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Biophysicochemical Variability Evaluation of *Jatropha curcas* L. Collections for Biodiesel Feedstock

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Abstract. The seed oils of six *Jatropha curcas* biotypes were evaluated for their oil quality parameters and showed: oil content (38-41 %), acid value (0.14-6.94 mg/g), free fatty acid (0.07-3.47 %), iodine value (115.48-163.37 mg/g) and viscosity (0.6320-0.7431). Significant differences among biotypes were observed in oil yield and biochemical parameters. The variability among the biotypes indicate a good scope of genetic gain through selection.

Keywords: *Jatropha curcas*, biodiesel, chemical composition, seed oil

Introduction

In anticipation of the supposed alarming scarcity in the production of crude petroleum oil and at the same time an increase in the number of automobiles and other internal combustion engines, the use of biodiesel with the diesel and alcohol in petrol has been made mandatory to initially minimize the cost of import. *Jatropha curcas* is a challenge species that can reduce the load of diesel import into the country. Genus *Jatropha* belongs to the family Euphorbiaceae and contains approximately 175 succulents, shrubs and trees; some are deciduous like *Jatropha curcas* L. having different uses. Its fruits and seeds are used as combustibles, fodder and green manure, seed oil is used as fuel, in production of soaps and for medicinal purpose, whereas the whole plant is put to a number of purposes.

Performance of engine associated problems have been encountered due to several basic properties of vegetable oils, used as biofuel such as naturally occurring gums, high viscosity, acid composition, free fatty acid content and low cetane rating. Therefore, it is crucial to estimate those parameters before using vegetable oil or biodiesel as a fuel substitute. The present study envisages use of *Jatropha* as a fuel plant, wherein different *Jatropha* biotypes grown at different geographical locations with respect to their biophysicochemical parameters, have been compared.

Materials and Methods

Six biotypes of *J. curcas* were collected from different geographic locations of India (Table 1). Seed samples were kept in the oven at 70 °C for 5-6 h for removal of moisture. Then, seeds were grinded finely for extraction of oil. Conventional soxhlet technique was used to estimate oil content according to AOAC (1984). Then, the following physicochemical parameters were estimated according to Harris (1984).

Specific gravity. Specific gravity of the oil was determined using specific gravity bottle which was first weighed empty (W_B) then filled completely with the liquid and weighed (W_L). After cleaning, the bottle was filled completely with distilled water and again weighed (W_w). The temperature of the liquid was also recorded.

$$\text{Specific gravity} = \frac{W_L - W_B}{W_w - W_B}$$

Refractive index. Refractometer was cleaned with alcohol and ether. A drop of oil was placed on the prism. The prism was closed by the ground glass-half of the instrument. The dispersion screw was adjusted so that no colour line appeared between the dark and illuminated halves. The dark line was adjusted exactly on the wires and the refractive index was read on the scale.

Acid value. Acid value was determined according to AOCS (1988). About 0.1 g of oil was weighed accurately in a 250 mL conical flask, dissolved in 50 mL of neutralized isopropanol followed by addition of 1 mL phenolphthalein solution. The solution was titrated with N/10 KOH with constant shaking until a pink colour persists for 15 sec. The titre value in mL (a) was recorded.

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Table 1. Passport data of *Jatropha* biotypes under study

PGR No.	Collection No.	Crop	Native place	Seed character
050105	Pant Selection4	Jatropha	CP, Nagpur	Smooth, brown, chocholate
040001	Shu-Jatro-04004	Jatropha	Jagdalpur, Chattisgarh	Rough, brown, chocholate
30003-2	Shu-Jatro-03005	Jatropha	Jagdalpur, MP	Smooth, brown, chocholate
050106	Pant Selection31	Jatropha	CP, Nagpur	Rough, black, chockolate
050107	Pant Selection97	Jatropha	CP, Nagpur	Rough, brown, chockolate
-*	RJ3	Jatropha	Pali, Rajasthan	Smooth, black, chockolate

***** = not allotted till study.

$$\text{Acid value} = \frac{(a) \times 56.1 \times N}{\text{Wt. of substance (g)}}$$

Saponification value. About 2 g of the sample was weighed accurately in a 250 mL round bottom flask. Alcoholic KOH solution (25 mL) was added, a reflux air condenser was attached and the flask was kept in a boiling water bath for 1 h. While the solution was still hot, 1 mL of phenolphthalein solution was added and the excess alkali was titrated against 0.5 N HCL (a). The experiment was repeated without the oil or fat to obtain the blank value (b).

$$\text{Saponification value} = \frac{[(b)-(a)] \times 0.02805 \times 1000}{\text{Wt. of substance (g)}}$$

Iodine value. The sample was accurately weighed in a dry iodine flask, dissolved by addition of 10 mL of CCl_4, then iodine monochloride (20 mL) solution was added. The flask was closed with stopper, previously moistened with KI solution, and the mixture was allowed to stand in the dark for 30 min at a temperature of 15-25 °C. KI solution (15 mL) was pipetted into the cup top, the stopper was carefully removed and rinsed along with the sides of the flask with water (100 mL). The flask was shaken and then, its content was titrated with 0.1N $Na_2S_2O_3$ solution using starch mucilage as indicator towards the end of the titration (starch is added only when the colour of the reaction mixture was faint yellow). The volume of thiosulphate required (a) was noted. Simultaneously, the experiment was carried out without the oil or fat to get the blank value (b).

$$\text{Iodine value} = \frac{[(b)-(a)] \times 0.01269 \times 100}{\text{Wt. of substance (g)}}$$

Viscosity. One bulb of pipette was filled with distilled water up to more than half. Second bulb was attached with the rubber tube and water was sucked up to the upper mark of the bulb and then allowed to flow under its own weight. The time of flow of water from upper to lower mark was recorded by the stopwatch. Three readings were taken and mean was calculated. The process was repeated with the oil sample. Temperature of the sample was also recorded. Viscosity was calculated as:

$$\eta_1 / \eta_2 = d_1 t_1 / d_2 t_2$$

where:

d_1 = density of oil
d_2 density of water
t_1 = time of flow of oil
t_2 = time of flow of water
η_1 = viscosity of oil
η_2 = viscosity of water.

Free fatty acids. Free fatty acids were extracted from lipids by using heptane: isopropanol: acetic acid ratio of 40:10:1. Then the heptane phase was evaporated *in vacuo*. Free fatty acids were estimated using coloured complex with copper.

Stearic acid as a standard was mixed with chloroform (5 mL) and placed in centrifuge ochrage tube. Copper reagent (2.5 mL) was added, the tube was stoppered, shaked vigorously for 1 min and centrifuged for 5 min at 13,000 g. Aqueous phase was removed by suction with a fine hypodermic needle. A portion (2.5 mL) of chloroform layer was taken into a clean dry tube, diethyldithiocarbamate reagent (0.5 mL) was added and the tube was shaken well. Absorbance was recorded at 440 nm and standard curve was prepared (Fig. 1).

Fatty acid determination. Methyl esters of fatty acids were prepared by the method described by Singh *et al.* (2006). Mechanism of reaction has been shown in Fig. 2. This elute was then subjected to analysis of fatty acids by gas chromatography at the Oil Quality Lab., Department of Genetics and Plant Breeding of the College of Agriculture, Pantnagar.

The experimental design for testing of mean and interaction effects of biotypes was one factor randomized block design with 3 replicates. Standard error of mean was calculated by using the formula:

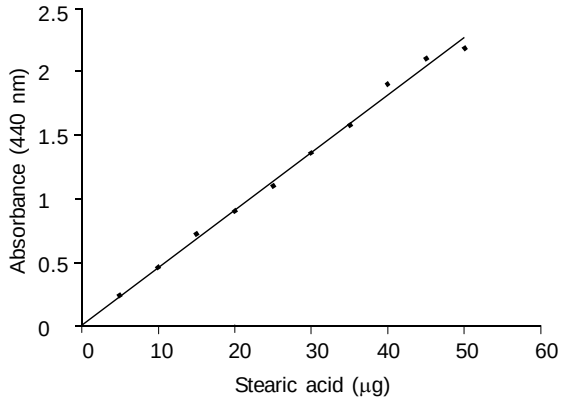

Fig. 1. Standard curve for free fatty acid determination.

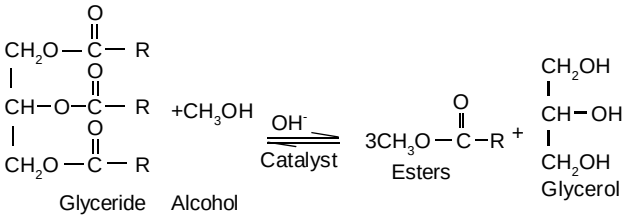

Fig. 2. Transesterification reaction for biodiesel production.

SE of mean = SD/√n

where:

SD = standard deviation and
n = no. of observations,

The critical difference (CD) was calculated as:

CD = SEm × √X × t value at 1% level of significance at *n*-1 degree of freedom.

Results and Discussion

Oil and all other biochemical parameters were widely distributed among different biotypes of *Jatropha* (Table 2); the oil content varied between 38 to 41% (w/v). The maximum oil recovery (41%) was recorded for RJ3 and minimum (38%) for Shu 3005. Among six biotypes of *Jatropha,* specific gravity of oil was maximum (0.9402) for Pant selection 97 and minimum (0.9123) for RJ3. Refractive index of all biotypes was similar. The acid value ranged from 1.22 to 6.94. The highest saponification and iodine values were recorded for RJ3. The viscosity ranged from 0.6032 to 1.1322 cP; Shu 3005 had maximum viscosity (1.1322 cP), while RJ3 had minimum (0.6032 cP). Free fatty acid, the major oil quality factor for biodiesel formation, was found to be maximum in Shu 4004 (3.47%), while minimum in Pant selection 97 (0.06%).

The oil from seeds of Jatropha was characterised for itsfatty acid composition using gas chromatography (Table. 3). All seed fats resembled simple "linoleic – oleic – palmitic – stearic" type.

The content of total saturated fatty acids varied from 8.66 to 23.54% (w/v) and total unsaturated fatty acids, from 75.72 to 89.03% (w/v). Among saturated fatty acids, palmitic acid content fluctuated between 6.85 to 15.82% (w/v). Maximum amount of palmitic acid was observed in Shu 3005, whereas, minimum in case of Pant Selection -31. Stearic acid content varied from 1.76 to 7.72% (w/v). Shu 3005 showed maximum content (7.72%) of stearic acid followed by Pant selection 4 (3.65%). In case of unsaturated fatty acids, oleic acid content varied from 49.91 to 61.25% (w/v), with maximum in Pant selection 4, and minimum in Shu 3005. Linoleic acid content ranged between 19.72 to 36.73%. Pant Selection-31 had maximum amount of linoleic acid, whereas, Pant selection-4 showed minimum amount. Linolenic acid was also in traces (0.05 - 0.27%).

The seed sources in most of the cases were significantly different in the yield and the quality parameters of oil showed a considerable amount of variability within the distribution range indicating a good scope of genetic gain through selection. The biochemical parameters compared well with the findings of Abigor *et al.* (1997) and Diwani *et al.* (2009), who reported oil (50.5%), saponification value (23.13), iodine value (103.42), acid value (1.41), free fatty acid (2.87) and specific gravity (0.94). Srivastava *et al.* (2002) and

Table 2. Biochemical parameters of oils of *Jatropha* biotypes

Biotype	Oil content (%)	Specific gravity (15 °C)	Refractive Index (mg/g)	Acid no. (mg/g)	Saponification value (mg/g)	Iodine no. (mg/g)	Viscosity (cP)	Free fatty acid (%)
Pant selection 4	40.5	0.9188	1.4719	4.87	145.70	128.20	0.9705	2.43
Shu 4004	39.5	0.9206	1.4736	6.94	152.29	132.52	1.0808	3.47
Shu 3005	38.0	0.9263	1.4741	5.13	144.44	115.48	1.1322	2.56
Pant selection 31	40.0	0.9125	1.4712	2.33	146.52	129.30	0.7431	1.16
Pant selection 97	39.0	0.9402	1.4695	0.14	138.36	130.95	0.6108	0.07
RJ3	41.0	0.9123	1.4708	5.54	156.20	163.37	0.6032	2.77

Table 3. Fatty acid profiling of *Jatropha* biotypes by gas chromatography

J.curcas	Palmitic acid (% w/v)	Stearic acid (% w/v)	Oleic acid (% w/v)	Linoleic acid (% w/v)	Linolenic acid (% w/v)
Pant selection 4	10.26	3.65	61.62	19.73	0.155
Shu 4004	10.45	3.36	52.92	26.60	0.151
Shu 3005	15.82	7.72	49.91	25.57	0.249
Pant selection 31	6.85	1.81	52.08	36.73	0.224
Pant selection 97	12.67	1.94	58.31	22.01	0.275
RJ3	9.23	1.76	59.51	22.42	0.051

Devappa *et al*. (2010) also reported similar findings for iodine value, saponification value and refractive index of *Jatropha* seed oil using chromatographic and spectroscopic techniques. *Jatropha curcas* is found in India mostly as small, discrete populations, which can be utilised for future improvement and breeding programme. Soil conditions, climate, altitude, rainfall and temperature play significant role in causing variations in biochemical parameters (Srivastava, 1999). Some of the variations found, may be associated with the discrete populations from which seeds were collected (Akbar *et al.*, 2009; Ginwal *et al.*, 2004). Ovando-Medina *et al*. (2009) determined various parameters of *Jatropha* biodiesel and estimated economy in usage of *Jatropha* oil as a fuel substitute.

Among oil quality parameters, oil content, acid value, free fatty acid content, iodine value, and viscosity were the major contributing characters whereas saponification value, refractive index and specific gravity were minor contribu-ting characters in variations among genotypes. Thus, it is concluded that *J. curcas* L. has variations among selected biotypes under study. The present study confirmed that considerable amount of genetic variability exists in *J. curcas* with respect to oil content and oil quality parameters, offering scope for selection and breeding. Further research is essential to enhance the knowledge base for genetic improvement in *Jatropha* for the quality oil production, which could be used as a potent fuel substitute in future.

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