The main plots included three sowing dates (November 5, November 25 and December 15) and subplots consisting of 10 levels of foliar application of micro-nutrients concentrations (2, 4, 6 g l⁻¹ Zn sulfate, 2, 4, 6 g l⁻¹ Mn sulfate, 2, 4, 6 g l⁻¹ boric acid, and the foliar application of distilled water as control treatment). The land-map design (3 main plots and 10 sub-plots) of the experiment was described in Supplementary Table 4. Foliar applications were at rosette (BBCH scale code-30) and the opening of the first flower (BBCH scale code-61; Weber and Bleiholder 1990). The studied cultivar was Hyola401. The first sowing date (Nov. 5) was considered the suitable sowing date (Kalantar Ahmadi et al. 2014). The air temperature was nearly 28 °C considered the threshold temperature (Morrison and Stewart 2002; Ghobadi et al. 2006).

2.4 Experiment Procedure

There was a 75-cm distance between ridges and two rows were sowed on each ridge. The needed area for each plot was 12 m². Each subplot included 4 ridges. After establishment, the plants were thinned to achieve 80 plants m⁻² at the 6-leaf stage. Before planting, 200 kg ha⁻¹ and 150 kg ha⁻¹ potassium sulfate and triple superphosphate were used, respectively. Nitrogen (N) was used to 180 kg ha⁻¹ (391 kg ha⁻¹

urea) while one-third of it was applied during the sowing period. Besides, split application of the remaining nitrogen was used at rosette (BBCH scale code-30) and the opening of the first flower (BBCH scale code-61; Weber and Bleiholder 1990).

2.5 Seed Yield, Oil, and Fatty Acids Content Assessment

Two center rows in each plot were harvested at maturity to measure seed yield in an area of 6 m². NMR (Nuclear Magnetic Resonance) German Broker Brand minispq20 model (Iso 5511, 1992) was used to assess the oil content of 5 g seed samples. To determine the oil percentage, after daily calibration of the machine with a reference sample and calibration of the product with pre-prepared standard samples, at least 3 g of seed canola was measured and transferred to the special cell of the device, then the oil percentage of the sample was recorded in the monitor for less than 1 min.

Fatty acid composition was determined using the gas chromatography (GS) method. Oil samples were extracted in triplicate from 100 g of seed canola based on the method explained by Azadmard-Damirchi et al. (2005). Fatty acid methyl esters were provided from the oil samples. Two ml of 0.01 M NaOH in methanol was added to the oil sample dissolved in 0.5 ml hexane and then kept in the water bath at 60 °C for 10 min. Next, B trifluoride in methanol (20% of BF₃ in methanol) was added and then it was held for 10 min in a water bath at 60 °C. Then, the sample was cooled under water and added 2 ml of sodium chloride (20% w/v) as well as added 1 ml hexane was added. After blending completely, the layer of hexane which contained the fatty acid methyl esters was detached by centrifugation. The fatty acid methyl esters were analyzed by GC according to the method illustrated by Azadmard-Damirchi and Dutta (2006). The glucosinolate was estimated using a spectrophotometer, equipped with a 50-m length CP-Sil 88 capillary column, the internal diameter of 0.25 Modified, and 0.2 μm of the static thickness (Makkar et al. 2007).

2.6 Statistical Analysis

Statistical analysis was performed using SAS (Version 9.2) for combined analysis of variance after conducting Bartlett's test and providing the homogeneity of the test variance in

Table 2 Temperature during flowering stage and number of days with maximum temperature more and less than threshold temperature (28 °C) at different sowing dates during 2 years of experiment (2015–2017)

| Sowing date | Flowering period duration (day) | | Minimum (°C) | | Maximum (°C) | | Mean (°C) | | Number of days with temperature [≤] 28 °C | | Number of days with temperature 28–30 °C | | Number of days with temperature ^{>} 30 °C | |
|-------------|---------------------------------|-------------|--------------|-------------|--------------|-------------|------------|-------------|--|-------------|--|-------------|---|-------------|
| | First year | Second year | First year | Second year | First year | Second year | First year | Second year | First year | Second year | First year | Second year | First year | Second year |
| Nov. 5 | 39 | 50 | 8.7 | 7.97 | 22.15 | 20.5 | 15.45 | 14.25 | 39 | 50 | 0 | 0 | 0 | 0 |
| Nov. 25 | 34 | 34 | 8.6 | 11 | 22.4 | 21.95 | 15.5 | 16.45 | 16 | 32 | 16 | 2 | 2 | 0 |
| Dec. 15 | 20 | 20 | 12.2 | 13.6 | 24.8 | 26.5 | 18.5 | 20.05 | 17 | 18 | 0 | 3 | 3 | 0 |

Table 3 Air temperature during silique, grain filling stages, and number of days with maximum temperature more and less than threshold temperature (28 °C) at different sowing dates during 2 years of experiment (2015–2017)

| Sowing date | Silique and grain filling duration (day) | | Minimum (°C) | | Maximum (°C) | | Mean (°C) | | Number of days with temperature [≤] 28 °C | | Number of days with temperature 28–30 °C | | Number of days with temperature ^{>} 30 °C | |
|-------------|--|-------------|--------------|-------------|--------------|-------------|------------|-------------|--|-------------|--|-------------|---|-------------|
| | First year | Second year | First year | Second year | First year | Second year | First year | Second year | First year | Second year | First year | Second year | First year | Second year |
| Nov. 5 | 80 | 90 | 12.87 | 14 | 27.32 | 28.2 | 20.1 | 21.34 | 45 | 69 | 3 | 9 | 32 | 12 |
| Nov. 25 | 74 | 70 | 15.06 | 13.36 | 29.86 | 28.86 | 22.46 | 21.1 | 29 | 40 | 3 | 10 | 42 | 20 |
| Dec. 15 | 69 | 60 | 16.3 | 14.4 | 31.85 | 30.03 | 24.07 | 22.23 | 19 | 26 | 3 | 9 | 47 | 25 |

each year. When the F -test indicated statistical significance at $P < 0.01$ or $P < 0.05$, the protected least significant difference (protected LSD) was applied to separate the means of the main effect and the significant interactions were performed by the least significant difference (LSD) test by slicing method at $P < 0.05$. The regression relationship between seed yield, oil content, and fatty acids with temperature during seed filling period was calculated by interaction year \times sowing date. To calculate the regression relationship between the studied traits and micronutrients, we used the mean of the simple effect of micronutrients during 2 years of the experiments. Minitab software (Version 16) was employed to do principal component analysis. In this study, principal components analysis was employed to select the effective features in the quantity and quality of the oil. In the composition technique, the variables X_1, X_2, \dots, X_p are combined with the components P , and a series of indicators or components Z_1, Z_2, \dots, Z_p that are not correlated with each other were obtained.

3 Results

Combined analysis showed that the simple effect of year and foliar application was significant in all the studied traits. The sowing date had a significant effect on all traits, except erucic acid. The interaction effect of year \times sowing date on palmitic acid, oleic acid, linolenic acid, linoleic acid, erucic acid, glucosinolate, and oil content was significant at 1% level. The interaction effect of sowing date \times foliar application of micro-nutrients was significant among all traits. The interaction effects of year \times sowing date \times foliar application of micro-nutrients did not have a significant effect on studied traits

(Supplementary Table 1). The interaction slicing analysis of foliar application at any sowing date showed significant results for the studied traits (Supplementary Table 2). Mean comparison of year \times sowing date revealed that the seed yield, oil content, and linoleic acid of all sowing dates in the second year were more than the first year (Table 4). Mean comparison of year \times sowing date showed that palmitic, oleic, linolenic, and erucic acids of all sowing dates in the first year were more than in the second year (Table 4). This matter was related to higher temperatures and the number of days with temperatures more than 30 °C in the first year compared to the second year (Table 3). Days with temperatures above 30 °C were not observed during the flowering stage in the second year (Table 2).

3.1 Seed Yield

An increase in temperature due to delay in the sowing date led to lower seed yield by 6.74% and 36.14% in the second (Nov. 25) and the last (Dec. 15) sowing dates, respectively. The mean comparison of the interaction effect of sowing date \times foliar application revealed that the highest seed yield was (4411.01 kg ha⁻¹) allocated to the foliar application of B (4 g l⁻¹) at the first sowing date (Nov. 5). The seed yield increased by 32% with foliar application of B (4 g l⁻¹) at the first sowing date (Nov. 5) compared to the control treatment. The lowest seed yield (1624 kg ha⁻¹) was associated with the last sowing date and use of distilled water (Table 5). Slicing revealed that all micro-nutrient foliar spray treatments except Zn (2 g l⁻¹) and control treatment were in the same statistical group for the second sowing date. In other words, compared to other foliar spray treatments, Zn (2 g l⁻¹) had the lowest effect on the increase in the seed yield (Table 5).

Table 4 Mean comparison of the two way-interaction of year \times sowing date on studied traits during 2 years (2015–2017)

| Year | Sowing date | Seed yield (kg ha ⁻¹) | Oil content (%) | Palmitic acid (%) | Oleic acid (%) | Linolenic acid (%) | Linoleic acid (%) | Erucic acid (%) | Glucosinolate ($\mu\text{mol g}^{-1}$) |
|-------------------------|-------------|-----------------------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|--|
| First year (2015–2016) | Nov. 5 | 3125.9 \pm 92.1c | 43.71 \pm 0.18b | 3.49 \pm 0.02c | 53.91 \pm 0.18d | 4.51 \pm 0.07c | 21.76 \pm 0.21d | 0.019 \pm 0.08a | 5.05 \pm 0.08c |
| | Nov. 25 | 2970.7 \pm 99.2c | 41.67 \pm 0.21d | 3.65 \pm 0.02b | 57.53 \pm 0.24b | 5.72 \pm 0.13b | 22.23 \pm 0.14c | 0.018 \pm 0.01a | 6.74 \pm 0.11b |
| | Dec. 15 | 2015.8 \pm 79.9e | 39.22 \pm 0.22f | 3.87 \pm 0.06a | 59.12 \pm 0.13a | 6.62 \pm 0.10a | 18.4 \pm 0.13f | 0.015 \pm 0.07b | 9.68 \pm 0.17a |
| Second year (2016–2017) | Nov. 5 | 3832.3 \pm 127.3a | 45.52 \pm 0.19a | 2.7 \pm 0.03e | 50.26 \pm 0.22e | 3.29 \pm 0.07d | 25.47 \pm 0.18a | 0.008 \pm 0.06c | 3.52 \pm 0.14d |
| | Nov. 25 | 3505.1 \pm 145.9b | 42.77 \pm 0.20c | 3.09 \pm 0.02d | 54.77 \pm 0.26c | 4.43 \pm 0.12c | 23.77 \pm 0.14b | 0.01 \pm 0.04c | 5.25 \pm 0.13c |
| | Dec. 15 | 2422.7 \pm 113.7d | 40.92 \pm 0.21e | 3.61 \pm 0.04b | 57.11 \pm 0.13b | 5.59 \pm 0.11b | 19.57 \pm 0.11e | 0.013 \pm 0.08b | 7.08 \pm 0.14b |
| LSD (0.05) | | 320.67 | 0.57 | 0.09 | 0.53 | 0.29 | 0.42 | 0.001 | 0.35 |

Lettering is showing the difference among treatment means. Means in each column, followed by least one letter in common are not significantly different ($P \leq 0.05$) by LSD test. The value after (\pm) is standard error

Table 5 Mean comparison of the two way-interaction of sowing date X micro-nutrients on studied traits during 2 years (2015–2017)

| Sowing date | Micro-nutri- ents | Seed yield (kg ha ⁻¹) | Oil content (%) | Palmitic acid (%) | Stearic acid (%) | Oleic acid (%) | Linolenic acid (%) | Linoleic acid (%) | Erucic acid (%) | Glucosinolate ($\mu\text{mol g}^{-1}$) |
|-------------|----------------------|-----------------------------------|-----------------|----------------------|---------------------|-----------------|-----------------------|----------------------|-----------------|---|
| Nov. 5 | Zn1 | 3017.55 ± 167.27c | 44.2 ± 0.41c | 3 ± 0.18de | 1.6 ± 0.07a | 51.67 ± 0.84 cd | 3.89 ± 0.31c | 22.83 ± 0.88e | 0.015 ± 0.02c | 4.57 ± 0.39c |
| | Zn2 | 3776.5 ± 173.02b | 44.85 ± 0.41bc | 3.1 ± 0.18c | 1.7 ± 0.09a | 52.32 ± 0.84b | 3.96 ± 0.30c | 23.53 ± 0.88d | 0.014 ± 0.02d | 4.26 ± 0.39d |
| | Zn3 | 3287.83 ± 172.12bc | 46.15 ± 0.41a | 3.2 ± 0.18b | 1.73 ± 0.07a | 53.62 ± 0.84a | 4.31 ± 0.31a | 24.58 ± 0.87b | 0.012 ± 0.02e | 4.1 ± 0.395e |
| | Mn1 | 2326.72 ± 132.53bc | 43.05 ± 0.46b | 2.95 ± 0.18e | 1.61 ± 0.08a | 50.52 ± 0.84e | 3.75 ± 0.30d | 22.68 ± 0.87e | 0.017 ± 0.02b | 4.8 ± 0.39b |
| | Mn2 | 3491.33 ± 358.45bc | 44.4 ± 0.44c | 3.05 ± 0.18 cd | 1.7 ± 0.10a | 51.87 ± 0.84c | 3.95 ± 0.30c | 23.98 ± 0.86c | 0.015 ± 0.02c | 4.46 ± 0.39c |
| | Mn3 | 359.99 ± 227.67bc | 45.05 ± 0.40b | 3.1 ± 0.18c | 1.77 ± 0.11a | 52.52 ± 0.84b | 4.12 ± 0.31b | 23.98 ± 0.89c | 0.013 ± 0.02d | 4.24 ± 0.39d |
| | B1 | 3452.16 ± 206.76bc | 43.95 ± 0.40c | 3.04 ± 0.18 cd | 1.68 ± 0.10a | 51.42 ± 0.84d | 3.67 ± 0.30d | 23.56 ± 0.86d | 0.012 ± 0.02e | 3.96 ± 0.39e |
| | B2 | 4411.01 ± 379.19a | 45.15 ± 0.40b | 3.21 ± 0.18b | 1.77 ± 0.11a | 52.62 ± 0.85b | 3.96 ± 0.27c | 24.18 ± 0.87c | 0.010 ± 0.02f | 3.73 ± 0.39f |
| | B3 | 3577.61 ± 358.52bc | 46.05 ± 0.45a | 3.38 ± 0.17a | 1.82 ± 0.11a | 53.52 ± 0.84a | 4.16 ± 0.28b | 24.88 ± 0.91a | 0.010 ± 0.02f | 3.66 ± 0.39f |
| Nov. 25 | Control | 2993.61 ± 264.82c | 43.25 ± 0.40d | 2.93 ± 0.18e | 1.68 ± 0.12a | 50.72 ± 0.85de | 3.26 ± 0.25e | 21.93 ± 0.86f | 0.019 ± 0.02a | 5.05 ± 0.43a |
| | Zn1 | 2672.44 ± 312.16b | 42 ± 0.43c | 3.27 ± 0.12d | 1.58 ± 0.08c | 54.27 ± 0.66 g | 5.05 ± 0.32d | 22.34 ± 0.35e | 0.016 ± 0.01c | 6.26 ± 0.39c |
| | Zn2 | 3307.88 ± 0.226.86a | 42.3 ± 0.34bc | 3.36 ± 0.12 cd | 1.65 ± 0.07c | 56.52 ± 0.64d | 5.23 ± 0.32c | 23.33 ± 0.42c | 0.014 ± 0.02d | 5.95 ± 0.39d |
| | Zn3 | 3121.66 ± 316.28a | 43.5 ± 0.37a | 4.49 ± 0.13b | 2.33 ± 0.25a | 57.22 ± 0.64bc | 5.95 ± 0.32a | 23.32 ± 0.35c | 0.013 ± 0.02e | 5.79 ± 0.39de |
| | Mn1 | 3469.39 ± 335.49a | 41.07 ± 0.60d | 3.25 ± 0.12d | 1.79 ± 0.17bc | 54.14 ± 0.63 g | 4.94 ± 0.29d | 21.64 ± 0.35f | 0.019 ± 0.02a | 6.49 ± 0.39b |
| | Mn2 | 3432.44 ± 288.52a | 41.77 ± 0.27 cd | 3.33 ± 0.13 cd | 1.86 ± 0.17bc | 55.67 ± 0.66e | 5.2 ± 0.29c | 22.38 ± 0.36e | 0.017 ± 0.03b | 6.25 ± 0.39c |
| | Mn3 | 3273.22 ± 290.39a | 42.57 ± 0.37b | 3.4 ± 0.12c | 1.98 ± 0.16b | 56.99 ± 0.65c | 5.67 ± 0.29b | 23.21 ± 0.39c | 0.017 ± 0.03b | 5.93 ± 0.39d |
| | B1 | 3486.77 ± 311.13a | 41.7 ± 0.42 cd | 3.29 ± 0.13d | 1.61 ± 0.07c | 56.55 ± 0.69d | 4.01 ± 0.33e | 23.13 ± 0.37c | 0.012 ± 0.01f | 5.65 ± 0.39e |
| | B2 | 3693.27 ± 165.71a | 42.53 ± 0.44bc | 3.49 ± 0.13b | 1.74 ± 0.07bc | 57.14 ± 0.63dc | 5.16 ± 0.33 cd | 23.68 ± 0.36b | 0.010 ± 0.01 g | 5.42 ± 0.39f |
| Dec. 15 | B3 | 3468.83 ± 289.69a | 43.38 ± 0.27a | 3.6 ± 0.14a | 2.23 ± 0.23a | 58.06 ± 0.64a | 5.76 ± 0.33b | 24.32 ± 0.35a | 0.009 ± 0.01 h | 5.35 ± 0.39f |
| | Control | 2452.83 ± 186.86a | 41.37 ± 0.68d | 3.22 ± 0.12d | 1.72 ± 0.10c | 54.92 ± 0.64f | 3.83 ± 0.28f | 22.65 ± 0.36d | 0.014 ± 0.02d | 6.93 ± 0.39a |
| | Zn1 | 2106.39 ± 107.12b | 39.58 ± 0.45d | 3.54 ± 0.08e | 2.79 ± 0.18ab | 57.32 ± 0.51d | 6.13 ± 0.26c | 18.51 ± 0.33b | 0.016 ± 0.01b | 8.66 ± 0.59b |
| | Zn2 | 1968.88 ± 110.28b | 40.24 ± 0.44c | 3.63 ± 0.08d | 2.87 ± 0.18ab | 58.6 ± 0.51a | 5.58 ± 0.29b | 18.73 ± 0.37b | 0.015 ± 0.01c | 8.4 ± 0.59d |
| | Zn3 | 1865 ± 94.32b | 41.54 ± 0.44a | 4.09 ± 0.17a | 2.93 ± 0.22ab | 58.08 ± 0.50b | 7.09 ± 0.27a | 19.61 ± 0.30a | 0.014 ± 0.01d | 8.2 ± 0.59d |
| | Mn1 | 2772.27 ± 415.95a | 38.5 ± 0.37e | 3.44 ± 0.08f | 2.72 ± 0.17bc | 57.98 ± 0.57b | 5.83 ± 0.25d | 18.51 ± 0.30b | 0.018 ± 0.01a | 8.9 ± 0.59a |
| | Mn2 | 2330.66 ± 337.81ab | 39.83 ± 0.39 cd | 3.54 ± 0.08e | 2.81 ± 0.18b | 57.65 ± 0.49c | 6.08 ± 0.25c | 18.56 ± 0.38b | 0.016 ± 0.01b | 8.42 ± 0.70c |
| | Mn3 | 2321.72 ± 217.67ab | 40.42 ± 0.47bc | 3.96 ± 0.11b | 2.94 ± 0.22ab | 58.6 ± 0.51a | 6.66 ± 0.25b | 19.48 ± 0.29a | 0.016 ± 0.01b | 8.32 ± 0.70 cd |
| | B1 | 2321.72 ± 187.76ab | 39.61 ± 0.41d | 3.65 ± 0.08 cd | 2.87 ± 0.20ab | 57.85 ± 0.49bc | 5.56 ± 0.25e | 18.59 ± 0.36b | 0.012 ± 0.01e | 8.17 ± 0.70d |
| B2 | 2561.33 ± 161.49ab | 40.8 ± 0.43b | 3.73 ± 0.08c | 2.93 ± 0.19ab | 58.61 ± 0.49a | 5.9 ± 0.25d | 19.51 ± 0.30a | 0.011 ± 0.01f | 8.15 ± 0.70de | |
| B3 | 2460.1 ± 248.60ab | 41.78 ± 0.36a | 3.93 ± 0.07b | 3.05 ± 0.20a | 58.86 ± 0.50a | 6.13 ± 0.25c | 19.63 ± 0.37a | 0.009 ± 0.01 g | 7.98 ± 0.70e | |
| Control | 1624 ± 198.22b | 38.38 ± 0.55e | 3.92 ± 0.15b | 2.55 ± 0.35c | 57.61 ± 0.49 cd | 5.14 ± 0.26f | 18.5 ± 0.32b | 0.014 ± 0.01 cd | 8.6 ± 0.85bc | |

Letters refer to mean comparison for the overall interactions based on slicing method at the 5% probability level according to LSM means. The value after (±) is standard error Zn₁, Zn₂ and Zn₃; 2, 4, and 6 g l⁻¹ ZnSO₄; Mn₁, Mn₂, and Mn₃; 2, 4, and 6 g l⁻¹ MnSO₄; B₁, B₂, and B₃; 2, 4, and 6 g l⁻¹ H₃BO₃; Control: distilled water

At the last sowing date (Dec. 15), foliar application of Mn (2 g l^{-1}) increased the seed yield by 41% compared to the control treatment (Table 5) and played a greater role in increasing the seed yield.

3.2 Oil Content

Foliar application of Zn (6 g l^{-1}) and B (6 g l^{-1}) at all levels of sowing dates produced the highest oil content (Table 5). When the high concentration of Zn, B, and Mn applied oil content was increased, the Zn and B had stronger effects in comparison to Mn (Table 5). On the first sowing date, the effect of Zn (6 g l^{-1}), Mn (6 g l^{-1}) and B (6 g l^{-1}) on increasing the oil content was 6.28%, 4%, and 6.08%, respectively. Nevertheless, under high-temperature conditions (Dec. 15), foliar application of Zn (6 g l^{-1}), Mn (6 g l^{-1}), and B (6 g l^{-1}) enhanced oil content by 7.6%, 5.04%, and 8.13%, respectively.

The regression results of oil content and seed yield with seed filling period (Fig. 1a) as well as the number of days with temperature less than 28°C (Fig. 1b) were positive. In addition, increasing the duration of seed filling and the number of days with a temperature of less than 28°C led to less oil content and seed yield. This happened while the regression relations between mean temperature (Fig. 1c) with oil content and seed yield were negative. In other words, the increase in temperature caused a reduction in oil content and seed yield. The oil's sensitivity to the higher temperature is more intense than that of the seed yield, in a way that with increasing the temperature from 27 to 29°C ; the seed yield did not show any change, while it caused a linear significant decrease in oil (Fig. 1c). The regression relationship between the seed yield and micronutrients showed that increasing the concentration of Zn, Mn, and B up to 4 g l^{-1} improved the seed yield (Fig. 2a) but the effects of micronutrients on oil content followed linear regression and high concentration of micronutrients (6 g l^{-1}) had a positive effect on the enhancement of oil content. Also, B had the greatest effect on increasing oil content (Fig. 2b). For a unit increase in Zn, Mn, and B concentrations, the oil content increased 0.44, 0.31, and 0.46%, respectively (Fig. 2b).

3.3 Palmitic Acid

Mean comparison of sowing date \times foliar application of micro-nutrients on palmitic acid during 2 years showed that the highest (4.09%) amount of palmitic acid belonged to the third sowing date (Dec. 15) and use of Zn (6 g l^{-1}). The lowest one was related to the first sowing date (Nov. 5) and foliar application of distilled water (Table 5). Under non-application of micro-nutrients, the amount of palmitic acid decreased at all sowing dates (Table 5). Increasing the

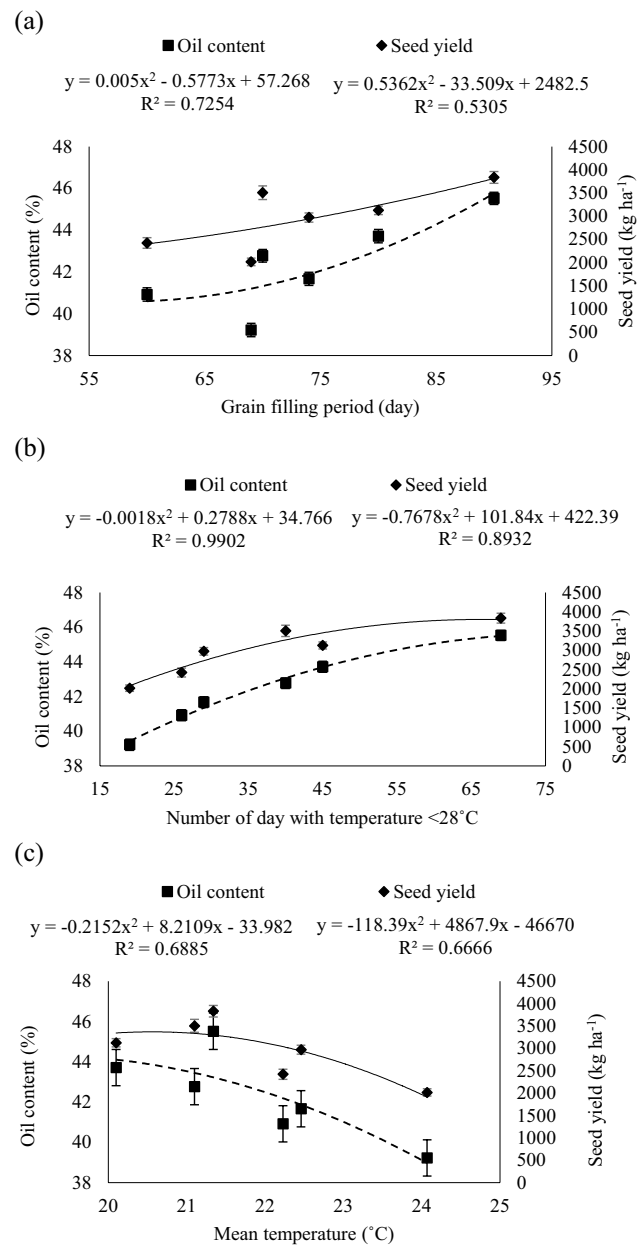


Fig. 1 Regression relationship of oil content and seed yield with seed-filling period (a), number of days with temperature $<28^\circ\text{C}</math> (b), and mean temperature (c) during seed-filling period. Means of interaction year \times sowing date were used (Each point shows mean three replications)$

amount of the application of micro-nutrients had a stronger effect on palmitic acid at all sowing dates. Results of slicing revealed that the maximum palmitic acid was related to foliar application of B (6 g l^{-1}) at the first (Nov. 5) and second (Nov. 25) sowing dates, but at the last sowing date (Dec. 15), the application of Zn (6 g l^{-1}) had the most influence on palmitic acid (Table 5). It seems that the reaction of palmitic acid was different from the foliar spray of micronutrients on different sowing dates.

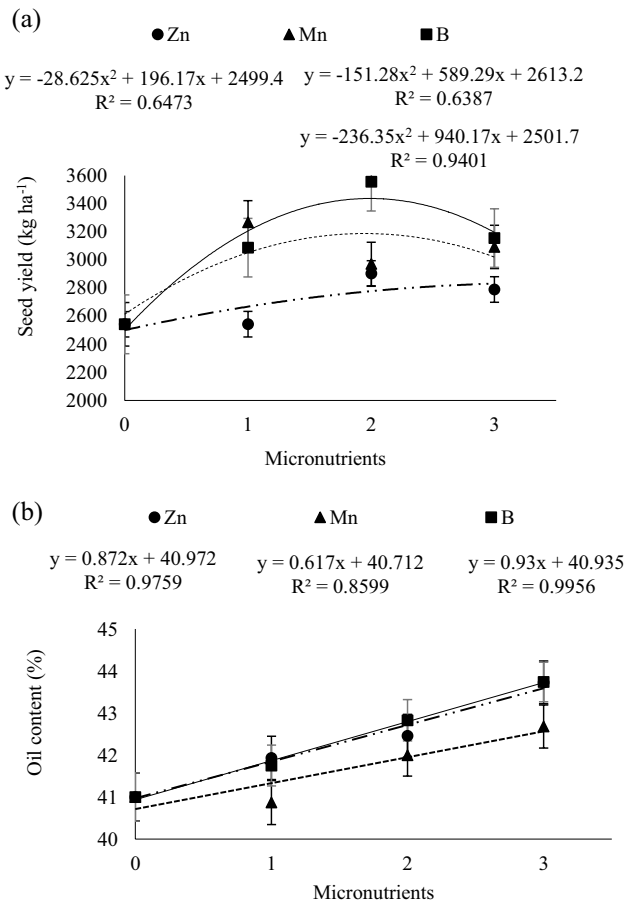


Fig. 2 Canola seed yield (a) and oil content (b) regression reaction to different micronutrients foliar application treatments (Each point shows mean three replications during 2 years) marked by (0): denoting control using distilled water, (1): 2 g l⁻¹ of ZnSO₄, MnSO₄, and H₃BO₃; (2): 4 g l⁻¹ of ZnSO₄, MnSO₄, and H₃BO₃; (3): 6 g l⁻¹ of ZnSO₄, MnSO₄, and H₃BO₃, respectively

3.4 stearic Acid

Results of slicing showed that the application of micronutrients did not have a significant effect on stearic acid at the first sowing date (Table 5). By delay in sowing date, the amount of stearic acid affected micro-nutrients and the highest amount of stearic acid was obtained by application of Zn (6 g l⁻¹) and B (6 g l⁻¹). With the value of 3.05%, foliar application of B (6 g l⁻¹) revealed the maximum amount of stearic acid at the third sowing date (Table 5).

There was a polynomial relationship between palmitic and stearic acid with maximum temperature (Fig. 3a) and the number of days with temperatures less than 28 °C (Fig. 3b). Increasing the maximum temperature led to more palmitic and stearic acids (Fig. 3a) while the number of days with a temperature of less than 28 °C caused less saturated acids (palmitic and stearic acids) (Fig. 3b). Palmitic acid was more sensitive to temperature changes

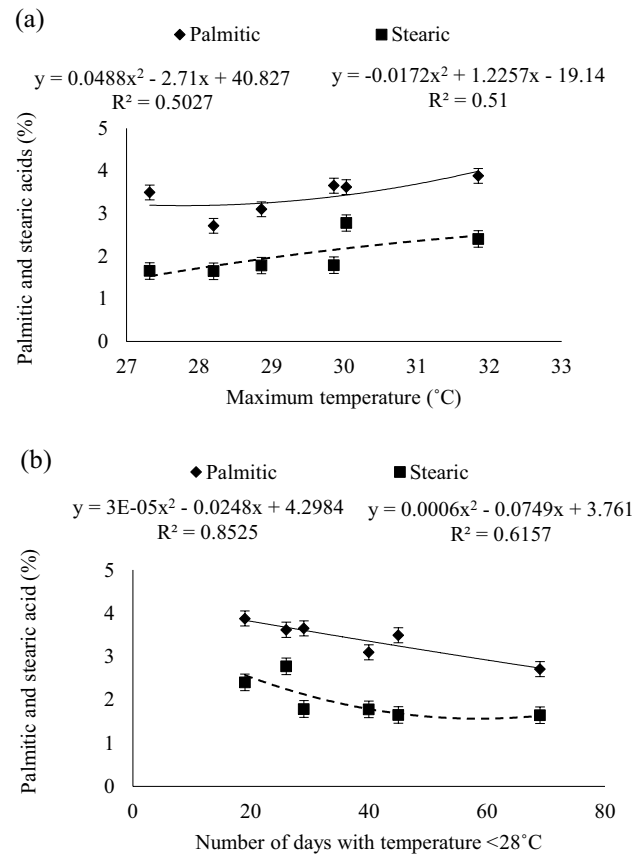


Fig. 3 Regression relationship between palmitic and stearic acids with maximum temperature (a) and number of days with temperature < 28°C during seed-filling period (b). (Each point shows mean three replications)

than stearic acid (Fig. 3a). Increasing the number of days with a mean temperature of less than 28 °C significantly reduced palmitic acid. An increase in the temperature of more than 30 °C caused a significant increase in palmitic acid but it did not cause any significant changes in the amount of stearic acid (Fig. 3b). The regression relationships between micronutrients and palmitic acid were polynomial. Higher micronutrients concentration led to more palmitic acid and B had the greatest effect on palmitic acid (Supplementary Fig. 3a). After the evaluation of the relationships between stearic acid and micronutrients, a positive relationship was found between them. For each unit increase in Zn, Mn, and B concentrations, stearic acid increased by 0.12, 0.08, and 0.13%, respectively. Changes in micro-nutrients application on palmitic acid content followed a similar trend, so it was not significantly affected by the 2 g l⁻¹ rate of application (Supplementary Fig. 3a). B had the most significant effect on stearic acid content and an increasing trend was observed at all levels of its application (Supplementary Fig. 4b).

3.5 Oleic Acid

In the first year, high temperature due to delay in sowing date increased the oleic acid by 6.71% and 9.66% in the second (Nov. 25) and the last (Dec. 15) sowing dates, respectively, while in the second year these amounts were 9% and 13.62%, respectively (Table 4). As shown in Table 5, the highest oleic acid (58.86%) at the third sowing date (Dec. 15) belonged to B (6 g l⁻¹) application. The lowest amount of oleic acid (50.52%) was obtained at the first sowing date (Nov. 5) and application of 6 g l⁻¹ of B (Table 5). Slicing findings showed that the effects of micro-nutrients could be different at various sowing dates. On the timely sowing date (Nov. 5), the application of Zn (6 g l⁻¹) and B (6 g l⁻¹) produced the maximum oleic acid. Also, in the second sowing date, the maximum oleic acid belonged to B (6 g l⁻¹) treatment. On the delayed sowing date (Dec. 15), the highest oleic acid obtained by foliar application of Zn (4 g l⁻¹), Mn (6 g l⁻¹), and B (6 g l⁻¹), and all of them belonged to the same statistical group (Table 5). The interaction sowing date × foliar application revealed that higher temperature during the seed filling period in the last sowing date and the use of Zn (4 g l⁻¹), Mn (6 g l⁻¹), and B (6 g l⁻¹) augmented the oleic acid by 1.68%, 1.68% and 2.12%, respectively (Table 5).

3.6 Linolenic Acid

The highest linolenic acid (7.09%) was observed at the last sowing date (Dec. 15) and foliar application of Zn (6 g l⁻¹). The lowest ones belonged to the first sowing date (Nov. 5) and the application of distilled water (Table 6). Slicing showed that foliar application of Zn (6 g l⁻¹) produced the maximum linolenic acid in all sowing dates. Increasing temperature during seed filling period in the last sowing date decreased the linoleic acid by 15.44% and 23.16%, respectively in the first and second years (Table 4). Mean comparison for the interaction effect of sowing date and micro-nutrients showed that linoleic acid (24.88%) was higher in the first sowing date and foliar application of B.

3.7 Linoleic Acid

Mean comparison between treatments according to the slicing method showed that the highest oleic acid was obtained by foliar application of B (6 g l⁻¹) at the first and second sowing date. However, in the last sowing date, the use of B (4 and 6 g l⁻¹), Mn (6 g l⁻¹), and Zn (6 g l⁻¹) had also some positive effects on increasing linoleic acid (Table 5). The interaction sowing date × foliar application demonstrated that the use of Zn (6 g l⁻¹), Mn (4 g l⁻¹), and B (6 g l⁻¹) increased the oleic acid by 10.78%, 8.54%, and 11.85%, respectively in the first sowing date. Application of

Zn (4 g l⁻¹), Mn (6 g l⁻¹), and B (6 g l⁻¹) increased the oleic acid by 2.91%, 2.14%, and 6.86%. In the last sowing date (Dec. 15), use of Zn (6 g l⁻¹), Mn (6 g l⁻¹) and B (6 g l⁻¹) elevated the oleic acid by 5.66%, 5.03%, and 5.75%, respectively (Table 5).

The regression relationship between oleic acid and seed filling period showed that the maximum oleic acid was obtained at nearly the seed filling period by 60–65 days. Extending the grain-filling period increased the percentage of linoleic acid and decreased the oleic and linolenic acids (Fig. 4a). The oleic acid changes were nearly constant during 55–65 days (Fig. 4a), but by increasing the length of the seed filling period and decreasing the mean temperature (Fig. 4b), the oleic acid percentage decreased. The accumulation trends of oleic and linoleic acids were not similar to each other. Longer seed filling periods up to about 75 days led to an initial increase and a subsequent decrease in oleic acid content but this was not true for linoleic acid in which case its content increased indefinitely regardless of how long the seed filling period was extended (Fig. 4a). The regression revealed that the highest linolenic acid was obtained at the seed filling period of 60–65 days (Fig. 4a). The highest linoleic acid was achieved at a mean temperature of nearly 20–22 °C (Fig. 4b). The relationship

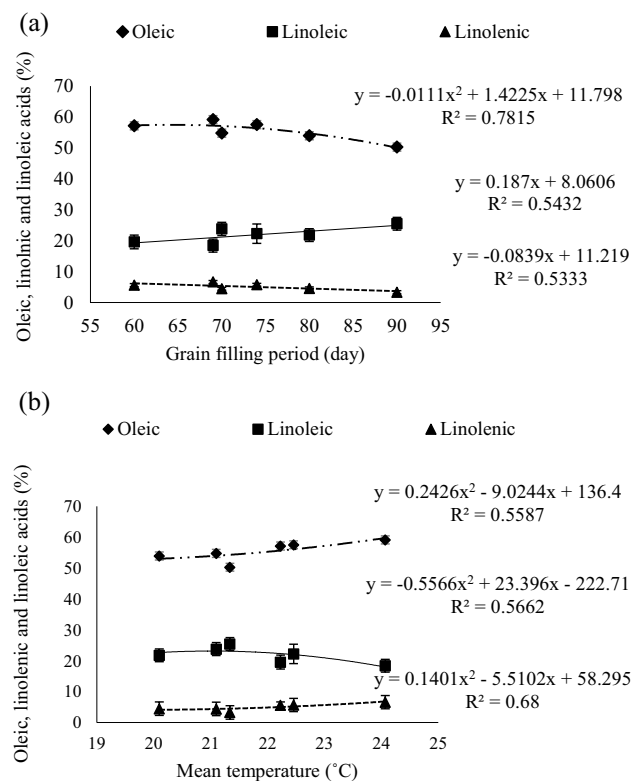
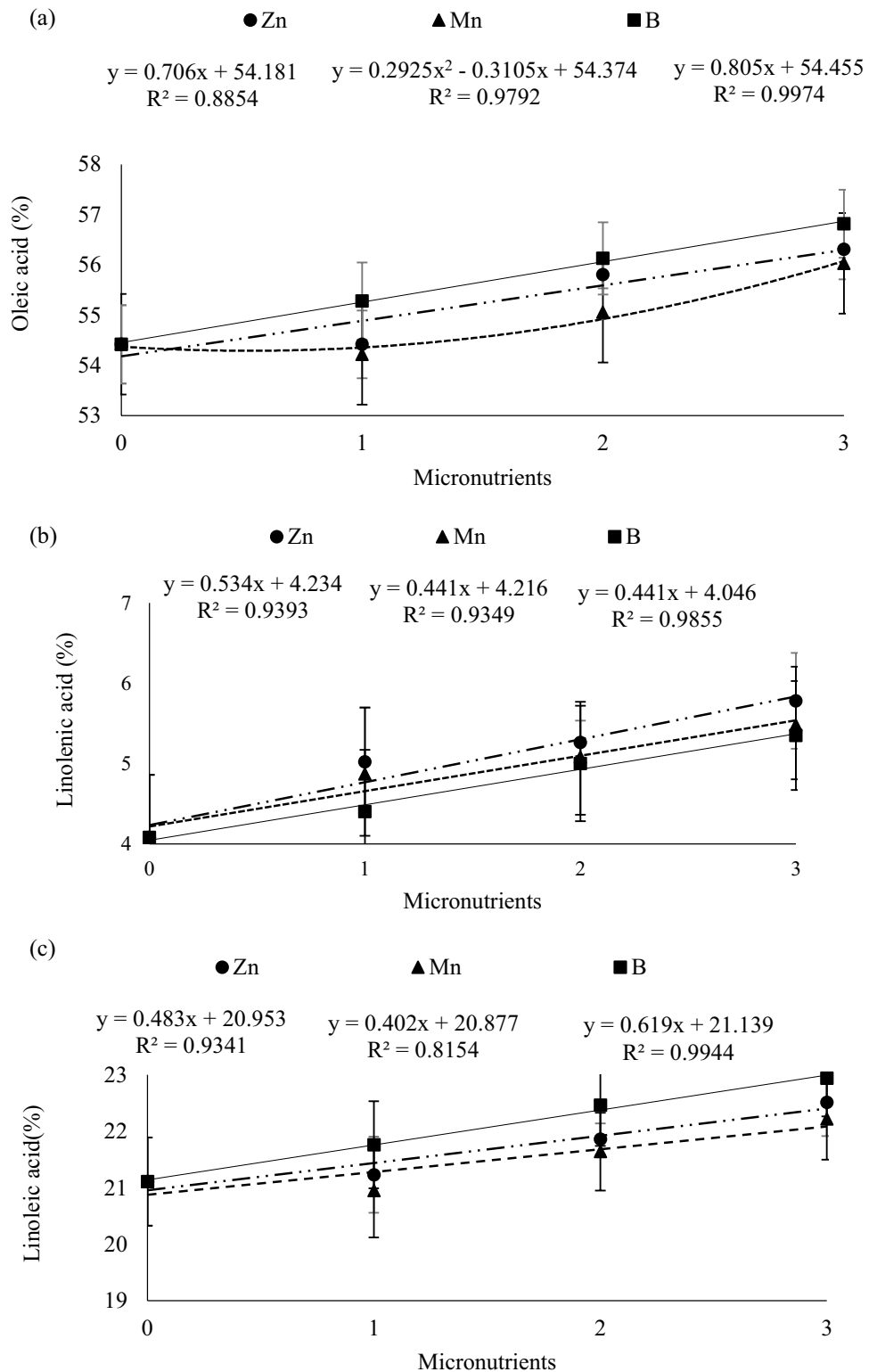


Fig. 4 Regression relationship of oleic, linolenic, and linoleic acids with seed-filling period (a) and mean temperature (b) during seed-filling period (each point shows mean three replications)

was significant between maximum temperature with oleic, linolenic and linoleic acid during the seed filling period and this regression showed 0.67, 0.63, and 0.75% of the variation in oleic, linoleic, and linolenic, respectively.

Positive correlations were observed between micronutrients and unsaturated acids (oleic, linolenic and linoleic acids). Between micro-nutrients, B was more effective on oleic acid (Fig. 5a) and linoleic acid (Fig. 5c), but Zn had more effect on linolenic acid (Fig. 5b). Enhancement

Fig. 5 Oleic acid (a), linolenic acid (b), and linoleic acid (c) regression reaction to different micronutrients foliar application treatments (each point shows mean three replications during two years) marked by (0): denoting control using distilled water, (1): 2 g l⁻¹ of ZnSO₄, MnSO₄, and H₃BO₃; (2): 4 g l⁻¹ of ZnSO₄, MnSO₄, and H₃BO₃; (3): 6 g l⁻¹ of ZnSO₄, MnSO₄, and H₃BO₃, respectively



in oleic, linolenic, and linoleic acids as a result of foliar application of Zn, Mn, and B was strongly and positively correlated with a higher concentration of the mentioned elements (Fig. 5a–c).

3.8 Erucic Acid

Mean interaction between sowing date and foliar application showed that the highest erucic acid (0.244%) was related to the combination of the first sowing date (Nov. 5) and foliar application of distilled water (Table 5). Slicing revealed that the use of Mn (2 g l^{-1}) produced the maximum amount of erucic acid at the second (Nov. 25) and the third sowing date (Dec. 15). The minimum amount of produced erucic acid at all sowing dates belonged to the foliar application of B (6 g l^{-1}).

3.9 Glucosinolate

Response of glucosinolate amount to the foliar application of micro-nutrients at different sowing dates was various. Slicing showed that a delay in sowing reduced glucosinolate amount, and foliar application of B (6 g l^{-1}) led to making the lowest amount of it at all sowing dates (Table 5). The absence of micro-nutrients increased glucosinolate amount at the first (Nov. 5) and second (Nov. 25) sowing dates, but on the last sowing date (Dec. 15), the use of micro-nutrients did not decrease glucosinolate in comparison to the control treatment (Distilled water) (Table 5).

Regression showed that the effect of the seed filling period on erucic acid was significant (Fig. 6a), but the mean temperature (Fig. 6c) did not have a significant effect on erucic acid. The relationship between erucic acid and glucosinolate with seed filling period was described by polynomial equations (Fig. 6a). A longer seed filling period due to lower average temperatures (Fig. 6a) and more days with less than 28°C temperature (Fig. 6b); decreased the glucosinolate amount while increasing average temperatures of up to 22°C led to a decrease and subsequent increase in erucic acid content. An increase in the seed filling period up to 80 days led to an increase in erucic acid content while longer grain-filling periods harmed it. The regression analysis showed that increasing the concentration of Zn and Mn mitigated the amount of erucic acid. Reduction of the applied concentration of B at nearly 4 g l^{-1} led to less erucic acid, but a larger concentration boosted the production of the erucic acid. There was a strong negative relationship between glucosinolate amount with Zn and B. For each unit increase in Zn and Mn concentrations, glucosinolate decreased 0.28 and 0.24, respectively. Application of B in amounts greater

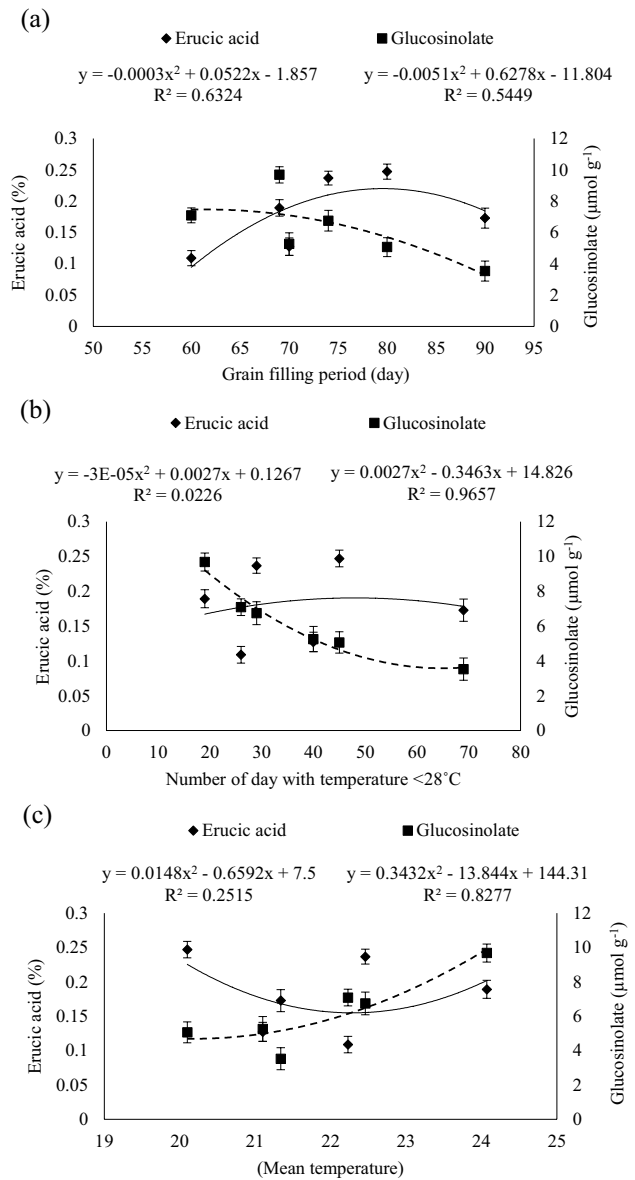


Fig. 6 Regression relationship of erucic acid and glucosinolate with seed-filling period (a), number of days with temperature $<28^\circ\text{C}$ (b), and mean temperature (c) during seed-filling period (each point shows mean three replications)

than 2 g l^{-1} led to more glucosinolate. All micro-nutrients reduced the amount of the erucic acid and glucosinolate but B was more effective in reducing them (Supplementary Fig. 4a, 4b).

3.9.1 Relationship Between Oil Content and Fatty Acids

Oil content had a negative relationship with stearic and palmitic acids. For each unit of increase in stearic and palmitic acids, the oil content decreased by 0.16 and 0.17%, respectively (Supplementary Fig. 5a). There was a positive

relationship between oil content and linoleic acid. For each unit of increase in linoleic acid, the oil content increased by 1.07%. The relationship between oil content with oleic and linolenic acids was negative. With more oleic and linolenic acids, the oil content was reduced by 1.4 and 0.52%, respectively (Supplementary Fig. 5b). An increase in temperature led to less oil content but it increased the oleic and linolenic acid while it decreased the linoleic acid. Therefore, increasing the oil percentage which occurs at lower temperatures has less saturated fatty acids and more linoleic acid.

3.9.2 Principal Component Analysis

Principal component analysis showed that all changes between data were expressed by both the first and the second principal components (69% and 17% for PCA₁ and PCA₂, respectively). Palmitic acid, stearic acid, oleic acid, linolenic acid, and glucosinolate had a positive coefficient and seed yield, oil content, and linoleic acid had a negative coefficient in PCA₁. As shown in Fig. 7, erucic acid had a positive coefficient in PCA₂, while linoleic acid had a slightly negative coefficient in PCA₂. Therefore, a biplot diagram along with the vector of the investigated traits was drawn to determine the best sowing date and application level of micronutrients based on PCA₁ and PCA₂ (Fig. 7). The distance between the treatment levels and the trait vector indicates their relationship with the desired trait and also the length of the standard deviation of

the trait (Mishra et al., 2017). In this cosine diagram, the angle between the vectors of two attributes represents their correlation coefficient. Therefore, the proximity of the vectors of oleic acid and linolenic acid traits indicates a high correlation between these traits. At an obtuse angle between glucosinolate, stearic acid, palmitic acid, oleic acid, and linolenic acid with seed yield, oil content, and linoleic acid indicated inconsistent changes among these traits (Fig. 7). Considering PCA₁, treatments in the third sowing date had the highest Eigenvalue and had the maximum amount of oleic acid and linolenic acid (data not shown). Therefore, PCA₁ showed the oil quality and choice based on it leads to achieving oil with high quality. Based on this, the highest quality of the oil is obtained on the third sowing date, and comparing micronutrient levels, S₃Zn₂ and S₃Zn₃ treatments had the highest oil quality. Also, vectors of seed yield, oil content, and linoleic acid had a negative correlation with the mentioned traits. Treatment levels on the first sowing date had the lowest PCA₁ and the highest oil production (seed yield and oil content) as well as S₁B₁ and S₁B₂ produced the maximum oil content.

4 Discussion

Heat stress during and after flowering affects silique development and decreases the seed yield as well as photo-assimilates for triacylglycerol biosynthesis and oil accumulation

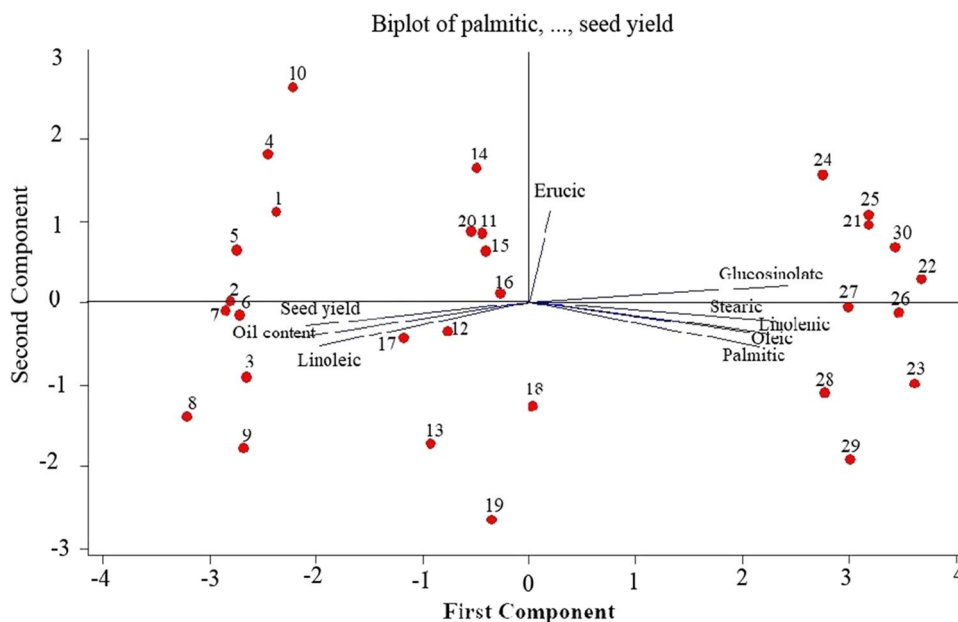


Fig. 7 Graphical biplot for 3 sowing dates and 10 levels of micronutrients on the basis of first and second principal components. 1: S1Zn1, 2: S1Zn2, 3: S1Zn3, 4: S1Mn1, 5: S1Mn2, 6: S1Mn3, 7: S1B1, 8: S1B2, 9: S1B3, 10: S1Control. 11: S2Zn1, 12: S2Zn2, 13: S2Zn3, 14: S2Mn1, 15: S2Mn2, 16: S2Mn3, 17: S2B1, 18: S2B2, 19: S2B3, 20: S2Control. 21: S3Zn1, 22: S3Zn2, 23: S3Zn3, 24: S3Mn1,

25: S3Mn2, 26: S3Mn3, 27: S3B1, 28: S3B2, 29: S3B3, 30: S3Control. Note: S1, S2 and S3 indicate different sowing dates (Nov. 5, Nov. 25 and Dec. 15, respectively) while Zn, Mn and B numbered as 1 to 3 show the foliar application of ZnSO₄, MnSO₄ and H₃BO₃ at concentrations of 2, 4 and 6 g l⁻¹, respectively. Distilled water was used as control

in seeds (Pokharel et al. 2020). Delaying in sowing date in autumn caused to coincide the stages of flowering and seed filling stages to high-temperature stress in April and May. The maximum temperature in flowering stage were increased by 0.9 and 4.2 °C and the grain filling period were enhanced by 1.6 and 3.2 °C on the second and third sowing dates, respectively. Therefore, mean duration of the flowering stage were decreased by 10 and 25 days and the grain filling period duration was reduced by 13 and 20 days on the second and third sowing dates, respectively. The difference between studied traits can be attributed to different temperature during 2 years, especially during silique stage and grain filling period in delayed sowing dates (Dec. 15). Therefore, the appropriate temperature during silique and grain filling period let to optimal growth of canola and more seed yield in the second year compared with the second year. These results are in line with Shiranirad et al. (2021) and Zinn et al. (2010) who report that high temperature can have harmful effects on reproductive stages and decrease seed yield of canola.

The process of seed construction in oil crops was done after pollination and fertilization, in seed formation stage (Miklaszewska et al. 2021). Heat stress deprives the plants of performing to their genetic potential (Ahmad et al. 2021a) and some researchers reported that sugar limitation (Farooq et al. 2017) photo-assimilates reduction for triacylglycerol biosynthesis (Pokharel et al. 2020), slows down of oil biosynthesis pathway activity (Iyer et al. 2008; Baud and Lepiniec 2010), and reduces seed oil percentage in high-temperature stress. Decreasing of the grain filling period from 90 to 60 days, reduced seed yield and oil content were 10 and 37%, respectively. By reducing the number of days with temperature less than 28 °C from 69 to 19 days, oil content and seed yield were decreased by 14 and 48%, respectively. The rate of reduction for seed yield and oil content was 36% and 11% in 24 °C compared with 20 °C. Therefore, the sensitivity of seed yield was more than oil content to high-temperature stress. Thus, a delay in the sowing date due to high temperature during the seed filling period decreased the phenological period and reduced the seed yield and oil content.

Seed oil content was decreased by sowing delaying and high-temperature intensity. Micronutrients have a favorable effect on essential oil production because they act as a cofactor of various antioxidative enzymes (Kumar et al. 2022; Bhat et al. 2020); it is involved in the mechanism of stress tolerance of higher plants (Bhat et al. 2020). Increasing of micronutrients concentration by foliar spraying linearly improved the oil content. However, Zn and B had more effective than Mn on oil content. The results supported the hypothesis that foliar application of micronutrients could be effective on improving seed qualitative and quantitative characteristics of canola and on alleviating the harmful

effects of high temperature. Plants employ adaptive mechanisms to detoxify heat stress damage (Ahmad et al. 2021b). Previous experiments have also pointed to the beneficial role of micronutrients (Zn, B, and Mn) in improving the seed yield and oil content (Ashkiani et al. 2020; Manaf et al. 2017; Jankowski et al. 2019; Sehgal et al. 2018). As it was mentioned in the results, exposure to high-temperature stress may aggravate the seed yield and oil content under no application of micronutrients and application of Zn, Mn, and B increased seed yield and oil content in all sowing dates.

The use of Zn increases the mass weight of canola and as a result increases the production of carbohydrates. Ultimately, the oil content rises on the optimum sowing date (Mozafari et al. 2022) and these findings are in line with present results. The stability of enzymes controlling oil accumulation may have differential sensitivities to high-temperature stress (Aksouh-Harradj et al. 2006) and it was determined that increasing temperature leads to a reduction in the oil content (Hammac et al. 2017). Therefore, Zn deficiency can decrease the oil content under a delay in the sowing date (Mozafari et al. 2022; Shoja et al. 2018). B helps to alleviate high-temperature stress in plants since it can participate in several physiological processes, such as chlorophyll biosynthesis, sugar mobilization, and pollen viability (Sarwar et al. 2019; Calderón-Páez et al. 2021), and the positive role of B in improved oil content and seed yield under heat stress may attribute to cell wall synthesis (Bellaloui et al. 2015) which leads to producing oil content (Ahmed et al., 2013) and fatty acids (Bellaloui et al. 2013). The increase in the oil content with B application may be due to a richer assimilate, accelerated total photosynthesis, and more effective translocation of photoassimilates (Mehmood et al. 2021).

Although oil content and composition are genetically determined in canola, they are affected by temperature during seed development (Pokharel et al. 2020). Palmitic and stearic acids are the main saturated acids (Badawy et al. 2016), and the results of this study indicated that delayed sowing date increased these saturated acids. The results of present study were similar to those previously reported that fatty acids profile changed and saturated fatty acids increased under high-temperature conditions (Elferjani and Soolanayakanahally 2018; Pritchard et al. 2000). The response of palmitic acid to foliar application of Zn at the first sowing date was more than on the second (Nov. 25) and third (Dec. 15) sowing dates in which the temperature was higher during the seed filling period. Micro-nutrients play a crucial role in metabolic processes, enzyme activities and Zn improved metabolism of fatty acids, especially stearic and palmitic acid (Shahsavari et al. 2014; Bybordi and Mamedov 2010). As found, a higher concentration of B increases the amount of palmitic and stearic acids, which could be due to the greater ability of canola to absorb this element to enhance these saturated acids. The results of

this research were similar to other findings where higher temperature increased the saturated fatty acids (Aslam et al. 2009; Elferjani and Soolanayakanahally 2018; Pokharel et al. 2020; Pritchard et al. 2000).

Lipid biosynthesis is related to enzymes which are sensitive to high temperatures and reduces oil synthesis (Elferjani and Soolanayakanahally 2018). High temperature affected oil formation and its composition for oilseeds (Schulte et al. 2013). The enzymes that have double bonds (unsaturation) in the fatty acyl chains (Narayanan et al. 2020), can be targets of high-temperature stress (Lanna et al. 2018). Fatty acids including Oleic, linoleic, and linolenic were affected by the high temperatures during grain filling period (Matías et al. 2021). How temperature affects fatty acid profiles depends on the severity and timing of higher temperatures. Extremely high temperatures (38 °C for 5 h, 25–29 day after flowering) elevated oleic acid and reduces linoleic acid (Aksouh-Haradj et al. 2006) and these results were consistent with the finding of the present study that high temperature occurred during the seed filling period at the delayed sowing date (Nov. 15 and Dec. 15). The effect of heat stress due to a delay in the sowing date increased the oleic acid by 8% and 11% in the second (Nov. 25) and third (Dec. 15) sowing dates, but the linoleic acid was reduced by 2.6% and 19%, respectively. The positive effect of Zn, Mn, and B on augmenting the oleic acid was 3.5, 3, and 4.5% in comparison with the control treatment, respectively. From this, it can be concluded that the effect of temperature on changes in fatty acids such as oleic acid is greater than a foliar application of micronutrients. An increase in oleic acid might be due to the limitation of the conversion of oleic acid as a precursor of linoleic acid, thereby the percentage of oleic acid increased and linoleic acid decreased under high temperatures (Dornbos and Mullen 1992). Cytololic desaturation of oleic acid to form linoleic is mediated by the enzyme oleate desaturase and the activity of this enzyme was put forward as an interpretation for shifts in oleic acid/linoleic acid ratio under drought and heat stresses (Flagella et al. 2002).

The use of Zn, Mn, and B (6 g l⁻¹) increased the amount of the oleic acid at three sowing dates. The positive effect of micronutrients in increasing the amount of oleic acid under heat stress conditions (Dec. 15) was slight compared to the optimum sowing date (Nov. 5). Regarding micro-nutrients, it was reported that Zn lessens the negative effect of late-season temperature stress on oleic and linoleic (stating resistance to high temperature) and as a result improves the oil quality (Ashkiani et al. 2020). The increase in oleic acid and change in other fatty acids by foliar application of B may be due to the effect of B on the activity of the fatty acid desaturases which control the accumulation and conversion of unsaturated fatty acids (Bellaloui et al. 2010, 2013). The role of Mn in the metabolism of lipid is more complex and it increases the amount of typical thylakoid membrane constituents such

as polyunsaturated acids (Salama et al. 2015). Moreover, the direct involvement of Mn in the biosynthesis of fatty acids may be a contributing factor (Broadley et al. 2012).

Erucic acid is one of the most important fatty acids that affects the quality of canola oil and human health, and its amount is influenced by genotype, nutrition and environment conditions (Mokhtassi-Bidgoli; Shiranirad et al. 2021). We found that a delay in the sowing date led to decrease the erucic acid in the first year, but increased it in the second year, which shows that in addition to the sowing date, other environmental parameters can alter the erucic acid. Gharechaei et al. (2019) found that the delay in sowing dates led to a decrease in erucic acid. Unlike erucic acid, an increase in temperature caused by a delay in the sowing date led to more glucosinolate amounts. Hot temperature (40 °C) from 29 to 34 days after flowering produces higher glucosinolate than a moderate temperature 21/16 °C (day-time maximum/night-time minimum) (Aksouh et al. 2001). A significant reduction in the erucic acid concentration of canola in reaction to a foliar spray of B and Zn was reported by Mei et al. (2009) and the results of the present study are in line with the findings of Mei et al. (2009). High-B availability could produce lower glucosinolate (Tian et al. 2020) and this is in agreement with our findings. In this research, the content of erucic acid (less than 2%) and glucosinolate (less than 30 μmol g⁻¹) had been within the standard range (Amiri et al. 2020).

5 Conclusion

Oil production is a regular process and total oil content results from the synthesis of different fatty acids, including saturated and unsaturated acids. It was observed that delaying the sowing date caused an increase the air temperature from 27 to 32 °C during the seed filling period, which had a different effect on the seed yield and oil quality. High temperature reduced the seed yield and seed oil percentage but increased the oil quality by an enhancement in oleic acid by 11%. Foliar application of zinc (6 g l⁻¹) and boron (6 g l⁻¹) was more effective to increase oleic acid in optimum sowing date (Nov. 5), but under high-temperature conditions (Dec. 15), foliar application of zinc (4 g l⁻¹), manganese (6 g l⁻¹), and boron (6 g l⁻¹) had the same effect in increasing oleic acid. In short, the present experiment suggested that foliar application of micronutrients helped to mitigate high-temperature adversities in seed yield and oil content. Generally, the following treatments are recommended for hot and dry regions: Nov. 5 sowing date × foliar application of boron (4 g l⁻¹) due to greater seed yield as well as oil content and Dec. 15 sowing date × foliar application of boron (6 g l⁻¹), Zn (4 g l⁻¹), and manganese (6 g l⁻¹) because of high oleic acid as qualitative indexes.

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Author Contribution Seyed Ahmad Kalantar Ahmadi: conceptualization, methodology, field practices, writing- original draft preparation; Jahanfar Daneshian: software, writing-review and editing.

Declarations

Competing Interests The authors declare no competing interests.

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