

Cocklebur (*Xanthium strumarium* L.) seed oil and its properties as an alternative biodiesel source

Cüneyt CESUR¹, Tanzer ERYILMAZ², Tansu USKUTOĞLU¹, Hülya DOĞAN^{3,*}, Belgin COŞGE ŞENKAL¹

¹Department of Field Crops, Faculty of Agriculture and Natural Science, Bozok University, Yozgat, Turkey

²Department of Biosystems Engineering, Faculty of Engineering and Architecture, Bozok University, Yozgat, Turkey

³Department of Seed Science, Vocational School of Technical Sciences, Bozok University, Yozgat, Turkey

Received: 07.08.2017 • Accepted/Published Online: 25.12.2017 • Final Version: 12.02.2018

Abstract: Cocklebur (*Xanthium strumarium* L.) seed oil is available for biodiesel production as a wild plant species. In the present study, the cocklebur seeds collected from Muslubelen, Yozgat, Turkey, at 1440 m in 2014 were sown in the Bozok University Agriculture Faculty Topçu Research Area during 2015 and 2016. The maximum crude oil ratio was obtained as 35%. Oleic and linoleic acid contents of cocklebur seed oil were determined as 11.37% and 76.97%, respectively. Some important fuel properties such as density, flash point, water content, copper strip corrosion, iodine value, linolenic acid, methyl ester, and sulfated ash content values were measured and compared to the EN 14214 and ASTM D6751 standards. These values were found to be within the standard ranges. Cost analysis of the cocklebur plant was also performed and compared to other field crops. According to our results, it seems that agronomic production of cocklebur will be possible in marginal areas because its genetic properties enable cocklebur to grow in dry areas. Therefore, production of cocklebur as an industrial plant may contribute to energy shortages without competing with food production. In areas devoid of sufficient irrigation possibilities, agriculture based on grain and cereal decreases the biodiversity. Production of drought-tolerant plants such as cocklebur in these areas will have positive effects on conservation and development of biodiversity in the ecosystem.

Key words: Cocklebur seeds, fatty acids, transesterification, biodiesel, fuel properties

1. Introduction

Renewable energy sources have become an important part of world energy policy to reduce greenhouse gas emissions caused by fossil fuels. Among these energy sources, biodiesel is an environmentally friendly fuel and its utilization is increasing throughout the world (Wen et al., 2009; Gokdogan et al., 2015). Biofuels have important benefits such as meeting energy demands, contributing to agricultural production, reducing poverty in rural areas, and alleviating unemployment (Scholz and Silva, 2008).

Biodiesel can be produced from both edible and nonedible oils (Azam et al., 2005; Atabani et al., 2015). Particularly, utilization of nonedible oils in biodiesel production is very strategic when the food shortage in the world is considered (Hasheminejad et al., 2011; Bastante et al., 2016).

Cocklebur belongs to the genus *Xanthium* of Asteraceae (Compositae). That genus has over 30 different species worldwide. It is an annual wild plant species found all over the world and is commonly seen in North America, Brazil, North Africa, China, India,

and Malaysia (Caius, 1986; Hsu et al., 2000; Kim et al., 2003; Lee and Owen, 2003; Chen et al., 2015). Cocklebur is a wild weed known as “domuz pıtrağı”, “pıtrak diken”, “kaymakam pamuğu”, and “kazık otu” in Turkey (Auld et al., 1988; Ulug et al., 1993).

In order to fight the weed, people spend not only energy but also time (Balabanlı et al., 2006; Serim et al., 2015; <http://www.fs.fed.us/database/feis/plants/forb/xanstr/all.html>). On the other hand, cocklebur as a wild weed produces 67% more biomass than cotton. For this reason, it can be utilized as a substantial sustainable energy source (Byrd and Coble, 1991). It is proposed as a raw material for biodiesel production because of its low cost, high oil content, and low acid degree. Cocklebur seed oil is a nonedible oil reaching up to 42.34%, and it has production potential of 100,000 t/year in China (Chang et al., 2013). Due to the high adaptability and competitive properties of this plant, dry areas will be used more productively (Wassom et al., 2003). The aim of this study is to investigate the biodiesel production potential of crude oil from cocklebur (*X. strumarium* L.) seeds.

* Correspondence: hulya.dogan2026@gmail.col

2. Materials and methods

The seeds of cocklebur (Figure 1) were collected from Muslubelen (altitude approximately 1440 m), Yozgat, Turkey, on 15 September 2014 (Figure 2). The seeds were sown (60 cm × 20 cm) in Yozgat (Topçu), Turkey, at the end of March 2015 and 2016. After sowing, N (150 kg/ha) was applied to the soil when the plants had 3 or 4 leaves. After the application of fertilizer, the soil was irrigated once. Furthermore, weeds were cleaned from the area. When seeds were mature, plants were harvested. The cocklebur seeds were separated from fruits. Seeds obtained in both years were combined for the analysis.

2.1. Obtaining crude oil and analysis of the fatty acid composition

The seeds of cocklebur plants were pressed using a cold press machine and crude oil (Figure 3) was obtained.

The moisture ratio of the cocklebur seeds was detected as $5.57 \pm 0.10\%$. The oil content of the cocklebur seeds was measured by Soxtec devices.

The fatty acid composition profile of cocklebur seed oil was analyzed using the Agilent 6890N Network GC System combined with an Agilent 5975C VL MSD network mass selective detector and HP Innowax capillary column of 60.0 m × 0.25 mm × 0.25 µm. One microliter of sample was injected into the GC system. The column heat was held initially at 60 °C for 1 min after injection, then increased to 185 °C with 1 °C/min heating ramp for 10 min and increased to 200 °C with 5 °C/min heating ramp without hold, and the injector temperature was set to 250 °C. The carrier gas was helium with column flow rate of 0.3 mL/min at a 30.0:1 split ratio. Table 1 shows the summary of GC operating conditions.



Figure 1. Cocklebur seed.



Figure 2. Cocklebur plants in the experimental area.



Figure 3. Cocklebur oil during distillation.

Table 1. Summary of the devices used to determine the properties.

Property	Device	Range	Unit	Accuracy	Manufacturer	Standard
Density / specific gravity	Kem Kyoto DA-645	Density: 0.00000–3.00000 Temperature: 0–93	g cm^{-3} $^{\circ}\text{C}$	± 0.00005 ± 0.03	Kem Kyoto Electronics, Japan	EN ISO 3675 EN ISO 12185
Kinematic viscosity	Polyscience 7306A12E	Ambient temperature –150 Measuring tube: 1.2–10 Measuring tube: 5–50 Timekeeper: 0–2400	$^{\circ}\text{C}$ $\text{mm}^2 \text{s}^{-1}$ $\text{mm}^2 \text{s}^{-1}$ s	± 0.05 ± 0.5 ± 1 ± 0.01	Polyscience, USA	EN ISO 3104
Flash point	Rapid Tester RT-1	–30 to +300	$^{\circ}\text{C}$	± 1	Paul N. Gardner Company, USA	EN ISO 2719 EN ISO 3679
Water content	Kem Kyoto MKC-520 Karl-Fischer moisture titrator	10–300,000	μg	± 0.1	Kem Kyoto Electronics, Japan	EN ISO 12937
Calorific value	IKA C200 bomb calorimeter	0–40,000	J	± 0.1	IKA, UK	DIN 51900
pH	Labkits ELE-PHP3BW	pH range: 0–14 Temperature range: 0–100	pH $^{\circ}\text{C}$	± 0.01 ± 1	Labkits, Hong Kong	-
Copper strip corrosion	Koehler K25330	Bath temperature range: Ambient temperature – 190	$^{\circ}\text{C}$	± 1	Koehler Instrument, USA	EN ISO 2160
Color	Lovibond PFXi 195/2	0.5–8	-	± 0.0004	Lovibond, UK	ASTM D 1500
Cloud, pour, freezing, and cold filter plugging points	Labkits PT-SYD-510F1 multifunctional low temperature tester	–70 – Ambient temperature	$^{\circ}\text{C}$	± 0.5	Labkits, Hong Kong	EN ISO 3015 EN ISO 3016 ASTM D 6371

2.2. Procurement of biodiesel

The biodiesel evaluated in this experimental work was transesterified from fatty acid methyl ester of the cocklebur seed oil. The transesterification process was performed by using a laboratory-scale biodiesel processor in the Biofuel Laboratory, Faculty of Engineering and Architecture at Bozok University, Yozgat, Turkey. The transesterification process was conducted in a base-catalyzed environment with sodium hydroxide as a catalyst and methanol as an alcohol. Transesterification was carried out in a 500-mL reaction flask equipped with reflux condenser, magnetic stirrer, and thermometer. The catalyst (0.35 wt.%) was dissolved in an aqueous methanol solution (20% v/v) by stirring in a small flask. About 200 mL of the cocklebur seed oil was added to the reaction flask and heated to 60 °C. When the temperature reached 60 °C, the alcohol/catalyst mixture was added to the oil while stirring the mixture continuously at 600 rpm. The final mixture was maintained by stirring for 1 h at 60 °C and then allowed to settle under gravity for 8 h in a separating funnel. The cocklebur oil biodiesel and glycerol phases are in the upper and lower layers, respectively. After transesterification, the glycerol layer was separated with a separating funnel. The bottom layer of glycerol was removed, and then the upper layer of the cocklebur oil biodiesel was heated at 55 °C with a magnetic stirrer in order to remove impurities such as unreacted methanol, oil, and catalyst. The heated biodiesel was taken into the separating funnel and washed four times with warm distilled water (55 °C, 20% v/v). After the final washing, the biodiesel and water mixture was again allowed to settle under gravity for 8 h and then the lower layer of waste water containing impurities was drained out. To remove the excess alcohol and water, the washed methyl ester was taken into a magnetic stirrer with a heater at 120 °C. The drying process was maintained at 120 °C for 2 h. Thus, cocklebur oil biodiesel was produced from the cocklebur seed. All chemicals used in biodiesel production such as methanol (99.9%) and NaOH (99%) were purchased from Merck.

2.3. Fuel property measuring devices

Physicochemical properties of the cocklebur oil biodiesel such as kinematic viscosity, density, calorific value, flash point, water content, color, copper strip corrosion, pH, sulfated ash content, phosphorus content, cloud point, pour point, cold filter plugging point, and freezing point were measured using devices given in Table 1.

2.4. Ash and element content of biodiesel

Ash content was determined according to the ASTM D482 standard. The ash samples, which were washed with 4 mL of 0.1 N HCl and filtered through Whatman filter paper, were diluted 5 times with 2% nitric acid solution and submitted directly to the Thermo Scientific iCAP-Qc device. Results were multiplied by the dilution factor.

2.5. Calculation of saponification number, iodine value, and cetane number

The saponification number (SN), iodine value (IV), and cetane number (CN) were calculated empirically and used to establish its suitability as a biodiesel within standard specifications.

The SN and IV were calculated from the fatty acid compositions with the help of Eqs. (1) and (2) (Eryilmaz and Yesilyurt, 2016), respectively:

$$SN = \sum (560 \times A_i) / MW_i \quad (1)$$

$$IV = \sum (254 \times D \times A_i) / MW_i \quad (2)$$

where A_i is the percentage of each component, D is the number of double bonds, and MW_i is the molecular mass of each component.

Cetane number was calculated from Eq. (3) (Eryilmaz and Yesilyurt, 2016):

$$CN = 46.3 + 5458 / SN - 0.225 \times IV \quad (3)$$

2.6. Cost analysis of cocklebur production

The cost analysis of cocklebur production was investigated and the results were compared to other field crops. The formulas for determining the cost analysis are given below.

Fixed costs = General administrative expense + Field rental (4)

Sum of changing costs = Tillage (1st + 2nd + 3rd + 4th + ...) + Sowing + Seed cost + Fertilization cost + Top fertilization + Top fertilization cost + Weeding + Pruning + Labor + Disinfestation + Drug value + Watering cost + Harvest + Shipping charge (5)

$$\text{Sowing} = \text{Machine rental} + \text{Labor} \quad (6)$$

$$\text{Seed cost} = \text{Seed (kg/ha)} \times \text{Seed price} \quad (7)$$

$$\text{Fertilization cost} = \text{Fertilization (kg/ha)} \times \text{Fertilization price} \quad (8)$$

$$\text{Top fertilization} = \text{Machine rental} + \text{Labor} \quad (9)$$

$$\text{Top fertilization cost} = \text{Fertilizer amount (kg/ha)} \times \text{Fertilizer price (kg)} \quad (10)$$

$$\text{Weeding} = \text{Machine rental} + \text{Labor} \quad (11)$$

$$\text{Disinfestation} = \text{Machine rental} + \text{Labor} \quad (12)$$

$$\text{Sum of production costs} = \text{Fixed cost} + \text{variable costs} \quad (13)$$

$$\text{Net revenue} = \text{Production value} + \text{Byproduct revenue} - \text{Sum of production costs} \quad (14)$$

$$\text{Production cost} = \text{Sum of production cost} + \text{Byproduct revenue} \quad (15)$$

3. Results and discussion

3.1. Oil content and fatty acid composition of the cocklebur seeds

In the present study, oil content was determined as 35.24%. In other *Xanthium* species oil content can reach up to 42% (Cheng et al., 2013; Rozina et al., 2017). A comparison of fatty acid compositions of cocklebur oil, safflower oil, canola oil, and soybean oil is given in Table 2. Detected oil components in cocklebur seeds were palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), α -linolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$), and cis-11-eicosenoic acid ($C_{20:1}$). Fatty acid composition showed similarities to that found by Rozina et al. (2017). While the most abundant acid component is linoleic acid at 76.97%, the second highest component was oleic acid at 11.37%. Linoleic acid, which is not synthesized in the human body, is one of the few rare fatty acids (Baydar and Erbaş, 2014). Linoleic acid particularly provides a balance for heart and vessel health and cholesterol levels. The linoleic acid content (76.97%)

of *X. strumarium* seed oil was close to that of safflower, but higher than that of soybean and canola oils. On the other hand, it is found that the linolenic acid ratio is 0.74% in *X. strumarium*. The linolenic acid ratio of cocklebur oil is lower than that of canola and soybean oil (Table 2).

3.2. Physicochemical properties of cocklebur oil biodiesel

Physicochemical properties of cocklebur oil biodiesel are given in Table 3. When the determined oil that can be used in the production of biodiesel is compared with the fatty acid components, the fatty acid components of cocklebur oil appear to be suitable for biodiesel production (Pinho et al., 2009; Lovato et al., 2014). The properties of the cocklebur biodiesel were compared to EN 14214 and ASTM D6751. The kinematic viscosity of cocklebur biodiesel at 40 °C is higher than EN 14214 and ASTM D6751 limit values. The density, flash point, water content, copper strip corrosion, iodine value, linolenic acid methyl ester, and sulfated ash content values of cocklebur oil biodiesel are within the standard ranges. The cocklebur oil biodiesel can be used as a blending fuel for diesel fuel because of its high kinematic viscosity at 40 °C. The kinematic viscosity of cocklebur biodiesel at 70 °C, 3.788 mm²/s, is within the standard values, enabling the utilization of cocklebur biodiesel in diesel engines. However, the phosphorus content and cetane number of cocklebur oil biodiesel are higher than the standard values.

Table 2. Fatty acid compositions of cocklebur oil, safflower oil, canola oil, and soybean oil (Anand et al., 2011; Koh and Ghazi, 2015; Eryilmaz and Yesilyurt, 2016).

No.	Fatty acid	Molecular weight	Structure	Systematic name	Formula	Cocklebur oil (this study)	Safflower oil	Canola oil	Soybean oil
1	Myristic	228	14:0	Tetradecanoic	$C_{14}H_{28}O_2$	0	0.05	0	0.1
2	Palmitic	256	16:0	Hexadecanoic	$C_{16}H_{32}O_2$	6.51	5.28	3.49	11.0
3	Palmitoleic	254	16:1	Hexadec-9-enoic	$C_{16}H_{30}O_2$	0.08	0.05	0	0.1
4	Stearic	284	18:0	Octadecanoic	$C_{18}H_{36}O_2$	3.80	1.79	0.85	4.0
5	Oleic	282	18:1	cis-9-Octadecenoic	$C_{18}H_{34}O_2$	11.37	29.88	64.4	23.4
6	Linoleic	280	18:2	cis-9-cis-12 Octadecadienoic	$C_{18}H_{32}O_2$	76.97	62.29	22.3	53.2
7	Linolenic	278	18:3	cis-9-cis-12 Octadecatrienoic	$C_{18}H_{30}O_2$	0.74	0.08	8.23	7.8
8	Arachidic	312	20:0	Eicosanoic	$C_{20}H_{40}O_2$	0.19	0.40	0	0.3
9	Gondoic	310	20:1	11- Eicosenoic	$C_{20}H_{38}O_2$	0.31	0.14	0	0
10	Behenic	340	22:0	Docosanoic	$C_{22}H_{44}O_2$	0	0	0	0.1
Saturated fatty acids						10.50	7.52	4.34	15.5
Monounsaturated fatty acids						11.76	30.07	64.4	23.5
Polyunsaturated fatty acids						77.71	62.37	30.53	61.0
Total						99.97	99.96	99.27	100

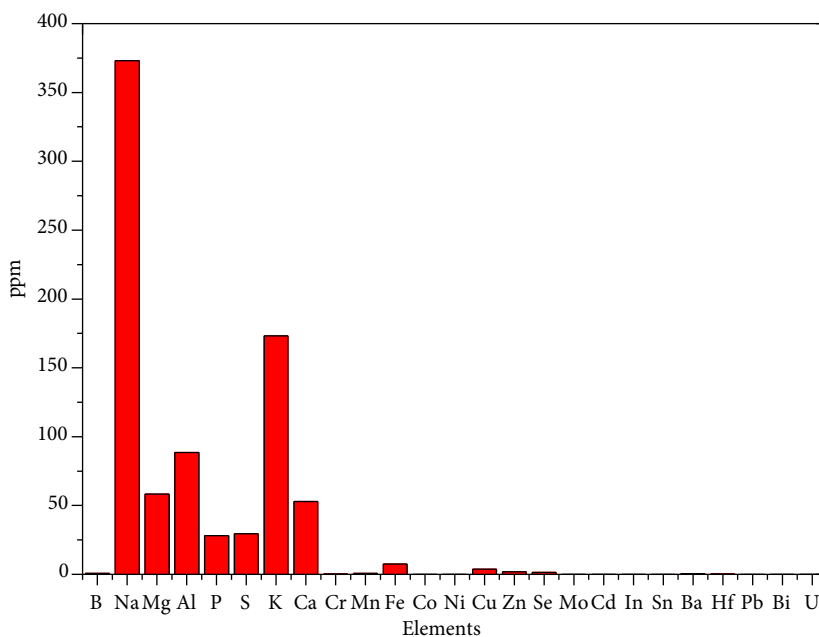
Table 3. Physical properties of cocklebur oil biodiesel.

No.	Property	Unit	EN 14214	ASTM D6751	Cocklebur oil biodiesel
1	Kinematic viscosity at 40 °C	mm ² /s	3.5–5.0	1.9–6.0	6.877
2	Kinematic viscosity at 70 °C	mm ² /s	-	-	3.788
3	Kinematic viscosity at 100 °C	mm ² /s	-	-	2.492
4	VI	-	-	-	237.146
5	Density at 15 °C	kg/m ³	860–900	-	896.89
6	Calorific value	MJ/kg	-	-	38.527
7	Flash point	°C	101 min	130 min	166
8	Water content	mg/kg	500 max	500 max	394.64
9	Cloud point	°C	Report	Report	-1
10	Pour point	°C	Report	Report	-19
11	Cold filter plugging point	°C	Report	Report	-8
12	Freezing point	°C	-	-	-22
13	Color	-	-	-	1.5
14	Copper strip corrosion		No. 1	No. 3	1a
15	pH	-	-	-	7.023
16	Cetane number	-	51 min	47 min	42.3
17	Iodine value	g I ₂ /100 g	120 max	-	144.78
18	Saponification value	-	-	-	190.94
20	Linolenic acid methyl ester	wt. %	12.0 max	-	0.746
21	Sulfated ash content	m/m %	0.02 max		0.019
22	Phosphorus content	m/m %			28.191

3.3. Element content of the cocklebur oil biodiesel

The element content of the cocklebur oil biodiesel is given in Figure 4. As can be seen, the contents of B, Cr, Mn, Co,

Ni, Mo, Cd, In, Sn, Ba, Hf, Pb, Bi, and U in the cocklebur oil biodiesel were lower than 1 ppm. The most abundant element was measured as Na at 373.18 ppm.

**Figure 4.** The element contents of the cocklebur oil biodiesel.

In Table 4, the cost analysis of cocklebur production is given and compared to different field crops grown in arid areas. The cost analysis of cocklebur production was calculated with Eqs. (5)–(16) while the data used for other crops were supplied by the Yozgat Directorate of Provincial

Food Agriculture and Livestock. In the present study, the economical cultivation of cocklebur plant was revealed. With this study and further research, the commercial market for cocklebur will also be formed as its economic value is fully determined.

Table 4. Cost analysis of cocklebur production.

Operations		Unit	Wheat	Barley	Sunflower	Safflower	Lentil	Chick pea	Cocklebur
Tillage	1st	(TL/ha)	71.42	71.42	71.42	71.42	71.42	71.42	71.42
	2nd	(TL/ha)	28.57	28.57	28.57	20	28.57	28.57	28.57
	3rd	(TL/ha)	28.57	28.57	-	-	-	-	-
	4th	(TL/ha)	-	-	-	-	-	-	-
Planting	Machine rental + labor	(TL/ha)	28.57	28.57	37.14	28.57	28.57	28.57	28.57
	Seed	(kg/ha)	240	220	5	20	120	140	40
	Seed price	(TL/kg)	0.40	0.31	1.18	0.85	1.42	1.71	-
	Seed cost	(TL/ha)	96	69.14	59.42	17.14	171.42	240	-
	Fertilizer amount	(kg/ha)	200	200	100	50	80	80	50
	Fertilizer price	(TL/kg)	0.37	0.37	0.37	0.34	0.37	0.37	0.34
	Fertilizer cost	(TL/ha)	74.28	74.28	37.14	17.14	29.71	29.71	17.14
Top fertilization	Machine rental + labor	(TL/ha)	14.28	14.28	-	-	-	-	28.56
	Fertilizer amount	(kg/ha)	16	10	-	-	-	-	15
	Fertilizer price	(TL/kg)	0.27	0.27	-	-	-	-	0.34
	Top fertilization cost	(TL/ha)	43.42	27.14	-	-	-	-	51.42
Weeding	Machine rental + labor	(TL/ha)	-	-	-	-	-	-	28.57
	Pruning		-	-	-	-	-	-	-
	Labor	(TL/ha)	-	-	-	-	-	-	-
Disinfestation	Machine rental + labor	(TL/ha)	14.28	14.28	22.85	5.71	14.28	14.28	-
	Drug value	(TL/ha)	8.57	8.57	14.28	5.71	11.42	28.56	-
Watering cost (Fuel or energy + labor)		(TL/ha)	-	-	-	-	-	-	-
Harvest		(TL/ha)	31,42	31.42	25.71	37.14	40	40	28.57
Shipping charge		(TL/ha)	25,71	25.71	20	14.28	25.71	25.71	14.28
Other expenses		(TL/ha)	8,57	8.57	5.71	5.71	8.57	11.42	5.71
Byproduct revenue		(TL/ha)	114,28	120	-	-	-	-	114.28
Yield		(kg/ha)	2800	3000	1500	1000	900	1000	6830
Price		(TL/kg)	0,24	0.20	0.45	0.42	1.14	1.14	0.14
Product value		(TL/ha)	680	617.14	677.14	428.57	1028.57	1142.85	975.71
Revolving fund interest			8,3	7.5	5.6	3.9	7.5	9	3.9
SUM OF CHANGING COSTS		(TL/ha)	474,28	431.42	322.85	222.85	428.57	517.14	302.85
General administrative expenses		(TL/ha)	9,42	8.57	6.57	4.57	8.57	10.28	3.42
Field rental		(TL/ha)	57,14	57.14	57.14	28.57	57.14	57.14	28.57
SUM OF FIXED COSTS		(TL/ha)	65,71	65.71	62.85	34.28	65.71	68.57	32
SUM OF PRODUCTION COSTS		(TL/ha)	540	497.14	385.71	254.28	494.28	585.71	334.85
Production cost		(TL/kg)	0,15	0.12	0.25	0.25	0.55	0.58	0.06
Net revenue		(TL/ha)	254,28	240	291.42	174.28	534.28	557.14	755.14

As can be seen in Table 4, the net revenue of the cocklebur plant is predicted as 755.14 Turkish lira (TL)/ha. According to the predictions, it seems that the cultivation of cocklebur plant for biodiesel feedstock will be profitable from an agricultural perspective under these conditions (Chang et al., 2017). The start of the cultivation of cocklebur as an alternative industrial plant will provide multiple benefits such as combating erosion for a sustainable environment and developing biodiversity and good agricultural practices.

3.4. Conclusions

Cocklebur is a plant growing almost everywhere in the world due to its high competitive power and endurance in various climate and soil conditions. In this study, it was determined that cocklebur seeds containing 35% crude oil can be grown easily in arid areas and can be used as an energy source. Moreover, its cultivation would contribute to biodiversity in arid areas and to sustainable

environmental management with good farming practices. The cocklebur seed crude oil could be transesterified using NaOH as a catalyst and methanol as an alcohol. Physicochemical properties of cocklebur oil biodiesel were compared to the EN 14214 and ASTM D6751 standards. Some important fuel properties such as density, flash point, water content, copper strip corrosion, iodine value, linolenic acid, methyl ester, and sulfated ash content values were within the limits of the standards. However, the phosphorus content and cetane number of cocklebur oil biodiesel were higher than the standard values. Cocklebur oil biodiesel can be used as a blending fuel for diesel fuel because of its higher kinematic viscosity. When the data from this study are evaluated altogether, cocklebur is a promising plant for supplying energy for the future. Cocklebur is agriculturally profitable when compared to other field crops grown in arid areas.

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