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The effect of different climatic zones on fatty acid profile of *Ricinus communis* seed oil

Zeynep Ergun[1] **Mozhgan Zarifikhosroshahi**[2,*]

[1]Adana Alparslan Turkes Science and Technology University, Faculty of Engineering, Department of Bioengineering, Adana, Türkiye
[2]Çukurova University, Agriculture Faculty, Department of Horticulture, Adana, Türkiye

[*]Corresponding Author: mn_zarifi@yahoo.com

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Abstract
Castor bean has not been well studied in different genotypes and geographic zones despite its application in industry and medicine. Recently, the use of castor beans as biodiesel and industry makes this plant a point of interest for researchers. However, more studies are needed for evaluating genotypes from different ecologies. The effect of climatic zones, Adana and Mersin, on the fatty acid profile of chaster bean seed oils was investigated. It was found that locations significantly influenced the fatty acid content. The main fatty acid was ricinoleic acid with 84.63% and 86.87% in both Adana and Mersin locations, respectively. Despite ricinoleic acid, Adana had higher concentrations of Palmitic acid (1.97%), Stearic acid (2.1%), Oleic acid (4.4%), and Palmitoleic acid (2.29%) whereas Linolenic acid (5.83%), and Ricinoleic acid (86.87%) was high in Mersin. These results showed that climate affects the fatty acid contents of studied caster oil. This study will help in the selection of proper caster oil cultivars not only in these regions but in other regions of the world as well.

Keywords
Ricinus communis, Fatty acids, Location, Ricinoleic acid

Introduction
The limited conventional energy resources besides the harmful and destructive effect of diesel fuels on the environment have accelerated the search for alternative fuels. Concerns about global warming of pollutants lead to using biodiesels as a solution. Biodiesel is an alternative non-toxic, environmental-friendly, and biodegradable fuel obtained from renewable sources such as animal or vegetable oils. Therefore, it is very important to evaluate plant species with potential applications as biodiesel. On the other hand, heavy metal pollution caused by releasing mine waste to open areas is another concern of ecological problem and makes it necessary to select the suitable plant species for planting on mine waste which accelerates the revegetation process, increasing biodiversity and stabilizing nutrient cycling (Olivares et al 2013). One of these special plant species is the castor bean.

Castor bean also known as castor oil plant (*Ricinus communis*) belongs to the Euphorbiaceae family. The origin of the castor plant is debatable, but the strong evidence shows that its origin is in the tropical belt of India and Africa. However, today, the castor plant is cultivated worldwide in many tropical and subtropical regions and even warm temperate regions (Kaur and Bhaskar 2020; Yusuf et al 2015). Castor bean is a fast-growing perennial shrubby plant 8-10 meters high. It can be self- and cross-pollinated and worldwide studies reveal low genetic diversity among castor bean germplasm. The roots of the castor bean are fibrous and scarcely ramified; the trunk is upright and highly branched, and its color varies from bright green, pale

green, or red to purple and is covered with a waxy layer that makes the plant high drought-resistant. The leaves are alternate green or reddish formed in long stalks, alternate and palmate, divided into 5-12 lobes. The stems have different colors. The flowers are monoecious the flowers formed in the upper parts are female and the ones form in the lower part are male. The fruits are set in upper branches in three boxes, these boxes separate into ripen fruits and each contains one oval, bean-like seed which is swelled in the center. The seeds are caruncle and have a warty appendage at one end (Jena and Gupta 2012). The Castor bean is a precious plant cultivated for industrial and medicinal purposes. Phytochemicals extracted from different organs of caster bean have numerous pharmacological uses such as antibacterial, cytotoxicity, antioxidant and anticancer, antiasthmatic, anti-inflammatory, laxative-cathartic reagent, and many other applications (Javanshir et al 2020; Vasco-Leal et al 2020, Franke et al 2019; El-Naggar et al 2019; Ribeiro et al., 2016; Jena and Gupta 2012, Salimon et al 2010).The castor bean also was reported as an efficient pesticide application by researchers (Carolina et al 2019, Adeniyi et al., 2018; Soni and Dhiman, 2017; Rampadarath and Puchoa, 2016). Castor oil is extracted from the grains, and the residue, and can be used for nematode control (Gahukar, 2017) and, as fertilizer (Lima et al., 2011). The industrial application of castor bean is in cosmetic production, production of PU foams, elastomers, surface coating materials, adhesives, and interpenetrating polymer networks (IPNs), in paint, print, and textile industries, production of soap, preparation of brake fluids as a lubricant, (Yusuf et al 2015). Castor bean is also an ideal plant for phytoremediation (Rehn et al 2020; Yeboah et al 2020, Palanivel et al 2020, Olivares et al 2013). However, castor bean is a tolerant plant grown in every ecological condition especially in semi-arid and arid regions even in metal-polluted sites (Rajkumar and Freitas 2008). Studies showed that not only *R. communis* is resistant to urban roadside air pollution, but also antioxidant activity and total free amino acids have tremendously increased in this condition (Khalid et al 2019).It is proved by many studies that chaster bean is an ideal biodiesel alternative for sulfur-based diesel due to its unique oil structure (Roy et al 2020; Carrino et al 2020; Awais et al 2020; Osorio-González et al 2020, Chan et al 2010, Perdomo et al 2013). However, what makes the castor bean a unique plant is the structure of its seed oil. Castor oil is rich in hydroxy fatty acid with one double bond, ricinoleic acid (cis-12- hydroxyoctadeca-9-enoic acid), the only commercial source of a hydroxylated fatty acid. Ricinoleic acid counts for unique properties of castor oil including high specific gravity, high boiling point(3130C), excellent solubility in alcohols, and unusual versatility. The presence of a hydroxyl group on C-12 of ricinoleic acid makes castor oil unusually polar, thus promoting hydrogen bonding. Unlike other oils, it is mixable with alcohol, but only slightly soluble in petroleum ether at room temperature. It is reported that the oil content of castor seed is about t 46-55% that 87-90% of its content is ricinoleic acid (Gupta et al. 1951; Foglia et al. 2000; Puthli et al. 2006; Ogunniyi 2006; Conceicao et al. 2007). Akpan et al., 2006; Ogunniyi, 2006; Conceicao et al., 2007), with only about 4.2% linoleic, 3.0% oleic, 1.0% each stearic, and palmitic, 0.7% dihydroxy stearic, and 0.3% each linolenic and eicosanoic acids (Dave, 2002). This high level of purity (by single fatty acid content) makes the oil unique among all naturally occurring fats and oils. Although caster seeds contain a toxic protein called ricin and toxic allergen and are poisonous to humans and animals, none of the toxic components is carried into the oil (Nangbes et al 2013). However, oil content and fatty acids composition in castor seed vary based on genetic characteristics, genotypes, geographical origin/climatic conditions, agricultural operations such as irrigation and foliar application of nutrients, and the oil extraction method(s) used (Sadeghi-Bakhtavari & Hazrati2020; Yusuf et al 2015). Therefore, it is important to have knowledge about different genotypes grown in different geographical zones. Because as mentioned above phytochemical composition of castor bean oil is affected strongly by geographical origin/climatic conditions.

The purpose of this study was to evaluate the effect of different ecological zones on the composition of fatty acids in naturalized wildly grown castor beans from Turkey which there is little information regarding its use, adaptation, and characterization.

Materials and methods

Plant Material

Ricinus communis plants were collected from Mersin, Atakent Municipality Kapızlı Camp, 8 m (36˚24′14″N, 34˚04′33″E), and Adana Cukurova University, Ali Nihat Gökyiğit Çarkıpare Şarıçam, 112 m, (37˚03′02″N,35˚21′14″E) with a one-week interval. Then seeds were removed and dried at 65˚C for 8 h. For each location, three samples were used.

Oil extraction

The oils of all samples were extracted immediately after harvest. The oil of samples was extracted via an automatic soxhlet device (Gerhardt GmbH & Co. KG). Ten grams of dried seeds were used for oil extraction. Hexane (Merck KGaA, Darmstadt, Germany) was used as a solvent and extracted oil was weighted for the determination of the oil percent in the samples. The oil content of seeds was expressed as g 100 g-1 in dry samples. Obtained fresh oil was analyzed determination of fatty acids composition.

Determination of fatty acids

Esterification of Fatty acids was done using the method described by Perdomo et al (2012). One hundred mL of oil was mixed with 1 mL of NaOH methanolic solution. Then, samples were heated up to 100˚C for 25 min followed by adding 6 mL of HCl methanolic solution. After heating the obtained solvent again up to 80˚C for 10 min, 75 mL of equimolar hexane was added. The upper phase was removed and mixed with 9 mL of NaOH solution, and after 1 min standing solution was used for injection to gas chromatography (GC, Perkin Elmer, Auto system GLX, Shelton, USA). Chromatographic separation was performed using a Supelco SPTM-2380 (30 m 0.25 mm inner diameter, 0.25 mm film thickness) column equipped with a flame ionization detector (FID). The injector and detector temperatures were 280˚C and

260˚C respectively. The carrier gas was helium with a flow rate of 0.5 mL/min. The initial temperature of the oven was adjusted at 120˚C for 2 min, increased at 58ºC/min to 220ºC, and held for 10 min. Data was collected and quantified with a TotalChrom Navigator and the results were expressed as percent concentration (Demirtas et al. 2013).

Statistical Analysis

The experiment was conducted as a completely randomized design using two replications. The results were expressed as average and standard deviation. The correlation analysis was done among fatty acids. The principal component analysis (PCA) was done using the XLSTAT software program (Addinsoft, USA).

Figure 1. A view of *Ricinus communis* plants and seeds (A: Mersin, B: Adana)

Results and Discussion

The fatty acid composition of castor oil from two studied geographic zones is shown in Table 1. The results showed that the major part of seed oil includes ricinoleic acid in both samples. These results are in agreement with previous studies on castor beans (Gupta et al. 1951; Foglia et al. 2000; Puthli et al. 2006; Ogunniyi 2006; Conceicao et al. 2007; Salimon et al, 2010; Pedremo et al 2013; Yusuf et al 2015; Sadeghi-Bakhtavari & Hazrati 2020).

Table 1. The fatty acid composition of castor oils from studied geographic zones [%]

Compound name	Adana	Mersin	Mean Difference
Total fat	46.36	47.41	
Capric acid (C10:0)	0.06±0.00	N.D.	0.06
Caprylic acid (C8:0)	0.08±0.00	0.07±0.00	0.01
Undecanoic acid (C11:0)	0.08±0.01	N.D.	0.075
Myristic Acid (C14:0)	0.09±0.01	0.04±0.00	0.045
Palmitic acid (C16:0)	1.97±0.04	1.44±0.01	0.53
Margaric Acid (C17:0)	0.04±0.01	0.04±0.01	0
Stearic acid (C18:0)	2.10±0.02	1.23±0.01	0.87
Arachidic acid (C20:0)	0.08±0.01	0.05±0.00	0.025
Behenic acid (C22:0)	0.31±0.01	0.04±0.00	0.265
Tricosanoic acid (C23:0)	0.04±0.02	0.02±0.00	0.015
Σ SFA	**4.81**	**2.92**	**1.895**
cis-Pentadecan Acid (C15:1)	0.02±0.00	0.03±0.00	-0.01
Palmitoleic acid (C16.1)ω−7	0.07±0.03	0.02±0.00	0.05
Oleic acid (C18:1n9c)ω−9	4.40±0.01	3.86±0.03	0.54
Eicosenoic acid (C20:1n9c)ω−9	0.50±0.01	0.49±0.00	0.005
Ricinoleic acid [C18:1(OH)]	84.63±0.13	86.87±0.05	-2.235
Σ MUFA	**89.62**	**91.27**	**-1.65**
Linoleic acid (Cl 8:2n6c) ω−6	5.20±0.04	5.38±0.03	-0.185
γ-Linolenic Acid (C18:3n6) ω−6	0.03±0.00	0.01±0.00	0.02
a-Linolenic acid (C18:3n3) ω−3	0.37±0.01	0.45±0.01	-0.08
Σ PUFA	**5.59**	**5.84**	**-0.245**

Due to the unique characteristics of ricinoleic acid with high purity in castor bean oil, castor oil is characterized as an inexpensive and environment-friendly biodiesel and precursor in industry. However, results revealed that samples from Mersin had a higher amount of ricinoleic acid compared to Adana samples. In terms of total oil percentage, the seeds from Mersin (47.41%) location contained higher oil than Adana

(46.36%). While the oil contents of seeds in the present study were found to be higher than those reported by Sadeghi-Bakhtavari & Hazrati 2020 (40.75% in full water and 37.83% in underwater stress), the obtained results are confirmed by the findings of Ogunniyi, (2006), Goytia-Jime´nez et al. (2011) Román-Figueroa et al (2020) (46-55%). Under natural conditions, the average oil content in castor beans fluctuates between 42.0% and 49.0% but in Mediterranean conditions increased up to 55.0% (Román-Figueroa et al 2020). However, previous studies emphasized that oil content in castor oil is extremely affected by variety and environmental conditions, especially during the seed-filling stage as seen in this study (Salmon et al 2010, Onemli 2012). Başalma and Pashazade (2011) reported that castor oil needs an optimum range of temperature (20-26 ºC) and humidity. At temperatures lower than 15 ºC and higher than 35 ºC the oil content of seeds decreases.

Mersin and Adana show the typical characteristics of the Mediterranean climate in the same geographical area. However, the sampling locality in Mersin is expected to be more open to the sea effect and high water table. So, it can be generalized that the castor seeds in Mersin have grown in more warm, humid, less volatile (in terms of air temperature and humidity), and wetland conditions. Although the average temperature of Mersin and Adana is the same which is 19.1°C, the temperature volatility is higher in Adana. In detail, the difference between the average maximum and average minimum temperatures are 8.6 °C and 12.5 °C for Mersin and Adana, respectively. In addition, the monthly average rainfall is 615.8 mm in Mersin and 671.3 mm in Adana which is concentrated during the winter season. The altitude of the Mersin sample collection point is 6 m in a marsh area, while it is 173 m for Adana in rugged terrain. This may lead to higher water-deficit stress for the Adana sample collection point despite higher rainfall. Moreover, Mersin sample collection point is near the seaside but the distance between the Adana sample collection point and the sea is approximately 55-60 km. Sufficient water supply during the growing period leads to extended flowering and pollination periods. Therefore, the number of seeds, seed weight, and oil content increased. It was reported that water deficit stress reduces the concentration of linolenic and linoleic acid but increases the concentration of oleic acid in oilseed crops (Alyari et al., 2000). In line with that, higher oleic acid and lower linoleic and a-Linolenic acids contents were observed in the seeds from Adana, associated with higher water deficit. In water-deficit stress, the uptake of nutrients decreases causing an increase in soluble salts and osmotic pressure around the root system (Sadeghi-Bakhtavari & Hazrati 2020).

The total SFA is higher in Adana location than Mersin which is caused by the higher rate of palmitic, stearic, and behenic acid. While the rate of MUFA is higher in the Mersin location compared to Adana samples. Although oleic acid was higher in Adana, the higher rate of ricinoleic acid in Mersin caused Mersin to have higher Total MUFA. The rate of total PUFA in Mersin is also higher than Adana due to higher linoleic acid and a-linoleic acid (Table 1 and Figure 2).

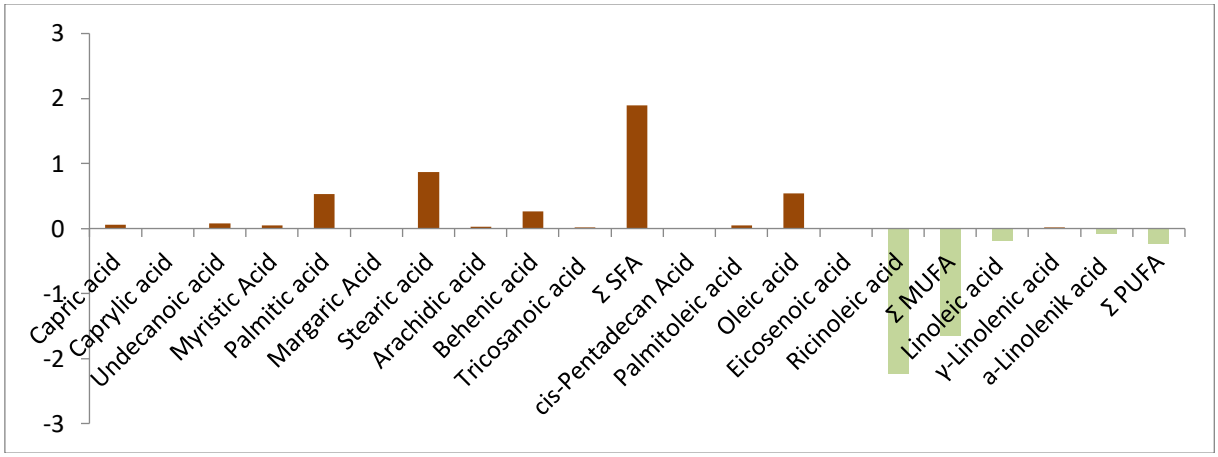

Figure 2. Differences among fatty acid compositions of seeds from Adana and Mersin-%.

In PCA analysis if the first two principal components (PCs) are able to explain a significant portion of the total variance, it is possible to visualize the experiment results. The variable chart indicates the correlations between the components and the variables. The correlations can be seen in the following matrix apparently (Table 2). The blue points are the observations and the vectors are the variables. In the figure 3, PC1 and PC2 can explain 86.61 % and 11,59% of the total variance, respectively. The location of the samples separated significantly from one another. As a result, using the *Ricinus communis* seeds from different locations as explant may create a difference in the fatty acids compositions even in the same climate and geographical area (Figure 3).

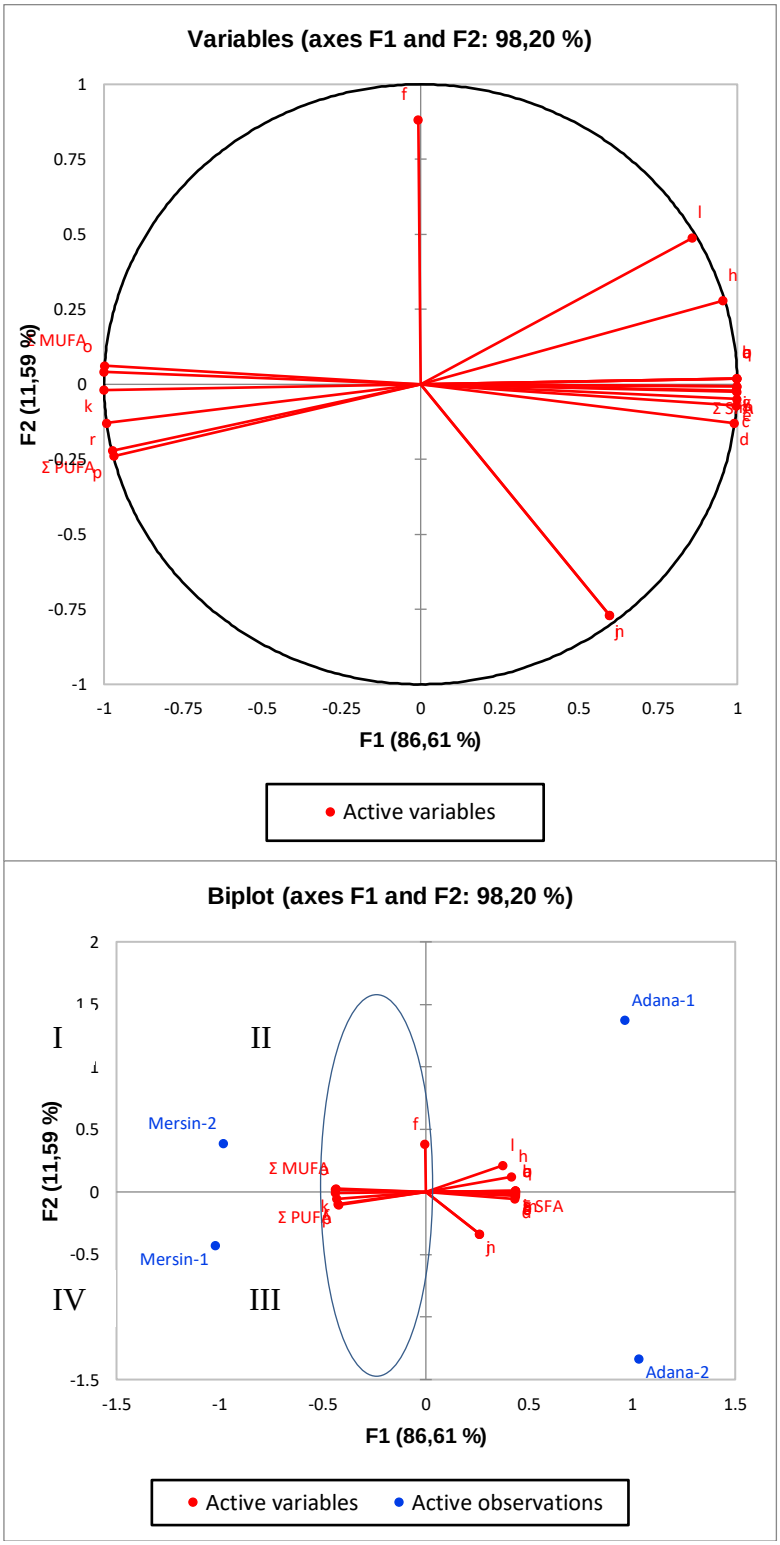

Figure 3. Correlation Circle Chart and Biplot Graph Obtained by The Principle Component Analysis (PCA) showing the interrelations of sample locations and fatty acids.

Capric acid a, Caprylic acid b, Undecanoic acid c, Myristic Acid d, Palmitic acid e, Margaric Acid f , Stearic acid g, Arachidic acid h, Behenic acid i, Tricosanoic acid j, cis-Pentadecan Acid k, Palmitoleic acid l, Oleic acid m, Eicosenoic acid n, Ricinoleic acid o, Linoleic acid p, γ-Linolenic Acid q, a-Linolenik acid r

On the Biplot graph Fatty acids observed in Area I: f, o, ΣMUFA, Area II: I, h, q, b, a, Area III: g, m, ΣSFA, c, I, e, d, n, j, Area IV: k, r, ΣPUFA, p

Table 2. Correlation Matrix (Pearson (n))

Variables	a	b	c	d	e	f	g	h	i	j	Σ SFA	k	l	m	n	o	Σ MUFA	p	q	r	Σ PUFA
a	**1**	**1.000**	**0.996**	**0.988**	**0.998**	**0.000**	**1.000**	**0.962**	**1.000**	**0.577**	**0.999**	**-1.000**	**0.870**	**0.998**	**0.577**	**-0.998**	**-0.997**	**-0.971**	**1.000**	**-0.992**	**-0.976**
b	**1.000**	**1**	**0.996**	**0.988**	**0.998**	**0.000**	**1.000**	**0.962**	**1.000**	**0.577**	**0.999**	**-1.000**	**0.870**	**0.998**	**0.577**	**-0.998**	**-0.997**	**-0.971**	**1.000**	**-0.992**	**-0.976**
c	**0.996**	**0.996**	**1**	**0.998**	**1.000**	**-0.066**	**0.998**	**0.932**	**0.998**	**0.651**	**0.999**	**-0.996**	**0.820**	**0.996**	**0.651**	**-0.999**	**-1.000**	**-0.950**	**0.996**	**-0.980**	**-0.955**
d	**0.988**	**0.988**	**0.998**	**1**	**0.996**	**-0.110**	**0.991**	**0.908**	**0.992**	**0.697**	**0.994**	**-0.988**	**0.783**	**0.990**	**0.697**	**-0.995**	**-0.997**	**-0.931**	**0.988**	**-0.967**	**-0.937**
e	**0.998**	**0.998**	**1.000**	**0.996**	**1**	**-0.056**	**0.999**	**0.942**	**0.999**	**0.630**	**1.000**	**-0.998**	**0.836**	**0.998**	**0.630**	**-1.000**	**-1.000**	**-0.955**	**0.998**	**-0.983**	**-0.960**
f	**0.000**	**0.000**	**-0.066**	**-0.110**	**-0.056**	**1**	**-0.023**	**0.192**	**-0.019**	**-0.577**	**-0.034**	**0.000**	**0.348**	**-0.055**	**-0.577**	**0.056**	**0.066**	**-0.236**	**0.000**	**-0.124**	**-0.219**
g	**1.000**	**1.000**	**0.998**	**0.991**	**0.999**	**-0.023**	**1**	**0.955**	**1.000**	**0.597**	**1.000**	**-1.000**	**0.858**	**0.999**	**0.597**	**-0.999**	**-0.998**	**-0.965**	**1.000**	**-0.989**	**-0.970**
h	**0.962**	**0.962**	**0.932**	**0.908**	**0.942**	**0.192**	**0.955**	**1**	**0.955**	**0.333**	**0.949**	**-0.962**	**0.972**	**0.953**	**0.333**	**-0.945**	**-0.937**	**-0.985**	**0.962**	**-0.979**	**-0.985**
i	**1.000**	**1.000**	**0.998**	**0.992**	**0.999**	**-0.019**	**1.000**	**0.955**	**1**	**0.599**	**1.000**	**-1.000**	**0.857**	**0.999**	**0.599**	**-0.999**	**-0.998**	**-0.966**	**1.000**	**-0.990**	**-0.971**
j	**0.577**	**0.577**	**0.651**	**0.697**	**0.630**	**-0.577**	**0.597**	**0.333**	**0.599**	**1**	**0.613**	**-0.577**	**0.101**	**0.598**	**1.000**	**-0.623**	**-0.642**	**-0.409**	**0.577**	**-0.501**	**-0.425**
Σ SFA	**0.999**	**0.999**	**0.999**	**0.994**	**1.000**	**-0.034**	**1.000**	**0.949**	**1.000**	**0.613**	**1**	**-0.999**	**0.847**	**0.999**	**0.613**	**-1.000**	**-0.999**	**-0.961**	**0.999**	**-0.987**	**-0.966**
k	**-1.000**	**-1.000**	**-0.996**	**-0.988**	**-0.998**	**0.000**	**-1.000**	**-0.962**	**-1.000**	**-0.577**	**-0.999**	**1**	**-0.870**	**-0.998**	**-0.577**	**0.998**	**0.997**	**0.971**	**-1.000**	**0.992**	**0.976**
l	**0.870**	**0.870**	**0.820**	**0.783**	**0.836**	**0.348**	**0.858**	**0.972**	**0.857**	**0.101**	**0.847**	**-0.870**	**1**	**0.856**	**0.101**	**-0.841**	**-0.827**	**-0.937**	**0.870**	**-0.907**	**-0.932**
m	**0.998**	**0.998**	**0.996**	**0.990**	**0.998**	**-0.055**	**0.999**	**0.953**	**0.999**	**0.598**	**0.999**	**-0.998**	**0.856**	**1**	**0.598**	**-0.999**	**-0.998**	**-0.957**	**0.998**	**-0.984**	**-0.962**
n	**0.577**	**0.577**	**0.651**	**0.697**	**0.630**	**-0.577**	**0.597**	**0.333**	**0.599**	**1.000**	**0.613**	**-0.577**	**0.101**	**0.598**	**1**	**-0.623**	**-0.642**	**-0.409**	**0.577**	**-0.501**	**-0.425**
o	**-0.998**	**-0.998**	**-0.999**	**-0.995**	**-1.000**	**0.056**	**-0.999**	**-0.945**	**-0.999**	**-0.623**	**-1.000**	**0.998**	**-0.841**	**-0.999**	**-0.623**	**1**	**1.000**	**0.956**	**-0.998**	**0.984**	**0.961**
Σ MUFA	**-0.997**	**-0.997**	**-1.000**	**-0.997**	**-1.000**	**0.066**	**-0.998**	**-0.937**	**-0.998**	**-0.642**	**-0.999**	**0.997**	**-0.827**	**-0.998**	**-0.642**	**1.000**	**1**	**0.951**	**-0.997**	**0.981**	**0.957**
p	**-0.971**	**-0.971**	**-0.950**	**-0.931**	**-0.955**	**-0.236**	**-0.965**	**-0.985**	**-0.966**	**-0.409**	**-0.961**	**0.971**	**-0.937**	**-0.957**	**-0.409**	**0.956**	**0.951**	**1**	**-0.971**	**0.993**	**1.000**
q	**1.000**	**1.000**	**0.996**	**0.988**	**0.998**	**0.000**	**1.000**	**0.962**	**1.000**	**0.577**	**0.999**	**-1.000**	**0.870**	**0.998**	**0.577**	**-0.998**	**-0.997**	**-0.971**	**1**	**-0.992**	**-0.976**
r	**-0.992**	**-0.992**	**-0.980**	**-0.967**	**-0.983**	**-0.124**	**-0.989**	**-0.979**	**-0.990**	**-0.501**	**-0.987**	**0.992**	**-0.907**	**-0.984**	**-0.501**	**0.984**	**0.981**	**0.993**	**-0.992**	**1**	**0.995**
Σ PUFA	**-0.976**	**-0.976**	**-0.955**	**-0.937**	**-0.960**	**-0.219**	**-0.970**	**-0.985**	**-0.971**	**-0.425**	**-0.966**	**0.976**	**-0.932**	**-0.962**	**-0.425**	**0.961**	**0.957**	**1.000**	**-0.976**	**0.995**	**1**

Values in bold are different from 0 with a significance level alpha=0

Conclusion

Castor bean is a precious plant in terms of various applications in medicine and industry. The oil extracted from seeds is high-quality to be considered biodiesel. The plant can grow almost everywhere in the world because it has high competitive power and endurance to various climate and soil conditions. More than half of the oil contains ricinoleic acid a unique saturated fatty acid with excellent properties which makes it a good candidate for application in industry and biodiesel. However, studies proved that the location where the plant is grown affects the rate of fatty acids and quality of the oil as the results obtained in this study from different locations. Therefore, this study will help international researchers on determining the optimum cultivation conditions for the exploitation of qualified oil for applications both in industry and biodiesel production.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Not applicable.

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