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Influence of seeding density on seed and oil yield, and fatty acid composition of white mustard (*Sinapis alba* L.)

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Influence of seeding density on seed and oil yield, and fatty acid composition of white mustard (*Sinapis alba* L.)

Abstract: The aim of this study was to determine the effects of seeding density on the seed yield of white mustard, the oil yield and fatty acid composition under the agroecological conditions of the northwestern Republic of Croatia. The field trials were conducted at the experimental station of the Faculty of Agriculture, University of Zagreb (45° 48' N, 16° 05' E) during two growing seasons. The trial included four seeding densities of white mustard: 50, 70, 90 and 110 germinable seeds m^{-2}. The trial was set up using a randomized block design with five replications. The highest seed yield was obtained with a seeding density of 110 germinable seeds m^{-2}, with no significant differences between seeding rates of 70 and 90 germinable seeds m^{-2}. The average oil content during researched years varied from 23.97 % in the seeding density of 50 germinable seeds m^{-2} to 24.37 % in the seeding density of 90 germinable seeds m^{-2}. The higher oil yield was achieved in 2014 due to the higher oil content in the seed that year. Regarding fatty acid composition, erucic acid was dominant along with oleic acid, linoleic acid, and linolenic acid.

Key words: alternative oilseeds, mustard, oil content, erucic acid, biodiesel, lubricants

Vpliv gostote setve na pridelek semena in olja ter sestavo maščobnih kislin pri beli gorjušici (*Sinapis alba* L.)

Izvleček: Namen raziskave je bil določiti vpliv gostote setve na pridelk olja in semena ter sestavo maščobnih kislin bele gorjušice v agroekoloških razmerah severozahodne Hrvaške. Poljski poskus je potekal na poskusni postaji Agronomske fakultete Univerze v Zagrebu (45° 48' N, 16° 05' E) v dveh rastnih sezonah. Poskus je obsegal štiri gostote semen bele gorjušice in sicer 50, 70, 90 in 110 kaljivih semen m^{-2}. Poskus je bilo zasnovan kot naključni bločni poskus s petimi ponovitvami. Največji pridelek semen je bil dosežen z gostoto setve 110 kaljivih semen m^{-2}, vendar brez značilnih razlik z gostotama setve 70 in 90 kaljivih semen m^{-2}. Poprečna vsebnost olja je v letih poskusa variirala od 23,97 % pri gostoti setve 50 kaljivih semen m^{-2} do 24,37 % pri gostoti setve 90 kaljivih semen m^{-2}. Največja vsebnost olja je bila dosežena v letu 2014 zaradi nasplošno največje vsebnosti olja v tem letu. Glede sestave maščobnih kislin je bila dominantna erucična kislina, ki so ji sledile oleinska, linoleična in linolenska kislina.

Ključne besede: alternativne oljarice, gorjušica, vsebnost olja, erucična kislina, biodiezel, lubricants

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1 INTRODUCTION

White mustard (*Sinapis alba* L.) is an annual plant from the Brassicae family that originates from the Mediterranean region (Sawicka & Kotiuk, 2007). It is an oilseed crop with great potential as a spring alternative crop in Europe as it has greater tolerance to stressful environments than rapeseed (Gunasekera et al., 2006a).

Besides seed production for processing to oil, white mustard has significant agronomy importance due to its ability to improve soil structure, its fertilizing and phytosanitary effects (Toboła, 2010) and its ability of heavy metal phytoextraction from the soil (Evangelou et al., 2007). Therefore, it is often used as green manure and intercrop.

White mustard seeds are also used as a condiment, hot dog mustard, salad dressing, natural food preservative, and food additive in the food processing industry (Rahman et al., 2018). In addition, the seeds are used as drug components in phytotherapy due to their analgesic, antiproliferative, antiviral and antimicrobial properties (Peng et al., 2013; Boscaro et al., 2018).

According to Ciubota-Rosie et al. (2013), mustard seeds have a high energy content with an oil content of 28–45 % and a relatively high protein content. Due to the high concentrations of erucic acid and the high content of glucosinolates in the fat-free seed residues, mainly sinalbin, traditional white mustard cultivars are not widely used in the production of foodstuffs (Jankowski et al., 2015). The strongest objection to the use of high erucic oil has been linked with its cardiotoxic potential (Galanty et al., 2023). High glucosinolates in livestock feed have adverse effects including reduced feed intake and growth, gastrointestinal irritation, goiter, anaemia, and hepatic and renal lesions (Bischoff, 2021).

Erucic acid is an oleochemical feedstock which is converted into erucamide and behenyl alcohol. Erucamide is used as a processing aid in the manufacture of polyethylene film, while behenyl alcohol is an emulsifier, viscosity regulator, or emollient in many cosmetic products (Hebard, 2016). Therefore, the oil extracted from the white mustard seed is used for industrial purposes, usually as a lubricant (Falasca & Ulberich, 2011). The vegetable oils are preferred over mineral oil as lubricating base oil due to their high biodegradability, renewability and low toxicity (Sajeeb & Krishnan, 2019) In addition, high erucic acid oils have a high degree of lubricity (Aukema & Campbell, 2011).

Recent studies also indicate the possibility of using white mustard oil as a feedstock for biodiesel production (Ciubota-Rosie et al., 2013; Sultana et al., 2014; Ambrosewicz-Walacik et al., 2015). An important advantage of using non-edible white mustard oil as an alternative feedstock for biodiesel production is that it does not compete with its use as food.

Plant density is one of the most important agronomic measures that determine grain yield, as it effects plant growth and development. It is well known that evenly distributed plants use land, light and other resources evenly and efficiently. A higher plant population per unit area, beyond an optimal limit, leads to competition among plants for natural resources, which results in weaker plants and can cause severe lodging (Kumar et al., 2004). According to Shekhawat et al. (2012), the optimal plant population density per unit area varies with the environment, genotype, sowing date, and growing season.

Important agronomic properties for oilseed crops, besides seed yield, are also oil yield and oil quality. A great influence on oil content and fatty acid profile has genetic base and environment and their interaction (Gunasekera et al., 2006b; Zhang et al., 2015), as an agronomic management (Shekhawat et al., 2012).

Therefore, the objective of this study was to determine the effects of seeding density on the yield potential of white mustard and to determine oil yield and quality under agroecological conditions in northwestern Croatia.

2 MATERIALS AND METHODS

The field trials were conducted at the experimental station of the Faculty of Agriculture, University of Zagreb (45° 48′ N, 16° 05′ E) during two growing seasons (2014 and 2017). The trial involved four seeding densities of white mustard: 50, 70, 90, and 110 germinable seeds m^{-2} i. e. 3 kg ha^{-1}, 4 kg ha^{-1}, 5 kg ha^{-1} and 6 kg ha^{-1} were used. A local landrace grown in the Brod-Posavina Country, Croatia was used for sowing. The trial was set up using a randomized block design with five replications. The plot size was 6.6 m^2 (5.5 m x 6 rows x 20 cm).

Sowing was performed on April 14, 2014, and March 23, 2017 by the "Wintersteiger" plot seeder. The previous crop in 2014 was spelt, and in 2017 year was a mixture of wheat and peas. Fertilization was carried out with basic tillage by application of 400 kg ha^{-1} NPK 7:20:30 fertilizer (28 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5 and 120 kg ha^{-1} K_2O). Topdressing was applied at the stage of six leaves with 100 kg ha^{-1} CAN (27 % calcium ammonium nitrate).

The weed control was carried out with 1.3 l ha^{-1} Butisan (active substance metazaklor 500 g l^{-1}) and with 0.8 l ha^{-1} Agil 100 EC (active substance propaqizafop 100 g l^{-1}).

Control of flea beetles (*Phyllotreta* spp.) was performed three times in vegetation with insecticides Chromorel D (active substance chlorpyrifos 500 g l^{-1} + cypermethrin 50 g l^{-1}) in the doze of 0,5 1 ha $^{-1}$, Karate Zeon (active substance lambda-cyhalothrin 50 g l^{-1}) in the dose of 0,15 1 ha $^{-1}$ and Rotor 1.25 EC (active substance deltamethrin 25 g l^{-1}) in the dose of 1 ha $^{-1}$.

The harvests were carried out with harvester "Winterstaiger" at the stage of horticultural maturity (July 24, 2014, and July 10, 2017) when seed moisture was below 12 %. The seed yield was calculated based on 9 % moisture and 2 % impurity content.

The oil content was determined in the Laboratory for Oil and Fat Technology at the Faculty of Food Technology and Biotechnology, University of Zagreb, on an average sample of five repetitions according to standard ISO 659:2009, the method according to Soxhlet (International Organization for Standards , 2009). Oil content was reported on a dry matter basis.

Fatty acids were determined by their methyl esters according to standard ISO 5509:2000 (International Organization for Standards , 2000) using a gas chromatograph (ATI Unicam 610, Cambridge, England) with capillary column TR-FAME (Thermo Scientific, Waltham, MA, USA) (30 m x 0.22 mm thickness the film of 0.25 µm; stationary phase: 70 % cyanopropyl-polisilfenilen siloxane) and FID detector (flow rate of 0.7 ml min^{-1}, helium carrier gas, injector temperature 250 °C, split: 1:75, detector temperature: 280 °C, the amount of sample injected: 1.0 µl) with the programmed column temperature 120 °C to 160 °C-4°C min^{-1}, 160 °C to 190 °C–10 °C min^{-1} at 190 °C was maintained for 10 min. Identifying individual fatty acids was carried out by comparing the retention time of methyl esters of certain fatty acids with the retention times of a standard mixture of methyl esters of fatty acids (F.A.M.E.) of known composition. Computer-selected method of normalization of peak areas was used to calculate the quantitative composition of fatty acids. Obtained data for fatty acid composition were analysed by descriptive statistics.

The obtained data for seed yield and oil yield were statistically analyzed through variance analysis using DSAASTAT (Onofri, 2007). The statistically significant differences were tested by the LSD test at 5 % probability.

2.1 SOIL CHARACTERISTICS

The trials were conducted on an anthropogenic eutric cambisol. The upper soil layer was neutral (pH in 1M KCl = 7.09), poorly supplied with humus (2.34 %), and well supplied with nitrogen (0.12 %) The soil was richly supplied with the plant-available phosphorous (AL-P_2O_5 = 37.03 mg 100 g soil^{-1}) and potassium (AL-K_2O = 16.20 mg 100 g soil $^{-1}$).

2.2 WEATHER CONDITIONS

A mean decade and monthly air temperatures and precipitation from March to July (during the growing season of mustard) in the years of research and a long-term average (1981–2010) for weather station Zagreb–Maksimir are given in Table 1.

In 2014 year, the total amount of precipitation during the growing season was higher by 54.2 % and the mean monthly temperature was higher by 1.2 °C than the long-term average.

The deficiency of precipitation in this growing season was noted only in March when precipitation was lower by 61 % than the long-term average. In the same month, the air temperature was higher by 3.7 °C than the long-term average. The higher air temperature was prolonged in April but with a sufficient amount of precipitation, which positively influenced germination and emergence. During the sensitive stages of flowering and seed development i. e. in May, July and July precipitations were higher by 112 %, 52 % and 121 % than the long- term average, respectively. On 26 May 2014 when the crop was at the stage of late flowering, hail occurred. During these months air temperatures were similar to the long-term average.

In 2017 year, the total amount of precipitation during the growing season was lower by 24.5 % and the air temperature was higher by 2.2 °C than the long-term average. Besides June, in all other months during the vegetation period was noted deficiency of precipitation. Where March, April, May and July were noted 63.4 %, 25.5 %, 48.7 % and 18.8 % lower precipitation than the long-term average, respectively. The mean monthly air temperatures during all months were higher than the long-term average, especially in June (by 2.9 °C) and July (by 2.5 °C) i.e., during stages of seed development and oil synthesis.

3 RESULTS AND DISCUSSION

3.1 SEED AND OIL YIELD

The average seed yields of white mustard obtained in the study are in agreement with the research of Harasimowicz-Hermann et al. (2019) and Serafin-Andrzejewska et al. (2020) under agroecological conditions in Poland. Higher yields, up to 2.0 t ha^{-1}, were reported under favourable environmental and cultivation conditions in Pakistan by Hassan & Arif (2012). No significant differences were found in seed yield between years. No signifi-

Table 1: Decade and monthly air temperatures and precipitation in 2014, and 2017, and long-term average

Months/decades		Air temperature, °C		Long-term average	Precipitation, mm		Long-term average
		2014	2017	1981-2010	2014	2017	1981-2010
March	I	8.3	8.6		6.3	19.6	
	II	11.4	8.9		0.1	0.1	
	III	11.7	12.4		14.6	0.1	
	I - III	10.5	10.0	6.8	21.0	19.8	54.1
April	I	14.1	14.5		13.8	0.7	
	II	10.8	10.5		19.2	21.3	
	III	15.0	12.1		37.4	22.3	
	I - III	13.3	12.4	11.4	70.4	44.3	59.5
May	I	14.3	14.0		33.3	22.4	
	II	13.5	19.0		55.0	12.0	
	III	19.2	19.9		56.7	0.8	
	I - III	15.7	17.7	16.5	145.0	35.2	68.6
June	I	20.2	20.6		5.0	38.6	
	II	20.3	22.3		42.1	2.2	
	III	20.2	24.7		99.9	67.0	
	I - III	20.2	22.5	19.6	147.0	107.8	97.4
July	I	20.7	24.6		14.9	18.8	
	II	23.1	23.7		44.0	0.4	
	III	21.6	23.8		98.9	38.8	
	I - III	21.8	24.0	21.5	157.8	58.0	71.4
Average/Total		16.3	17.3	15.1	541.2	265.1	351.0

cant interactions between years and seeding density were found for seed yield either. (Table 2).

The analysis of variance showed a significant influence of seeding density on seed yield. The highest seed yield was obtained with a seeding density of 110 germinable seeds m^{-2}, with no significant differences between seeding density of 70 and 90 germinable seeds m^{-2} (Table 3). Similarly, Sáez-Bastante et al. (2016) reported that under Mediterranean rainfed conditions in a semi-arid area in southern Spain, higher plant density of white mustard resulted in higher seed yield, but not proportionally. They found that seed yield was slightly lower at a density of 40 plants m^{-2} than when plant density was doubled (80 plants m^{-2}). Keivanrad & Zandi (2012) reported that a plant density of Indian mustard in Iran greater than 80 plants m^{-2} did not result in a significant increase in seed yield.

The oil content of white mustard varied across years and sowing rates from 23.97 % (50 germinable seeds m^{-2}) to 24.37 % (90 germinable seeds m^{-2}). The average oil contents are comparable to the results obtained by Stamenković et al. (2018) in Serbia and Sáez-Bastante et al. (2016) in Spain. While Ciubota - Rosie et al. (2013) in Romania found an oil content of 28 %.

Another important parameter in this study was the oil yield per hectare. The analysis of variance showed a significant difference in oil yield between the years. A higher oil yield was obtained in 2014. The higher oil yield in 2014 was due to the higher oil content of seeds in 2014 (25.25 %) compared to 2017 (22.97 %). Water availability and cooler temperature during the seed development stage are the main determinants of seed and oil yields

Table 2: Results of analysis of variance for researched properties of white mustard

Source of variation	Seed yield (kg ha^{-1})	Oil yield (kg ha^{-1})
Year	ns	*
Seeding density	*	ns
Year x seeding density	ns	ns

ns–not significant; * significant for $p < 0.05$

Table 3: The influence of seeding density on seed yield and oil yield of white mustard in 2014 and 2017 year

Source of variation	Seed yield (kg ha^{-1})	Oil content (% d. m)	Oil yield kg ha^{-1})
Year			
2014	1064	25.25	237 a
2017	1052	22.97	205 b
Seeding density (germinable seeds m^{-2})			
50	933 b	23.97	197
70	1028 ab	24.01	217
90	1081 ab	24.37	232
110	1168 a	24.10	238

Different letters significant for $p < 0.05$
d. m.–dry matter

(Marjanović-Jeromela et al., 2019). It can be concluded that the weather conditions in 2014 were more favourable for achieving higher oil content in the seeds. In 2014, the amount of precipitation during seed development (June and July to harvest) was higher by 62 % than during the same period in 2017. The air temperature was also 2.3 and 2.2 °C lower in June and July 2014 than in the same months in 2017 (Table 1).

Higher seeding density increased oil yield, but the difference wasn't statistically significant (Table 3). These results are in agreement with those of Sáez-Bastante et al. (2016), who also found no effect of plant density (16, 26, 40 and 80 plants per m^2) on the oil yield of white mustard.

3.2 FATTY ACID COMPOSITION

The fatty acid composition of white mustard oil in dependency on seeding density during 2014 and 2017 is shown in Table 4 and Table 5. The analysis of the fatty acid composition of white mustard oil shows that erucic acid (C22:1), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) are dominant fatty acids in this oil.

The analysed oil of white mustard also contained palmitic acid (C16:0), stearic acid (C18:0), arachidonic acid (C20:0) and gadoleic acid (C20:1). In addition to these fatty acids, eicosatrienoic acid (C20:2) was present in 2014, and palmitoleic acid (C16:1), heptadecanoic acid (C17:0) and behenic acid (C22:0) were present in 2017 (Table 4 and Table 5). According to the Official law regulation of R. Croatia (Official Gazette, 2019), only in 2017, the average content of oleic acid was slightly above the prescribed value, as was the presence of heptadecanoic acid. The content of another fatty acid in white mustard oil was within the prescribed values.

In general, the fatty acid composition of any type of oil significantly influences the physical properties, nutritional value, and oxidative stability of the oil. As erucic acid was dominant in the oil of white mustard in this study and reached a limit of more than 5 % required by the European Directive for human consumption and for foodstuffs containing added fats or oils Directive 76/621/EEC (Council of the European Union, 1976), this oil is not recommended for human consumption, but it is well suited for industrial purposes. Erucic acid (C22:1) is an important oleochemical product that has many uses in metallurgy, machinery, rubber, chemical industry, and other fields due to its hydrophobicity and water resistance (Wang et al., 2022). In addition, this oil can be used as a feedstock to produce biodiesel (Ciubota-Rosie et. al., 2013). According to Pinzi et al. (2009), a higher content of monounsaturated fatty acids and saturated fatty acids (such as oleic and palmitic acids) is considered more desirable than polyunsaturated fatty acids (linoleic and linolenic acids) in terms of biodiesel oxidation stability, cetane number and fuel cold weather performance.

In the 2014 year by increasing the seeding density from 50 to 110 germinable seeds m^{-2}, the content of erucic acid was gradually increased by 2.8 %, 3.6 % and 4.4 % compared to the seeding density of 50 germinable seeds m^{-2}. In 2017 it varied from 37.10% (70 germinable seeds m^{-2}) to 38.29 (90 germinable seeds m^{-2}).

The oleic acid content in 2014 varied between 20.90 % (50 germinable seeds m^{-2}) and 21.40 % (90 germinable seeds m^{-2}) depending on seeding density. In 2017, the average content of oleic acid was 3.10 % higher than in 2014, ranging from 23.55 % (110 germinable seeds m^{-2}) to 24.50 % (50 germinable seeds m^{-2}).

The linoleic acid content in 2014 with an increase in seeding density from 50 germinable seeds m^{-2} to 90 and 110 germinable seeds m^{-2} decreased from 10.30 % to 9.70 %. In 2017, the average content of linoleic acid was slightly higher compared to 2014 (by 1.3 %), with a variation from 11.02 % (70 germinable seeds m^{-2}) to 11.41% (50 germinable seeds m^{-2}).

The content of linolenic acid in 2014 with increasing seeding density from 50 to 110 germinable seeds m^{-2} resulted in decreases of 3.4 %, 4.4% and 4.6 % compared to the lowest seeding density. In 2017, linolenic acid content was on average 2.23 % lower than in 2014, with a variation from 9.41 % (70 germinable seeds m^{-2}) to 9.85 % (110 germinable seeds m^{-2}) depending on seeding density.

Fatty acid profile in mustard depends on genetic base (Sawicka et al., 2020) but also on weather conditions (Ciubota-Rosie et al., 2013). The analysis of white mustard oil showed differentiation of fatty acid profile among researched years. Therefore, in the 2017 growing season when air temperature during stages of seed development and maturation (May and June) was higher and precipitations lower, the content of oleic and linoleic acid increased while linolenic and erucic acid decreased. Wilkes et al. (2013) also observed the influence of the growing season on the content of oleic, linoleic and erucic acids in the oil of Indian mustard (*Brassica juncea* (L.) Czem.). According to these authors, oleic and linoleic acids were inversely correlated with the content of erucic acid, which tended to be higher in cooler growing conditions. Similarly, Pospišil et al. (2007) reported for rapeseed, where the highest content of oleic acid and the lowest content of linoleic and linolenic acid in the oil were observed in the growing season with higher monthly air temperatures and lower precipitation in May and June.

Temperature is a major environmental factor that regulates fatty acid desaturation in plants (Dar et al., 2017). Lower temperatures generally favour the accumulation of polyunsaturated fatty acids (PUFA), such as linolenic acid (Ciubota-Rosie et al., 2009). The results of Hou et al. (2006) indicate that the content of linolenic acid (18:3) in soybean seed oil is the most sensitive to environmental changes. According to Menard et al. (2017), plants modify the content of polyunsaturated fatty acids in their membranes and storage lipids to adapt to temperature changes. In developing seeds, this response is largely controlled by the activities of the microsomal

Table 4: Fatty acids composition of white mustard in dependency on seeding density, 2014 year

Fatty acid (% of total)		Seeding density (germinable seed m^{-2}) 50	70	90	110	Mean	SD	CV	Official Regulation of R. Croatia*
C16:0	Palmitic	3.20	3.00	3.00	3.00	3.00	0.10	3.28	0.5-4.5
C18:0	Stearic	1.30	1.00	0.90	0.90	1.03	0.19	18.47	0.5-2.0
C18:1	Oleic	20.90	21.30	21.40	21.00	21.15	0.24	1.13	8.0-23.0
C18:2	Linoleic (ώ-6)	10.30	10.00	9.70	9.70	9.93	0.29	2.89	10.0-24.0
C18:3	Linolenic (ώ-3)	15.00	11.60	10.6	10.40	11.90	2.13	17.92	6.0-18.0
C20:0	Arachidinic	0.60	0.60	0.60	0.60	0.60	0.00	0.00	ND-1.5
C20:1	Gadoleic	9.10	9.8	10.1	10.1	9.78	0.47	4.83	5.0-13.0
C20:2	Eicosatrienoic	0.7	0.7	0.7	0.7	0.70	0.00	0.00	ND-1.0
C22:1	Erucic	35.60	38.4	39.20	40.0	38.30	1.91	5.00	38.30

ND-Not detected; SD–standard deviation; CV–coefficient of variation
* Official Gazette-NN 11/2019 (2019)

Table 5: Fatty acids composition of white mustard in dependency on seeding density, 2017 year

Fatty acid (% of total)		Seeding density (germinable seed m^{-2}) 50	70	90	110	Mean	SD	CV	Official Regulation of R. Croatia*
C16:0	Palmitic	3.22	3.29	3.14	2.94	3.15	0.15	4.81	0.5-4.5
C16:1	Palmitoleic	0.17	0.17	0.16	0.19	0.17	0.01	7.29	ND-0.5
C17:0	Heptadecanoic	0.99	2.35	0.00	0.07	0.85	1.10	128.51	ND
C18:0	Stearic	0.92	0.95	0.99	0.90	0.94	0.02	1.85	0.5-2.0
C18:1	Oleic	24.50	24.45	24.35	23.55	24.21	0.45	1.84	8.0-23.0
C18:2	Linoleic (ώ-6)	11.41	11.02	11.17	11.29	11.22	0.17	1.49	10-24
C18:3	Linolenic (ώ-3)	9.74	9.41	9.66	9.85	9.67	0.19	1.93	6-18
C20:0	Arachidonic	0.60	0.61	0.61	0.60	0.61	0.01	0.95	ND-1.5
C20:1	Gadoleic	9.19	9.26	9.40	9.33	9.30	0.09	0.97	5.0-13.0
C22:0	Behenic	0.49	0.54	0.51	0.51	0.51	0.02	4.02	0.2-2.5
C22:1	Erucic	37.85	37.10	38.29	37.56	37.70	0.50	1.33	22.0-55.0

ND-Not detected; SD–standard deviation; CV–coefficient of variation
* Official Gazette-NN 11/2019 (2019)

ω-6- and ω-3-fatty acid desaturases FAD2 and FAD3. The enzyme fatty acid desaturase 2 (FAD2) catalyses the conversion of oleic acid (C18:1) to linoleic acid (18:2), which is further desaturated to linolenic acid (18:3) by the enzyme FAD3 (Dar et al., 2017). With increasing temperatures, the activity of FAD2 and FAD3 decreases and with it the content of polyunsaturated fatty acids in the seeds (Alsajri et al., 2020).

Also, it can be observed that in 2014 by increasing seeding density from 50 to 110 germinable seeds m^{-2} erucic acid was increased while linoleic and linolenic gradually decreased. These findings are in line with Karydogianni et al (2022) in Greece who found higher quantities of polyunsaturated fatty acid at low lower plant density (46 plants m^{-2}) in comparison to higher plant density (76 plants m^{-2}). While Sáez-Bastante et al. (2016) in Spain found that increased plant density had a positive effect on the content of linoleic and linolenic acid. The negative correlation between erucic acid (C22:1) and oleic acid (C18:1) and linoleic acid (C18:2) in oil of Indian mustard (*Brassica juncea*) according to Wilkes et al. (2013) reflects the biosynthetic pathway of these fatty acids and the amount and/or activity of enzymes involved in each step of the pathway. It is a well-known substrate competition in the catalysis of C18:1 CoA to erucic acid or PUFA biosynthesis (i.e. linoleic acid and linolenic acid). In the cytoplasm, C18:1 can either be elongated to C22:1 in a reaction involving a β-ketoacyl CoA synthase (also known as fatty acid elongase 1, FAE1) or to linoleic acid (C18:2) and subsequently to linolenic acid (C18:3) through the action of the membrane-bound, microsomal enzymes desaturase FAD2 (omega-6 desaturase) and FAD3 (omega-3 desaturase) (Lu et al., 2011).

4 CONCLUSIONS

In conclusion, under agroecological conditions in north-west Croatia, 70 germinable seeds m^{-2} seeding density is sufficient to achieve a high seed yield.

Higher oil yield was achieved in 2014 due to lower air temperatures and higher precipitation during the stage of seed formation and maturation. The high content of erucic acid in white mustard oil makes it suitable for industrial purposes, such as the production of lubricants and biodiesel.

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