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Quality assessment of oil and biodiesel derived from *Balanites aegyptiaca* collected from different regions of Rajasthan

Mahesh Kumar Saini [a], Paras Sharma [b], Jagdish Prasad [c], S.L. Kothari [a], Vinod Singh Gour [a,*]

[a] Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, 303002, India
[b] Food Chemistry Division, ICMR-National Institute of Nutrition, P/O Jamai Osmania, Tarnaka, Hyderabad, 500007, India
[c] Amity School of Applied Sciences, Amity University Rajasthan, Jaipur, 303002, India

A R T I C L E I N F O

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A B S T R A C T

Increasing demand for clean and sustainable energy resources has enhanced the importance of biodiesel. *Balanites aegyptiaca* is an underexplored plant with biodiesel potential and wide range adaptability. In the present study, variations in oil yield in samples of *Balanites aegyptiaca* collected from the different agro-climatic zone of Rajasthan have been explored. The fatty acid analysis was carried out using GC-FID and oil samples were compared for their fatty acid composition. Based on data of fatty acids, biodiesel parameters were estimated using a dry lab. The study reveals that out of the seven agro-climatic regions under study Jodhpur, Dungarpur, Udaipur, Kota and Alwar regions are the suitable regions to get high oil yield of *B. aegyptiaca* with statistically no significant difference in the oil yield. The oil is rich in oleic acid irrespective of the region. Oleic acid varied from ~39% to ~58% of the total fatty acids content. Density, kinematic viscosity, iodine value and cetane number of the biodiesel derived from the oil samples collected from all the regions follows the European biodiesel standards (EN 14214). The study will help in exploring the possibility of getting biodiesel from this salt and drought tolerant plant.

1. Introduction

To mitigate the effect of global warming and fulfill the rising demand for renewable and sustainable energy resources plant scientists have been attracted towards biodiesel (Dincer, 2000). By improving biodiesel availability in terms of quality and quantity, energy demand can be supplemented along with wind and solar energy. Thereby, we can decrease the reliance on fossil fuels (Atadashi et al., 2010). Apart from energy supply, use of plants/trees at a larger scale as a source of biodiesel will help in reducing pollution, soil erosion, carbon sequestration and enhancing soil health.

Biodiesel can be produced by transesterification process of fatty acids present in seed oil (Van Gerpen, 2005). Transesterification converts fatty acids into methyl/ethyl esters. These esters act as an efficient fuel.

Quality of biodiesel depends on several factors including the fatty acid composition of oil (Knothe, 2007). Various properties like carbon chain length, amount of saturated or unsaturated forms of fatty acids and magnitude of unsaturation of fatty acids characteristics the properties of biodiesel (Knothe, 2005). These chemical properties of feedstock oil will influence various properties of biodiesel including monounsaturated fatty acid (% MUFA), poly unsaturated fatty acid (PUFA%), degree of unsaturation, saponification value, iodine value, cetane number, cold filter plugging point, cloud point, pour point, oxidation stability, heating value, kinematic viscosity and density (Saraf and Thomas, 2007).

These properties will decide the efficiency of the biodiesel in terms of its calorific value, its use at an appropriate temperature range and the oxidative stability during storage.

Several plant species including Jatropha, karanj, mastwood bastard poon, mahua, koroch, rubber, neem, caster, peanut, soya bean, palm, sunflower, rapeseed, corn seeds, tallow etc. have been explored for their biodiesel potential (Atabani et al., 2013; Singh and Singh, 2010). *Balanites aegyptiaca* (L.) Del. has also been studied as a source of biomass for the biodiesel (Singh and Singh, 2010; Chapagain et al., 2009). *B. aegyptiaca*, also known as 'Hingot' in Hindi, 'Desert date' in English, belongs to Balanitaceae family, is found in several tropical countries but still underexplored. Xerophytic nature, survival in poor soil, low maintenance cost, tolerance of flood and salt, high annual fruit yield and fairly good carbon sequestration potential make this plant an interesting choice to study in detail for its biodiesel potential (Firmin, 1971; IPGRI,

* Corresponding author.
E-mail addresses: vinodsingh2010@gmail.com, vkgaur@jpr.amity.edu (V.S. Gour).

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1984; Rathore et al., 2005; Gour and Datta, 2015). The oil quality parameters, fatty acid composition, transesterification, and standard biodiesel analysis have been reported by Chapagain et al., 2009). According to them biodiesel derived from *B. aegyptiaca* meets the biodiesel international standards. Variation in oil yield in *Balanites aegyptiaca has* been reported (Chapagain and Wiesman, 2005). Deshmukh and Bhuyar (2009) have reported properties and engine performance of esters derived from *Balanites aegyptiaca* oil. Maximum ester yield was found to be 95% (Deshmukh and Bhuyar, 2009). According to Deshmukh and Bhuyar (2009) the calorific value of methyl esters derived from the oil of *B. aegyptiaca* is nearly 94% of the diesel fuel (Deshmukh and Bhuyar, 2009).

Looking at the high potential of *B. aegyptiaca* and the possibility to utilize poor soil (which is available at large scale) affected with salt and scarcity of water the present study has been carried out to look into variation in seed oil properties and biodiesel potential in the samples collected from different agro-climatic regions of Rajasthan state (India). Till now there is no study which reveals variation in fatty acid profiles in the seed oil collected from different agro-climatic regions of Rajasthan. The study will help in understanding variation in fatty acid profile and thereby identification of a region(s) to grow *B. aegyptiaca* in order to get better quality biodiesel to benefit the local population in tropical regions.

2. Experimental setup

2.1. Plant material

Rajasthan covers the largest geographical area of India with 3.42 Lakh Km^2. It has got huge variations in agro-climatic conditions hence the state is divided into ten agro-climatic regions (Soil Resource Atlas of Rajasthan, 2010). The fruit samples have been collected from seven agro-climatic regions namely Jaipur-Dausa, Churu-Sikar, Jodhpur-Pali, Udaipur, Kota, Alwar-Bharatpur, Dungarpur-Banswara (Fig. 1). Total fifty-six samples were collected.

2.2. Extraction of kernel

The nut was extracted from the fruit and then it was broken with the help of hammer in order to isolate kernel. The kernel was powdered using mortar-pestle.

The oil was extracted from kernel powder using soxhlet apparatus. A known amount of kernel powder was wrapped in chromatography paper and kept in soxhlet apparatus. As a solvent, n-hexane was used at 80 °C temperature. The solvent was recovered by distillation method. In order to remove traces of solvent, the oil sample was kept on water-bath at 80 °C until the smell of n-hexane got removed. The oil yield in percentage was calculated as follows

$$Oil\ \ yield(\%) = \frac{weight\ \ of\ \ oil\ \ (g)}{weight\ \ of\ \ kernel\ \ powder\ \ (g)} \times 100$$

Oil yield percentage has been obtained for 56 plants. This percentage data was not normally distributed on the basis of the histogram hence $\log_{10}$ transformation was applied. Out of the 56 data for oil percentage **three data were found to be outlier on the basis of 4-Sigma outlier technique.** Except outliers, the rest of the data were subjected to Ryan-Joiner normality test (RJ test). According to the test the RJ value was found to be 0.981 and *P* values was >0.100. This indicates that the data are not significantly different than the normal distribution.

The z value for the data was found to be 0.049 which is in the range of ±1.96. This also supports the normal distribution of the data. Then the transformed data was subjected to One-Way ANOVA to find if there is any significant difference among the seven zones. The ANOVA table indicates significant difference among the zones (Table 1). Then, to look

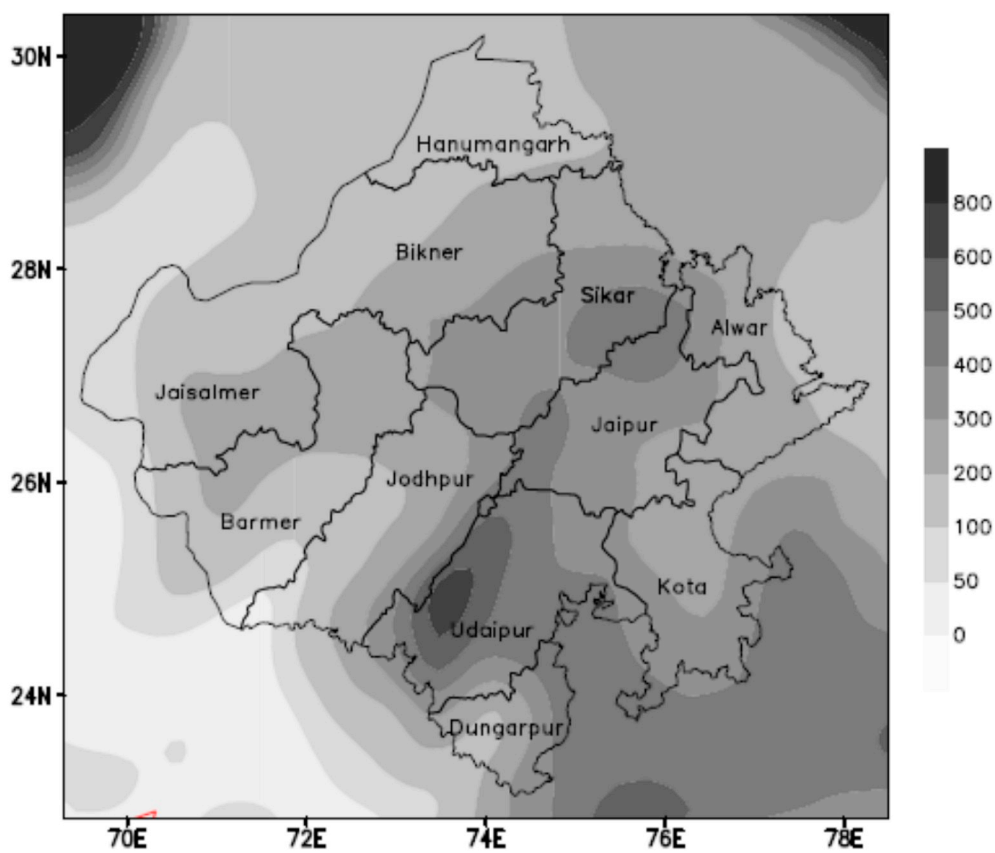

Fig. 1. Agro-climatic regions of Rajasthan.

into significant difference in oil yield between all possible pair of zones post hoc test and Duncan's Multiple Range Test (DMRT) was applied using SPSS ver 8.8.

2.3. Fatty acid analysis

An equal volume of oil from all the accessions of the same agro-climatic region was taken in a vial. The oil sample from each agro-climatic region, after derivatization to fatty acid methyl esters, were subjected to GC-FID (Agilent Series, 7890 Series, USA) in order to determine the fatty acid profile. The method used here for fatty acid analysis is AOAC official method 996.06 (AOAC, 1995). The same method has been used by O'fallon et al., 2007 and Dorni et al. (2018) (O'fallon et al., 2007; Dorni et al., 2018). Direct trans-esterification of the fatty acids present in oil was carried out using 2% sulphuric acid in methanol. In 40 ml of the oil sample (in a screw-capped Pyrex culture tube) 1 ml of C17 (1 mg/ml; Heptadecanoic acid; Sigma H3500) was added as an internal standard. Potassium hydroxide (0.7 ml) and 5.3 ml of methanol with 0.05% of butylated hydroxyl toluene (BHT) were added in the mixture. This mixture was then maintained in a water bath at 55 °C temperature for 90 min with vigorous shaking for 20 s at an interval of every 20 min. Then, the tubes were cooled under running tap water.

After that 2% sulphuric acid was added and the mixture was incubated at 55 °C temperature for 90 min. In this mixture 3 ml n-Hexane was added, vortexed and centrifuged for 5 min at 2000 rpm. The n-hexane layer, after centrifugation, was collected in a 5 ml test tube having 0.1 g of sodium sulphate. The tubes were again vortexed and the solvent was evaporated under a gentle stream of nitrogen. 1.5 ml of dichloromethane was added to the tube and mixed thoroughly. 0.5 ml of the dichloromethane with FAME was filtered through 0.22 mm PVDF syringe filter and injected to the gas chromatograph. SP 2560 (75 m × 0.18 mm x 0.14 mm) column was used for the gas chromatographic separation with following instrumental conditions: Injector temperature: 250 °C; Carrier gas: Hydrogen @ 0.6 ml/min; Split ratio: 1:100; Oven program: 140 °C (Hold 1.5 min) to 220 °C @ 3 °C (Hold 1.0 min) to 230 °C (Hold 3 min); Detector: Flame Ionization Detector; Temperature: 260 °C; Hydrogen flow rate: 40 ml/min; Zero Air: 400 ml/min; Injection Volume: 1 ml. The fatty acids present in the samples were quantified by percentage area calculation using Supelco 37 FAME Mixture (Sigma Cat. No. 47885-U) as reference standard. Results were expressed as percentage fatty acids of oil. Internal standard as well as Standard Reference Material (SRM)-1544 was used for analytical quality assurance.

2.4. Biodiesel analysis

On the basis of fatty acid content of the oil, the biodiesel analysis was carried out using the Biodiesel Analyzer v2.2 software (Mitra et al., 2015) to compare biodiesel parameters of oil samples which represent the different agro-climatic regions. Palmitic acid, stearic acid, linoleic acid and oleic acid were taken into consideration for biodiesel analysis.

3. Result and discussion

3.1. Variation in oil yield

Oil content variation has been observed in *Balanites aegyptiaca* where mean oil content varied from ~49% to ~69% (Fig. 2). The statistical analysis (ANOVA) of the transformed data indicates that agro-climatic zones have significantly different oil yield (Table 1). The post-hoc test (DMRT) of the transformed data categorized the oil yield in two groups (Fig. 2). Jodhpur, Dungarpur, Udaipur, Alwar and Kota have no significant difference in the oil yield and these regions form one group. However, within this group the highest mean oil percentage has been recorded from Dungarpur region with 69.07%, followed by Jodhpur region with 66.24%. The other group with lower oil yield is having only two agro-climatic regions namely Jaipur (49.12%) and Sikar (61.89%) with no significant difference in oil yield. Chapagain and Wiesman (2005) reported a similar pattern of oil yield in *Balanites aegyptiaca* where oil yield varied from the lowest value of 39.20% in Indian sample to the highest value 50.22% in Bet-Shean sample (Chapagain and Wiesman, 2005). However, Deshmukh and Bhuyar (2009) reported a highly stable oil yield in the range of 45–47% in *Balanites aegyptiaca* (Deshmukh and Bhuyar, 2009). The variation in oil content may be due to the environment and genetic make-up of the plants under study. Even there may be the effect of interaction between the genotype and the environment on oil yield. According to Tomar et al. (2011 the oil yield depends on many factors like annual rainfall, light and soil quality (Tomar et al., 2011). In contrast, Sunil et al. (2009) reported that the oil content in *Pongamia pinnata* is feebly influenced by the environmental conditions (Sunil et al., 2009). The maximum oil content (~69%) was observed in the samples collected from Dungarpur region (Fig. 2).

3.2. Fatty acid composition

The oil contains various fatty acids namely palmitic acid (C16:0), oleic acid (C18:1), Linoleic acid (C18:2) and stearic acid (C18:0) (Table 2). Palmitic acid varied from ~15% (in the samples collected from Alwar) to the maximum amount of ~20% (in the samples collected from Udaipur). Range of variation in stearic acid is very narrow from ~7% to ~9%. The highest amount of stearic acid was observed in the oil sample from Sikar. Oleic acid varied from ~39% to ~58%. The highest amount of oleic acid was found in the oil samples collected from Jaipur. Linoleic acid varied from ~15% to 26%. The highest amount of linoleic acid has been recorded from the Kota region. Irrespective of agro-climatic regions oleic acid was found to be the maximum contributing fatty acid. The second most abundant fatty acid was found to be as linoleic acid followed by palmitic acid. These findings indicate that the oil of *Balanites aegyptiaca* is rich in unsaturated fatty acid than the saturated fatty acids.

Quantitative variation in fatty acid composition in oil in *B. aegyptiaca* samples collected from different regions may be due to environmental factors, genetic factors and their interactions, similar results have been observed in *Jatropha curcas* (Kaushik and Bhardwaj, 2013; Sinha et at., 2015; Kumar and Das, 2018).

Table 1
Analysis of variance (ANOVA) table for the transformed data of percentage oil yield among seven agro-climatic regions.

ANOVA							
Transformed data of percentage oil yield							
			Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Between Groups	(Combined)		1.465	6	0.244	3.471	0.007
	Linear Term	Unweighted	0.522	1	0.522	7.419	0.009
		Weighted	0.716	1	0.716	10.174	0.003
		Deviation	0.749	5	0.150	2.130	0.079
Within Groups			3.166	45	0.070		
Total			4.632	51			

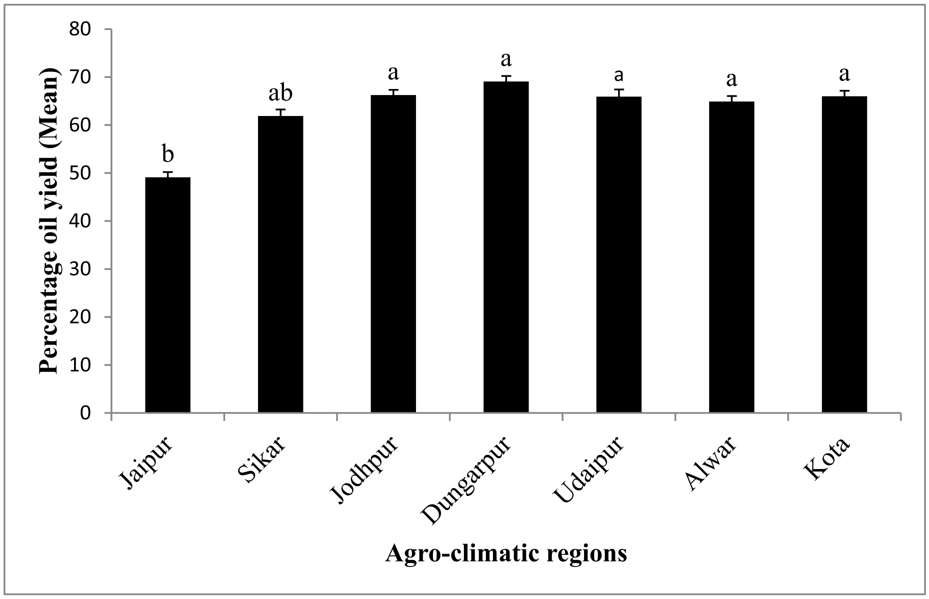

Fig. 2. Seed oil yield variation among seven agro-climatic regions of Rajasthan. The value presents mean oil percentage and the error bar presents standard error. DMRT – mean followed by different letters differ significantly at $p \le 0.05$.

Table 2
Percentage fatty acid composition in oil of *Balanites aegyptiaca* from different agro-climatic region.

Fatty acid	Jaipur	Sikar	Jodhpur	Dungarpur	Udaipur	Alwar	Kota
Palmitic acid (C16:0)	19.13	17.77	17.79	17.22	20.25	14.92	18.86
Stearic acid (C18:0)	7.34	9.11	9.08	7.1	8.62	7.35	7.48
Oleic acid (C18:1)	58.05	47.67	47.56	50.99	38.84	47.15	48.06
Linoleic acid (C18:2)	15.48	25.45	25.57	24.7	32.3	30.58	25.61

3.3. Properties of biodiesel

Biodiesel properties including saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, degree of unsaturation, saponification value, iodine value, cetane number, long-chain saturated factor, cold filter plugging point, cloud point, pour point, allylic position equivalent, bis-allylic position equivalent, oxidation stability, higher heating value, kinematic viscosity and density have been studied and recorded as presented in Table 3. Few important parameters have been discussed in the following paragraphs.

3.3.1. Kinematic viscosity

The kinematic viscosity is a fluid flow property. Kinematic viscosity of the biodiesel derived from *B. aegyptiaca* oil has been found to be quite stable ($3.8\ mm^2/s$ to $3.9\ mm^2/s$) as presented in Table 3. Kinematic viscosity of the biodiesel derived from *Jatropha curcas* and *Pongamia*

Table 3
Biodiesel properties on the basis of fatty acid profile in *Balanites aegyptiaca* from different agro-climatic region.

Biodiesel properties	Jaipur	Sikar	Jodhpur	Dungarpur	Udaipur	Alwar	Kota
SFA	26.470	26.880	26.870	24.320	28.870	22.270	26.340
MUFA	58.050	47.670	47.560	50.990	38.840	47.150	48.060
PUFA	15.480	25.450	25.570	24.700	32.300	30.580	25.610
DU	89.010	98.570	98.700	100.390	103.440	108.310	99.280
SV	202.226	202.069	202.075	201.996	202.693	201.593	202.334
IV	80.241	88.966	89.085	90.593	93.434	97.791	89.607
CN	55.235	53.293	53.266	52.937	52.205	51.371	53.114
LCSF	5.583	6.332	6.319	5.272	6.335	5.167	5.626
CFPP	1.063	3.416	3.375	0.086	3.426	−0.244	1.198
CP	5.070	4.355	4.366	4.066	5.660	2.856	4.928
PP	−1.317	−2.093	−2.082	−2.407	−0.677	−3.721	−1.471
APE	89.010	98.570	98.700	100.390	103.440	108.310	99.280
BAPE	15.480	25.450	25.570	24.700	32.300	30.580	25.610
OS	10.209	7.224	7.203	7.365	6.242	6.447	7.195
HHV	39.525	39.510	39.509	39.513	39.484	39.504	39.504
Kinematic Viscosity (mm^2/s)	3.964	3.904	3.903	3.896	3.843	3.859	3.888
Density (g/cm^3)	0.874	0.876	0.876	0.876	0.876	0.877	0.876

SFA: Saturated Fatty Acid (%), MUFA: Mono Unsaturated Fatty Acid (%), PUFA: Poly Unsaturated Fatty Acid (%), DU: Degree of Unsaturation, SV: Saponification Value (mg/g), IV: Iodine Value, CN: Cetane number, LCSF: Long Chain Saturated Factor, CFPP: Cold Filter Plugging Point (°C), CP: Cloud Point (°C), PP: Pour Point (°C), APE: Allylic Position Equivalent, BAPE: Bis-Allylic Position Equivalent, OS: Oxidation Stability (h), HHV: Higher Heating Value, Kinematic Viscosity (mm^2/s), Density (g/cm^3).
Source: http://www.brteam.ir/analysis/#result

pinnata were found to be 4.4 and 4.85 mm^2/s respectively at 40 °C (Atabani et al., 2013). According to ASTM D6751 biodiesel fuel standard, kinematic viscosity at 40 °C may vary from 1.9 to 6.0 mm^2/s (Moser, 2009). The kinematic viscosity for diesel fuel is 3.06 mm^2/s at 38 °C. High kinematic viscosity of biodiesel in comparison to diesel fuel hampers fuel atomization during injection and therefore, it requires modification in fuel injection systems (Singh and Singh, 2010). However, the present findings reveal that the kinematic viscosity of biodiesel derived from *B. aegyptiaca* is very close to that of diesel.

3.3.2. Iodine value

The iodine value (IV) or iodine number is the mass of iodine (g) that binds with the double bonds present in fatty acid carbon chain in 100 g biodiesel. As the unsaturation of fatty acids increases in biodiesel, the IV also increases. The biodiesel with high IV remains more vulnerable to oxidation when it comes in contact with air. According to European standards (EN 14214) for biodiesel, this value should be below 120. Iodine value beyond 130 would promote polymerization of fatty acid and these polymerized fatty acids will deposits on injector nozzles, piston rings and piston ring grooves. Biodiesel derived from *B. aegyptiaca* has IV ranging from 80 to ~98 as presented in Table 3. IV for biodiesel derived from *Jatropha curcas* has been reported as 93 (Makkar and Becker, 2009).

3.3.3. Cetane number

Cetane number (CN) is the primary indicator of fuel quality as it provides information about the fuel-ignition. Higher the cetane number, lesser will be time taken to start the compression engine ignition. European standards (EN 14214) recommend the minimum value of cetane number 51for biodiesel. Very low CN will cause lag in ignition time. In the winter season, biodiesel with low CN will not be able to warm the engine faster so the fuel combustion will be incomplete and the air pollution will increase especially due to release of hydrocarbon as a part of the smoke. In the present study, CN for biodiesel derived from *B. aegyptiaca* has been found to vary in a very narrow range (~51–55) (Table 3). CN of biodiesel derived from *Jatropha curcas* and *Pongamia pinnata* were found to be 57.1 and 58 respectively (Atabani et al., 2013).

3.3.4. Cold flow properties

Fuel flow properties at low temperature play an important role for engine ignition. At low-temperature fuel components get crystalized or suspended in the pipeline of the fuel system or clog the fuel filters and leads to delay in engine ignition. If this condition persists for a longer duration the engine may not ignite at all. The cold flow properties include cloud point (CP), pour point (PP), cold filter plugging point (CFPP) and viscosity of the fuel.

3.3.4.1. Cloud point (CP). The visibility of the crystals of fuel components in the fuel is regarded as a cloud. The maximum temperature at which the fuel starts the formation of wax crystals is known as the cloud point. Cloud point depends on the ratio of saturated and unsaturated fatty acid in the fuel. Higher the amount of unsaturated fatty acid higher will be the CP of the fuel and the fuel quality will be poor. The cloud point in the biodiesel derived from *B. aegyptiaca* varied from ~3 °C to ~6 °C (Table 3). Biodiesel derived from *Jatropha curcas*, *Madhuca indica,* and *Hevea brasiliensis* have been found to be 4 °C, 5 °C, and 4 °C respectively (Atabani et al., 2013).

3.3.4.2. Pour point (PP). After reaching the cloud point, as the temperature declines further, the fuel forms wax crystals and these crystals disrupt the free flow of fuel through the pipe system of ignition engine. This property of fuel also depends on the magnitude of saturated fatty acids in the fuel. The higher amount of unsaturated fatty acid in the fuel causes low pour point and that leads to a better quality of the fuel. The pour point of biodiesel derived from *Balanites aegyptiaca* varied from −3.721 °C (Alwar) to −0.677 °C (Udaipur) (Table 3). The pour point in biodiesel derived from Tallow and soya bean have been reported to be 9 °C and −7 °C respectively (Rao, 1991 and Fukuda et al., 2001).

3.3.4.3. Cold filter plugging point (CFPP). The cold filter plugging point is the lowest temperature at which 20 ml of fuel passes through a filter within 60 s by applying a vacuum of 2 kPa. CFPP does not reflect the actual limit of the fuel's operability temperature but and its value depends on the climatic conditions of the region. The value of CFPP for the biodiesel derived from *B. aegyptiaca* ranged from ~-0.2 °C to ~3.0 °C (Table 3). Variations in CFFP derived from different feedstocks have been reviewed by Atabani et al. (2013), according to them it may vary from −17 °C to 11 °C (Atabani et al., 2013).

3.3.5. Density of biodiesel fuel

Density is the mass per unit of volume. Since the density varies with temperature therefore according to the quality standards for biodiesel the density of biodiesel should be determined at 15 °C. Fuel density directly affects fuel performance, heating value, and viscosity. It is an important parameter to be taken into account for the storage, transportation and distribution of biodiesel.

The density of biodiesel depends on the size (molecular weight and shape) of methyl/ethyl esters of fatty acids. The density of biodiesel is typically higher. The density of biodiesel varies in a very narrow limit. Contamination of the biodiesel significantly affects its density; therefore, density is included as one of the criteria of purity.

The density of biodiesel derived from *B. aegyptiaca* was almost constant irrespective of the agro-climatic zone (Table 3). The density varied from 0.87 to 0.88 g/cm^3. Almost similar density (0.830 g/cm^3) in biodiesel derived from the seed oil of *Jatropha curcas* has been reported [30]. Dhar and Agarwal (2014) reported density of biodiesel derived from seed oil of *Pongamia pinnata* to be 0.881 g/cm^3 (at 30 °C). The density limits specified for biodiesel by ASTM 6571 ranges from 0.8 to 0.9 g/cm^3 (at 30 °C) (Dhar and Agarwal, 2014).

The biodiesel derived from oil of *B. aegyptiaca* growing in the state of Rajasthan the kinematic viscosity is quite close to that of diesel. IV, cetane number and density of biodiesel are in the range of European standards (EN 14214); European standards (EN 14214) and ASTM 6571 respectively. Cold flow properties are also in the acceptable range of practical applications of this biodiesel.

4. Conclusion

Seed oil yield varies in samples collected from different agro-climatic regions of Rajasthan and the highest oil yield (~69%) has been obtained from the samples collected from Dungarpur region. However, there is no significant difference in oil yield in the samples collected from Jodhpur, Dungarpur, Udaipur, Alwar and Kota. Hence these regions may be explored to get seed oil from *B. aegyptiaca*. The oil is rich in oleic acid irrespective of the region of sample collection. The biodiesel analysis indicates that biodiesel derived from the oil of *B. aegyptiaca* will have IV, CN, kinematic viscosity and density in the range of European biodiesel standards (EN 14214). A large-scale study is required to explore the economic feasibility of the production of biodiesel derived from this plant as an alternative/additional source of income for the farmers, as this plant can be grown on boundaries of farmland.

Declaration of competing interest

The authors have no conflicts of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101374.

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