Int.J.Curr.Microbiol.App.Sci (2017) *6*(8): 1344-1355

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 8 (2017) pp. 1344-1355**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.608.164

Physiological Characterization of *Jatropha curcus* L. a Biofuel Plant from Hyderabad Karnataka Region, India

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Keywords

Biodiesel, *Jatropha curcus* L, SDS-PAGE, Soxhlet extraction.

Article Info

Accepted:
17 June 2017
Available Online:
10 August 2017

A B S T R A C T

The Physiological investigation of plants chemical incorporated constituents and plant developmental parameters such as shoot length, root length, number of leaves, leaf area, wet biomass and dry biomass, plant seed ponders and so on different physiological examination of *Jatropha curcus* L plant was additionally completed with a specific end goal to evaluate the total of chlorophyll content. By considering all the above parameters the present investigation uncovers that the *Jatropha curcus* L. plant is useful for generation of better quality of biodiesel.

Introduction

Current name is *J. curcas* Linnaeus (Euphorbiaceae). Linnaeus was the first to name the physic nut *J. curcas* L. The genus name Jatropha derives from the Greek word jatr´os (doctor) and troph´e (food), which implies its medicinal uses. The physic nut, by definition, is a small perennial tree or largeshrub, which can reach a height of three to 5 m, but can attain a height of 8 or 10 m under favorable conditions. The plant shows articulated growth straight trunk, thick branchlets with a soft Wood and a life expectancy of up to 50 years. Flowering occurs during the wet season often with two flowering peaks, i.e. during summer and autumn. In the permanently humid regions, Flowering occurs throughout the year. *J. curcas* is native of tropical America, but is now found abundantly in many tropical and sub-tropical regions throughout Africa and Asia because of likely distribution by Portuguese ships via the Cape Verde islands and Guinea Bissau. *J. curcas* has spread beyond its original distribution because of its hardiness, easy propagation, drought endurance, high oil content, low seed cost, short gestation period, rapid growth, adoption to wide agro-climatic condition, bushy/ shrubby nature and multiple uses of different plant parts. Added to this, qualitative

sustainability assessment, focusing on environmental impacts and strengthened by some socioeconomic issues, is quite favorable as long as Jatropha is cultivated on wastelands/degraded lands. In view of these advantages many investors, policy makers and clean development mechanism (CDM) project developers are interested to tackle the twin challenges of energy supply and GHG emission reduction. The inflorescence is axillary paniculate polychasial cymes formed terminally on branches and are complex, possessing main and co-florescences with paracladia. Flowers are unisexual, monoeceious, greenish yellow colored interminal long, peduncled paniculate cymes. Male flowers: calyx segments 5, nearly equal, elliptic or obviate; corolla is campanulate, lobes 5, connate, hairy inside, exceeding the calyx, each lobe bear inside a gland at the base, stamens 10 in two series, outer five filaments free, inner five filaments connate, anthers dithecous erect, opening by longitudinal slit. Female flowers: sepals up to18 mm long, persistent; calyx as in male, corolla 4 scarcely exceeding the calyx lobes united, villous inside; ovary 3-locular, ellipsoid,1.5–2 mm in diameter, style bifid, ovules solitary in each cell. The inflorescences form a bunch of green trilocular ellipsoidal fruits yielding approximately 10 or more ovoid fruits. The exocarp remains fleshy until the seeds are mature. Wiehr and Droit described the microscopical anatomy of the seeds in detail, while Singh described that of fruits.

Currently due to gradual depletion of world petroleum reserves and the impact of environmental pollution of increasing exhaust emissions, there is an urgent need to develop alternative energy resources, such as biodiesel fuel. Bio-diesel is expanding very fast because of demand, necessary policy support and technological availability. India consumes approximately 40 million tons of diesel and ranked 5th in the world after the US, China, Russia and Japan in terms of fossil fuel consumption. Recently, Government of India launched National Mission on Bio-diesel with a view to and a cheap and renewable liquid fuel based on vegetable oils. However, shortage of raw material to produce bio-diesel is a major constraint. The total number of oil-bearing species range from 100 to 300 and of them 63 belonging to 30 plant families hold promise for bio-diesel production.

The National Biodiesel Board (NBB) is the national not-for-profit trade association representing the biodiesel industry as the coordinating body for advocacy, research and development in the NBB is undertaking a project to work with states to catalog information regarding their authority to regulate fuels; their status in adopting ASTM D6751 as the fuel specification for biodiesel; enforcement procedures; and to assess their capacity to analyze samples.

The National Biodiesel Accreditation Program is a cooperative and voluntary program for the accreditation of producers and marketers of biodiesel fuel called BQ-9000. The program, which began in 2004, is a unique combination of the ASTM standard for biodiesel, ASTM D 6751, and a quality systems program that includes storage, sampling, testing, blending, shipping, distribution, and fuel management practices.BQ-9000 helps companies improve their fuel testing and greatly reduce any chance of producing or distributing inadequate fuel. To receive accreditation, companies must pass a rigorous review and inspection of their quality control processes by an independent auditor. This ensures that quality control is fully implemented. In the interim period since the original Washington State Ferries biofuel test of 2004-05, there have been continued improvements in manufacturing and handling processes and the monitoring of these systems.

In 2004, the feed stocks available to the U.S. Biodiesel market were largely limited to soy and animal fat, and available only out of the Midwest. There are now other feed stocks in use such as Canola, and producers located in the Pacific Northwest broadening source options for local consumers. Additionally, there are now local testing laboratories established specifically for Biodiesel analysis. The biodiesel byproducts such as crude glycerol have been used as a carbon source to the microorganisms for the production of some industrially important chemical components (Waghmar and Naik, 2016).

Materials and Methods

Physical properties of *J. curcas* seeds

Moisture content

The dry seeds of *J. curcas* were used for all the experiments in this study. The seeds were cleaned manually to remove all foreign matter such as dust, dirt, stones and chaff as well as immature and broken seeds. The initial moisture content of the seeds was determined by oven drying at 105±1ºC for 24 h. The initial moisture content of the seeds was 0.02 ± 0.01 d.b. The samples of the desired moisture contents were prepared by adding the amount of distilled water as calculated from the following relation (Sacilik *et al.*, 2003):

$$Q = \frac{W_i (M_f - M_i)}{100 - M_f}$$

Where, Wi, is the initial mass of sample in kg; Mi, is the initial moisture content of sample in % d.b.; and Mf, is the final moisture content of sample in % d.b.

The samples were then poured into separate polyethylene bags and the bags sealed tightly. The samples were kept at 5°C in a refrigerator for a week to enable the moisture to distribute uniformly throughout the sample.

Before starting a test, the required quantity of the seed was taken out of the refrigerator and allowed to equilibrate to the room temperature for about 2 h (Singh and Goswami, 1996; Coskun *et al.*, 2006). All the physical and mechanical properties of the seeds were determined at five moisture contents in the range of 7.78–21.67% d.b. with four replications at each moisture content.

Plant seed studies

For the seed analysis totally we have taken 100 irrigated seeds of *J. curcas a*nd analyzed the following parameters with the help of instrument Vernier Caliper.

a) Seed length
b) Seed Width &
c) Seed Thickness

A Vernier caliper was used to measure the axial dimensions of randomly selected 100 seeds; length, width, and thickness. From the average of axial dimensions the geometric mean diameter Dg in mm was determined by using the following formula (Joshi *et al.*, 1993):

$$D_P = (abc)^{1/3}$$

where: a, the length is the dimension along the longest axis in mm; b, the width is the dimension along the longest axis perpendicular to a in mm; and c, the thickness, is the dimension along the longest axis perpendicular to both a and b in mm.

The sphericity was determined using (Mohsenin, 1970):

$$\Psi = \frac{(abc)^{1/3}}{a}$$

To identify Lipids in a given sample by thin layer chromatography

In biological material, lipids are found as lipoprotein complexes and these have to be extracted. Lipids, being soluble in non-polar organic solvents and proteins being soluble in polar aqueous solvents, the efficient lipid extraction can be achieved only when an aqueous solvents like ethanol or methanol is included in the non-polar organic solvents like chloroform and diethyl ether. This would help in breaking the lipoprotein complexes. Extracted lipid components can be separated on TLC based on their differential mobility along the porous stationary phase such as silica gel and these can be located by spraying the plates with either 2', 7'-dichlorofluorescein or 50% sulfuric acid.

Extraction of lipids from sample

1 g of the tissue was grinded in the extraction solvent {either ethyl ether: ethanol (3:1) or chloroform: methanol (2:1)} in pestle and mortar. Transfer the homogenate to a separating funnel. Shake the content vigorously and allow it to stand in the two phases have completely separated. Drain out the lower organic layer which contains the lipids. Evaporate the solvent under vacuum and keep the concentrated lipid extract protected from light under nitrogen atmosphere. Prepare the TLC Plates using silica gel as a adsorbent, Activate the TLC Plates at 110 c for 30 minutes, cool them in desiccators and spot the lipid samples, standers as well as unknown. Develop the plate in solvent system consisting of petroleum ether or hexane: ethyl ether: glacial acetic acid (80:20:1) till the solvent as travel up to 1 cm from the opposite side of the plate. Remove the plate and allow it to air dry. Locate the lipid spots by either of following methods. Spray the plate with 2', 7'-dichlorofluorescein and examine it under UV light. Lipids show up as green fluorescent regions against the dark background. Spray the plates carefully with 50% H_2SO_4 and heat it in an oven at 110˚ c for 10 minutes. Areas containing lipids get charred and appear as black spot.

Calculate the R_f value of the lipid components in the sample and identify them by comparing their R_f values with lipid standards.

$$R_F = \frac{\text{Distance traveled by unknown lipid}}{\text{Distance travelled by the solvents system}}$$

Electrophoresis (SDS- PAGE) Banding pattern of proteins in *J. curcas*

Proteins of leaves were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on the basis of their molecular mass by following Laemli (1970) method. Fresh plant tissues (100 mg) were ground in 1 ml phosphate buffer (pH 7.2), using pestle and mortar and were centrifuged at 10000 rpm for 15 min at 4°C and extracts were immediately stored at -20 degree.

Estimation of protein was done prior to loading by the following method of Lowry *et al.,* (1951) and the samples of different concentration were loaded onto the wells of gel that consists of two equal amount of protein samples were mixed with sample buffer in eppenddorf tubes and then layers, upper being the 5 % stacking gel and lower 12% (w/v) resolving gel containing 1 % SDS and 10 % ammonium per sulphate in the vertical electrophoresis apparatus.1x SDS running buffer (Tris –Glycine buffer) was used for running the gel and a voltage was applied to the gel. Proteins separated were stained with CBB stain and the gel was stored in 50% (v/v) methanol. The banding pattern of the gel was observed under UV Tran's illuminator and was photographed.

Descriptions of biomass allocation patterns in *J. curcas*

We established an experiment in a tropical compartment of the green house of Department of Biotechnology, Gulbarga University. *J. curcas* seeds were individually sown in the center of pots (height: 20.5cm; volume: 6.5 liter) filled with a 2:1 river sand: peat mixture. Eighty one pots randomly arranged in a Latin square design. The distance between columns and rows was 40 cm.

Growth conditions

All plants were allowed to grow for 64 days in optimal conditions (further called growth phase or GP). Diurnal variation range in air temperature was controlled and kept between 17°C and 27°C. The relative humidity was 70% and the pots were watered with a solar radiation-dependent drip irrigation system, keeping them at field capacity. The water contained a balanced nutrient mixture. The relative air humidity was lowered to 30-40%, while the temperature was kept as during the GP. In this artificial environment the CO_2 concentration averaged around 500-600 ppm during the whole experiment. In order to control the target pot mass the plants were manually watered standing on a balance. Watering was performed three times per week (on Monday, Wednesday and Friday), with the same nutrient-enriched water used in the GP. These measurements allowed to check the target pot mass and to recalculate the target mass at different moments during the experiment to correct for biomass increment. Infection by pests or diseases was regularly checked.

Measurements

On day 64, 78, 92 and 116 growth was monitored by recording the number of leaves (>1cm2), shoot length measured vertically from substrate surface till apical meristem) (cm), and root length were calculated.

Fresh weight of shoot and root was done and the dry mass of shoots and roots was determined after oven-drying at 105°C until constant weight. The base of the stem was visibly woodier than the rest of the stem + branches.

Growth parameters

The growth parameters were measured on every 64, 78, 92 and 116 day of the plant growth under normal arid condition of Gulbarga area.

Shoot length

The plants were uprooted carefully without damaging the root system. The shoot length of the plants was measured from the collar region to the tip of the plant, using a standard scale and values were recorded in centimeters.

Root length

After the growth period, the plants were uprooted carefully with damaging the root system. The root system was cleaned under a running tap water to eliminate the sand and soil particles adhering to it. The root length was recorded by measuring from crown region to the root tip using the measuring scale and values were recorded in centimeters.

Number of leaves

Number of fully opened leaves in all the treatments was counted manually.

Leaf area

The leaves were separated from the shoot system of harvested plant. The leaf was measured with leaf area meter.

Fresh shoot biomass

The shoot portion was separated from the root system and was blotted on the blotting paper to absorb the moisture. The weight of the shoot was estimated using an Electronic monopan balance and the values were expressed in grams.

Fresh root biomass

The plants were carefully uprooted and roots were washed in running tap water to clean the adhering soil particles. The washed roots blotted on blotting paper and the roots were weighed using monopan electronic balance. The values were expressed in grams.

Dry shoot biomass

After measuring the fresh shoot biomass of the plant, it was placed in paper cover and dried in hot air oven at 60°C for two days to attain constant weight. The dry weights of shoots were recorded in grams.

Dry root biomass

To obtain the dry root of biomass of the plant, the roots were placed in a paper cover and dried in a hot air oven at 60°C, for 48 hours and values were expressed in grams.

Physiological analysis of plant

Estimation of chlorophyll

The chlorophyll content of the plants was estimated by the method of Arnon (1949). The leaves were excised from the plant and washed with distilled water and blotted dry. One gram of leaf sample was homogenized with 80% acetone in pre chilled mortar. A pinch of $CaCO_3$ was added to facilitate easy grinding. The extract was then centrifuged at 5000 rpm for 15 min and supernatant was made up to 10 ml with 80% acetone. The supernatant was filtered through Watman no 1 filter paper and the clear supernatant were transferred to one centimeter glass cuvette. The observance was measured using specific absorption coefficient for chlorophyll 'a' and 'b' at 645 nm and 663nm using 80% acetone as a blank in shimadzu (UV- 240) double beam spectrophotometer. The following simultaneous equations were set up for measuring chlorophyll concentrations.

Chlorophyll a = (2.311 x OD at 663 nm)-(1.062 x OD at 645 nm)
Chlorophyll b = (2.757x OD at 645 nm)-(2.241 x OD at 663 nm).

Results and Discussion

Chemical analysis

Physical, mechanical and chemical properties of seed and kernel are needed for the design of equipment to handle, transport, process, store and assessing the product quality. In the oil industry, different processes must be done before oil extraction occurs. When *Jatropha curcas* fruits arrive for oil extraction different processes are conducted before: (a) dehulling, separating hull from nut, (b) deshelling, separating shell from kernel, (c) drying and then (d) oil extraction. The aim of this study was to investigate the physical, mechanical and chemical properties of Jatropha curcas L. seeds, as part of optimization of de-shelling and oil extraction of *J. curcas* L. The considered parameters oil content, iodine value, peroxide value, saponification value and acid value. These parameters will be useful in designing of handling and processing equipment.

Chemical properties

The chemical properties of oil are amongst the most important properties that determines the

present condition of the oil. Free fatty acid and peroxide values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil (Waghmare Naik, 2015)

Table 1 shows the physicochemical properties of the *J. curcas* seed oil compared to other biofuel oil like soya and castor seed oil. *J. curcas* seed oil in this study contained high oil content as per the data analyzed below. The iodine value of the *J. curcas* seed oil was 348.3 ± 0.22 (mg/g) which is higher than the other biofuel oil. The oil analysis shows a high iodine value may be due to its high content of unsaturated fatty acids. As a crude oil, the peroxide value of *J. curcas* seed oil showed a low value of 1.2 ± 0.44miliequivalence/kg. The high iodine value and oxidative stability showed that the seed oil upholds the good qualities of plant oil and semi-drying oil purposes (Eromosele *et al.,* 1997). The acid value and free fatty acid content of the Jatropha oil are low in general. The saponification value of *J. curcas* seed oil (214.86 ± 0.25 mg/g) was higher. The slightly higher value of unsaponifiable matter in the Soxlhet method may be due to the ability of the solvent to extract other lipid associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments (Bastic *et al.,* 1978; Salunke *et al.,* 1992).

Physical analysis

Moisture content of the seed

The initial moisture content of the seed was found to be 0.02 ±0.01% d.b. The four other moisture levels obtained after conditioning the seeds were 3.78 ±0.53, 4.01 ±0.72 and 2.82 ±0.34% d.b., respectively. The investigations were carried out at the above moisture levels to determine the effect of moisture content on the physical and mechanical properties of *J. curcas* seed.

Seed size and shape studies

Fig. 7 shows the suspension line of *J. curcas* seeds, air speed needed for suspending different weight fraction of seeds, and the 3 groups of 100 seeds were selected based on average weight. The variation of the seed length, width, thickness and geometric mean diameter was studied. Length increased from 17mm to 19 mm, the width from 10mm to 12mm, and the thickness from 0.8 to 0.9mm. These could be of important consideration in the theoretical determination of the seed volume at different moisture contents.

Descriptions of biomass allocation patterns in *J. curcas*

The seedlings of the wet treatment kept growing steadily (length, diameter at stem Base and volume) throughout the entire period. Biomass characteristics: shoot and root length did not differ significantly. During the treatment the stem length increase of seedlings in the wet treatment was twice as much as in the early treatment. Shoot length and leaf diameter were constantly increasing and the data (Table 3) supports the growth study of the plant.

The biomass allocation study of *J. curcas* plant shows that the plant as gained balanced marginal mean portion of the different plant parts relative to the total dry biomass are shown.

Chemical and physical properties

The data collected from the study of the physical and chemical properties of the test samples shown oil content of Jatropha seeds was determined at 13g dry weight. Oil content of Jatropha seeds (32.5%), was found higher than castor and Soya seeds which is 27.5% and 25%, respectively. High oil content of *J. curcas* indicated that *J. curcas* are suitable as

non-edible vegetable oil feedstock in oleo chemical industries (biodiesel, fatty acids, soap, fatty nitrogenous derivatives, surfactants and detergents, etc).

The iodine value is a measured of the unsaturation of fats and oils. Higher iodine value indicated that higher unsturation of fats and oils. The iodine value of Jatropha oil was determined at 348.3g. 12/100g standard iodine value for biodiesel was 120 for Europe's EN 14214 specification. The limitation of unsaturated fatty acids is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides. This can lead to the formation of deposits or to deterioration of the lubricating.

Fuels with this Characteristic (e.g. Sunflower oil, soybean oil and safflower oil) also likely to produce thick sludge in the sump of the engine, when fuel seeps down the sides of the cylinder into crankcase. The iodine values of *Jatropha curcas* place them in the semi-drying oil group. High iodine values of jatropha are caused by high content of unsaturation fatty acid such as oleic acid and linoleic acid. The iodine values of jatropha oil seed of suggest their use in production of alkyd resin, shoe polish, varnishes etc.

The usual method of assessment hydro peroxides (primary oxidation products) is by determination of peroxide value Peroxide value of Jatrophaoil seed showed a low value (as crude seed oil) of 1.2meq/kg, proving the oxidative stabilities of the seed oil relatively. The high iodine value and oxidative stability shows that the seed oil upholds the good qualities of semidrying oil purposes. Saponification values of the studied oil were 214.86.

High saponification value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries. Experimental result showed that a Jatropha oil seed has FFA content 14.025. The FFA and moisture contents have significant effects on the trans esterification of glycerides with alcohol using catalyst.

Fatty acid profile

Unsaturated total fatty acids are prevalent. This prevalence of the unsaturated fatt**y** acids and the high values of the iodine index indicate that the oil of *Jatropha curcas*is of the *unsaturated type.* The comparison of the composition in fatty acids of *Jatropha curcas* seed oil with that of vegetable oils indicates that this plant is rich in acids oleic (C18:1), linoleic (C18:2) and palmitic (C16:0. *Jatropha curcas* seed oil of Congo shows alinoleic rate of acid high C18:2 and a profile in fatty acid near to that of Mexico i.e. rich in oleic acid C18:1, linoleic acid C18:2 and palmitic acid C16:0. Other countries (Benin, Togo, Cape-Vert, Sao Tomé et Principe, Paraguay, India and Pakistan) present a profile in fatty acids very near. This comparison makes it possible to conclude that one notes little variation in the chemical composition of seeds different geographical origins.

Biomass allocation

Biomass allocation was effective study concerning the shoot, root and leaf area dimensions. It shows a predominant effect of the plant dimension and environment on growth above the genetic stability of the plant to survive in arid lands. The plant dimensions would show high variability among different accessions in different regions. Leaf size was best modeled by a power relation with $LL \times LW$ as independent variable. In the greenhouse, the CO_2 level was unintentionally higher than ambient level.

Table.1 Chemical analysis of seed oils of sixth month old plant

Sl. No	Parameters	Values of *Jatropha curcas*	Values of Soya oil	Values of Castor oil
1.	Oil Content	**32.5 ± 0.5 %**	25 ± 0.2 %	27.5 ± 0.5 %
2.	Acid value (mg KOH/g)	**1.4 ± 0. 25**	1.12 ± 0.5	2.24 ± 0.1
3.	Peroxide value (mg KOH/g)	**1.2 ± 0.44**	4.1 ± 0.20	6.2 ± 0.41
4.	Iodine value (mg KOH/g)	**348.3± 0.22**	226.4 ± 0.12	104.5 ± 0.15
5	Saponification value (mg KOH/g)	**214.86 ± 0.25**	235.7± 0.17	246.0± 0.21
6.	Free fatty acid %	**1.03 ± 0.10**	2.01 ±0.2	3.5 **±** 0.10

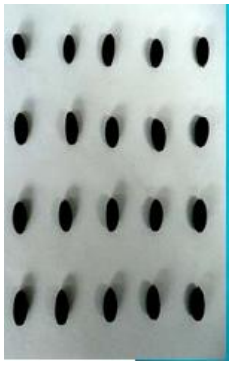
Table.2 Physical parameters studies of *J. curcas*

Sl. No	Parameters	64 day	78 day	92 day	116 day
1.	Number of leaves(cm)	80	115	122	140
2.	Shoot length in cm	90	97	111	123
3.	Leaf length in cm	13	14	14.5	17
4.	Leaf width in cm	13	14.8	15	17.5

Table.3 Biomass allocation studies of *J. curcas*

Sl. No	Parameters	Values in grams
1	Fresh shoot biomass	15.95 ± 0.3
2	Fresh root biomass	1.77 ± 0.5
3	Dry shoot biomass	9.05 ± 0.1
4	Dry root biomass	0.53 ± 0.3

Fig.1 & 2 Measurement of seeds by Vernier Caliper and Seed size and shape



It is generally known that growth of young trees is enhanced under increased atmosphericCO2 through increased carbon uptake which can lead to increased plant height, stem diameter, leaf area index and fine root density. This information can feed into stand biomass and water use modeling of *J. curcas* plantations.

In conclusion, in this present study the plant *J. curcas* has shown good results for physical and chemical parameters. This indicates the plant has high free fatty acids, high Iodine number, high moisture content and saponification value and low peroxide value etc., when compared with the other oil yielding plants like Soya and Castor. The growth and development of our plant is considerably good. Plant seeds studies suggests that seeds have good quality and quantity which given plenty of oil when extracted. TLC for lipids and SDS-PAGE for proteins shown good results.

With the reported greenhouse experiment on *Jatropha* seedlings we could successfully assess some important plant traits. The monitoring of the growth and biomass allocation showed that *J. curcas*, in optimal conditions, grows fast, produces a lot of biomass and achieves a high leaf area. Well and medium watered *Jatropha* plants showed medium biomass investment in leaves and low biomass investment in roots. Furthermore the experiment results in allometic relations which successfully predict aboveground dry biomass and leaf area of the seedlings based on stem diameter. These resulting relations suggest that *J. curcas* fits well into the universal allometric Model. *Jatropha biomass* study results are useful step towards modeling plantation stand biomass, water use and leaf area. Hence we can be concluded that this plant is eligible for the production of better quality of Biodiesel.

Acknowledgement

The authors are thankful to Gulbarga University Gulbarga for providing laboratory to carry out the experiments.

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How to cite this article:

Tathagat Waghmare, Prithviraj Bhandare, Basavaraj Belladal and Ramesh Londonkar. 2017. Physiological Characterization of Jatropha Curcus L' a Biofuel Plant from Hyderabad Karnataka Region. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 1344-1355.
doi: https://doi.org/10.20546/ijcmas.2017.608.164