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The Effect of Biofertilizer application on Chemical composition of oil from Micropropagated *Jatropha curcas L.*seeds

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Subject: Pharmacognosy

Abstract

The suitability of a plant oil for its application as biodiesel depends on its chemical and physical properties and the plant source. Oil quality and consistency are important aspects of biodiesel. Physical and chemical properties of *Jatropha* oil (JO) are significantly influenced by environment and hence exhibit high degree of variability in terms of seed size, weight and oil content. The aim of the present study is to evaluate the effect of biofertilizers on characteristic and chemical composition of oil from micro propagated plants. Application of biofertilizers *(Trichoderma viride, Azospirillum, Phosphobacterium)* increased oil content significantly. The physico-chemical properties of JO were assessed to prospect its potential in biodiesel. High percentage of saturated fatty acids and low unsaturated fatty acids make JO more stable. FTIR and ^{1}H NMR analysis depict that JO obtained from micro propagated plants supplemented with biofertilizers is best suited for biodiesel production on commercial scale.

Keywords: *Biodiesel, Biofertilizers, Jatropha curcas, Trichoderma viride, Azospirillum, Phosphobacterium, Micropropagation.*

Introduction

The possibility of a future fuel crisis and the increasing awareness of the health and environmental damage wrought by burning fossil fuels have motivated a lot of research on the development of clean and renewable alternative energy sources. Agriculture-based economies are putting their stake on biodiesel production from energy crops. With no competing food uses, this characteristic turns attention to *Jatropha curcas* which grows in tropical and subtropical climates across the developing world (1). It is significant to point out that, the non-edible vegetable oil of *Jatropha curcas* has the requisite potential of providing a promising and commercially viable alternative to diesel oil since it has desirable physicochemical and performance characteristics comparable to diesel. Cars could be run with *Jatropha curcas* without requiring much change in design. *Jatropha curcas* is a drought-resistant perennial, growing well in marginal/poor soil. It is easy to establish, grows relatively quickly and lives, producing seeds for 50 years. *Jatropha* the wonder plant produces seeds with an oil content is 35 - 40 and 50 - 60 % in the kernel .The oil can be combusted as fuel without being refined. It burns with clear smoke-free flame, tested successfully as fuel for simple diesel engine. The by-products are press cake a good organic fertilizer, oil contains also insecticide. The oil contains 21% saturated fatty acids and 79 % unsaturated fatty acids. There are some chemicals elements in the seed which are poisonous and render the oil not appropriate for human consumption. A more effective extraction technique would yield greater quantities of oil. This is partly inaccurate, since an effective extraction method would only yield the optimum quantity and not more than that. The optimum oil content in *Jatropha* plants varies between species and genetic variants. Climatic and soil conditions generally affect the yield of the oil. However, improper processing techniques such as prolonged exposure of the harvested seeds to direct sunlight can impair the oil yield considerably. The maximum oil content that has been reported in *Jatropha* seeds has been close to 47%. However, the accepted average is 40%.

Biodiesel is an alternative fuel made from renewable biological sources such as vegetable oils both (edible and non edible oil) and animal fats.

Vegetable oils are usually esters of glycol with different chain length and degree of saturation. It may be seen that vegetable contains a substantial amount of oxygen in their molecules. Practically the high viscosity of vegetable oils (30-200 Centistokes) as compared to that to Diesel (5.8-6.4 Centistokes) leads to unfavorable pumping, inefficient mixing of fuel with air contributes to incomplete combustion, high flash point result in increased carbon deposit formation and inferior coking. Due to these problems, vegetable oil needs to be modified to bring the combustion related properties closer to those of Diesel oil. The fuel modification is mainly aimed at reducing the viscosity and increasing the volatility. (2). Triglycerides (the major component of vegetable and animal oil) must be chemically altered into an ester via transesterification. It is the process of exchanging the organic group R″ of an ester with the organic group R′ of an alcohol. These reactions are often catalyzed by the addition of an acid or base catalyst. The reaction can also be accomplished with the help of enzymes (biocatalysts) particularly lipases. It is important to note that the acid or base are not consumed by the transesterification reaction, thus they are not reactants, but catalysts. Common catalysts for transesterification include sodium hydroxide, potassium hydroxide, and sodium methoxide. Almost all biodiesel is produced from virgin vegetable oils using the base-catalyzed technique as it is the most economical process for treating virgin vegetable oils, requiring only low temperatures and pressures and producing over 98% conversion yield. The composition and physico-chemical properties of oils have been found to vary depending on location of the plant and agricultural practices applied on the raw materials.

Biological activities are markedly enhanced by microbial interactions in the rhizosphere of plants [3]. Biofertilization is the most important factor affecting the yield, biochemical constituents, and oil quality [4]. Asia Nosheen [5] reported that *A. brasilense* and *A. vinelandii* could be highly effective in improving yield and nutritive value of canola oil. *A. brasilense* and *A. vinelandii* play an important role in decreasing the free fatty acid and significantly increased monounsaturated oleic acid content.

Mahfouz *et al*., [6]reported that the effects of biofertilization on growth, fruit yield, and oil composition of fennel plants. The biofertilizer treatments altered the composition of essential oils in fennel. The highest percentage of volatile oil resulted from inoculating the *Foeniculum vulgar* plants with *Azotobacter+Azospirilium* in the presence of a full dose of nitrogen, phosphorus, and potassium (714 kg ammonium sulphate + 714 kg calcium super phosphate + 190 kg potassium sulphate per ha) [7] .

Plant growth depends heavily on protein synthesis for the manufacture of photosynthetic, biosynthetic, and regulatory enzymes, and structural proteins . Secondary metabolism (Essential Oil synthesis) competes with primary growth processes for common substrates such as sugars and proteins. Abundant resources (Basal medium+ cytokinins) provided, increased growth and morphogenesis responses occurred without any reduction in secondary metabolism. Maricel Valeria [8] reported that the production of secondary metabolites was affected only by the addition of cytokinin in basal medium, which resulted in a ~40% increase in the total yield of essential oils (EOs). ability of growth regulators to influence plant growth and development, physiological and biochemical processes, and even gene regulation, there are very many ways in which applications of these compounds can potentially alter EO production [9].

The aim of the research was evaluation of effect of biofertilizers on physico- chemical composition of *Jatropha* oil from micro propagated *Jatropha curcas L.* plant seeds.

Materials & Methods

Field Experiment

This field experiment was carried out in Dolly Farms Periyakulam,Theni(Dt),Tamil Nadu. Micro propagated *Jatropha curcas* plants were planted in Dolly farms using soilless coir pith medium during the growing season of 2007. Micro propagated plants were individually treated with various types of fertilizers like, *Trichoderma viride, Azospirillum and Phosphobacterium. Jatropha curcas* seeds were harvested from micro propagated plants . The seeds were air-dried for 2–3days and stored until use . Seeds were cleaned and damaged seeds were discarded.

Oil Extraction From *Jatropha curcas* Seeds

Jatropha oil had been extracted from seeds by mechanically. Oil presses method were followed in mechanical extraction process. Oil extraction from *Jatropha* seeds were done in Gudalur, Theni(Dt) . Tamil Nadu. Crude oil were stored in bottles for further analysis.

Percentage of Oil Estimation by Soxhlet Method

This method described by Akbar *et al*., [10] with slight modification was used .The seed kernel 3 gm were grounded using a mechanical method and defatted in a Soxhlet Apparatus the extraction was carried out by using petroleum ether as a solvent .The process continued for 6 hours ,solvent was removed by vacuum evaporation and exposure to heat in a drying oven at 50 ºC. The amount of oil recovered was calculated as percentage of total oil present in *Jatropha curcas* seed kernels.

Transesterification of *Jatropha* Oil

Transesterification experiments were performed with alkali catalyst. *Jatropha* biodiesel prepared with methanol, the experiment was conducted with optimum molar ratio (6:1) keeping the catalyst concentration (1% KOH), reaction temperature (65°C) and reaction time (1 hour).[11]The crude *Jatropha* oil filtered ,measured and filled into the reactor .Methoxide solution was added to the oil . The mixture was agitated vigorously for 1 hour with the magnetic stirrer at the temperature of 65ºC.After completion of the reaction the mixture allowed settle for 12 hours. Two layers were formed in the reactor, with the glycerol phase settled at the bottom and mixture of methyl ester phase at the top. The glycerol is drained out through a nozzle at the bottom of the reactor. Methyl ester layer was purified by washing using warm water along with magnetic stirrer to removal of methanol, residual catalyst and soaps. Gentle agitation carried for avoid the emulsion. The mixture of biodiesel and water was stirred for 3 minutes and allowed to settle . Biodiesel (Amber yellow colour)with lower density on the top , water layer with catalyst residues was drained off from the bottom of the separating funnel. Completion of the washing process is determined by using the pH paper to measure the pH of the methyl ester . The accepted pH of biodiesel is 7(neutral), Separation funnel was used to run the fine separation process for biodiesel and remaining distilled water.

Physico-chemical Properties of *Jatropha* Oil

Physico-chemical properties of the *Jatropha* oil i.e. Specific gravity, Kinetic viscosity @40ºC,Pour point, Flash point, Carbon Residue, Ash content, Calorific value, Sulphur content, Iodine value, Saponification Copper strip corrosion and Total acid number of the *Jatropha* oil were measured by IS 1448(P-25) method in Bangalore Analytical Research Centre(P).Ltd.Bangalore.

Fourier Transforms Infrared Spectroscopy analysis of Fatty Acids

FTIR spectra were recorded using Shimadzu IR-Prestige 21 Fourier Transform Infrared Spectrometer with a DRS-800 diffuse reflectance attachment using KBr as reference. Thermal gravimetric analyses were carried out in a Shimadzu TGA-50 Thermal Gravimetric Analyzer. The heterogeneous base catalyst samples were vacuum-dried for four hours before being hermetically sealed in aluminum pans (Shimadzu). After the weights of the samples at room temperature were taken, each was heated at a rate of 10ºC per minute up to 520ºC under a 30-mL per minute nitrogen flow[14].FTIR was used to measure functional groups of *Jatropha* oil.

Lipid profiling of *Jatropha curcas* L. seeds using ^{1}H NMR spectroscopy

Jatropha curcas dried seed samples were extracted thrice with hexane using tissue homogenizer. Combined extracts were filtered and concentrated under reduced pressure. ^{1}H NMR spectra of hexane extracts of all the *Jatropha* samples were obtained on Bruker Biospin Avance 400 MHz NMR spectrometer using a 5 mm broad band inverse probe head, equipped with shielded z-gradient accessories. To facilitate the assignment of the peaks due to individual acids, the proton NMR spectra of pure stearic, palmitic, oleic, linoleic and linolenic acids were recorded. One-dimension a ^{1}H NMR experiments of extracts were obtained by using one-pulse sequence. Samples were dissolved in 600µ l deuterated chloroform and transferred to the 5-mm NMR tube. The deuterated chloroform chemical shift peak at 7.26 ppm was taken as internal reference. Typical parameters used were: spectral width: 4,800 Hz; time domain data points: 32 K; flip angle: 90;relaxation delay: 5 s; spectrum size: 32 K points; and line broadening for exponential window function: 0.3 Hz. One-dimensional ^{1}H NMR experiments were also performed with homo nuclear decoupling to olefinic CH@CH proton by using a single pulse sequence with relaxation delay of 5 s.[15]

Fatty acid composition of *Jatropha* methyl esters using GC-MS

Jatropha curcas methyl esters were analyzed by Gas Chromatography and mass spectrum (GC-MS) in Amphigene Research Laboratory , Thanjavur, Tamilnadu. *Jatropha curcas* methyl esters sample was analyzed in GC-MS for different components present in the extract.Column:Elite-5MS (5%Diphenyl/95% Dim ethyl poly siloxane), 30x0.25mm x 0.25μm df Equipment: GC Clarus 500 Perkin Elmer Carrier gas: 1ml per min, Split: 10:1 Detector: Mass detector Turbo mass gold-Perkin Elmer Software: Turbo mass 5.2 Sample injected: 2μl Oven temperature Programme-110°C-2 min hold Up to 200° C at the rate of 10 ° C/min-No hold Up to 280 ° C at the rate of 5° C / min-9 min hold Injector temperature 250° C Total GC running time 36 min Library used NIST Version-Year 2005 Inlet line temperature 200°C Source temperature 200°C Electron energy: 70 eV Mass scan (m/z): 45-450 Solvent Delay: 0-2 min Total MS running time: 36 minutes.

Results

The growth of micropropagated *Jatropha curcas* was greatly affected by the application of growth regulators and biofertilizer application. Significant increase of plant height, number of leaves, number of branches, seed yield and percentage of oil content by application of biofertilizers. Physico-chemical properties of *Jatropha* oil is given in Table1. Micropropagated *Jatropha* plants yielded 40.39of oil. Specific gravity of *Jatropha* oil measured as 0.96.Pour point measured as 6ºC. Flash point of a fuel is the temperature at which it ignites when exposed to a flame or spark. Flash point was noted as 237ºC.Saponification value presence of high percentage of fatty acids in the oil. *Jatropha* oil saponification value noted as 190. The iodine value is a measured of the unsaturation of fats and oils,its iodine value is 96.56. The ash content is 0.10 in 40ºc. The acid value was determined to be 21.32 mg KOH/g. Kinematic viscosity of *Jatropha* oil is noted 46.97 cSt at extraction temperature 40ºc. Sulphur content of *Jatropha* oil noted that 0.0083%. The FT-IR spectra of *Jatropha* oil noted in Fig.1. The methoxy ester carbonyl group in *Jatropha* oil was appeared as 1745.64cm-^{1}. Peaks at 2924.18- 2852.81 cm-1 indicated the CH_2 and CH_3 scissoring while 3007.12 cm-1 for C=C bending vibration of (Table.2). Wave number of spectrums before and after adsorption were 2924.18, 2852.81, 1745.64 and 1462.09 cm^{-1} equivalent to the functional groups -CH_2bond, -CH aliphatic, C=O bond carboxylic or ester and CH_2, CH_3, respectively. These functional groups were in the chemical structure of triglyceride in *Jatropha* seed oil. ^{cc} NMR spectroscopy of *Jatropha curcas* oil was noted in fig 2. it reported that the spectroscopic determination of yield of transesterification reaction utilizing ^{1}H NMR depicting its progressing spectrum. The area of the proton was determined by integration of respective peaks. The peak of triglycerol esters were noted in 4.3 ppm. Fatty acid methyl esters were observed in 3.6 and 3.95ppm.The characteristic peak of methoxy protons was observed as a singlet 3.65 and a triplet of α CH_2 protons at 2.8ppm.The allylic methylene protons signal at 2.05 ppm was used for percentage estimation of saturated and un saturated fatty acids. Diallylic signal at 2.78 ppm was used for estimation of mono and poly unsaturated fatty acids. Signals around 2.74-2.84 ppm was of diallylic methylene fatty acids appeared as multiplet.The ^{1}H NMR data verified that *Jatropha* oil conversion in to biodiesel was quite complete. Fatty acid composition of *Jatropha* methyl esters given in Fig.3. Caprylic, myristic, palmitoleic, palmitic, oleic, stearic, octonoic,lauraic acids were found. The major saturated fatty acids in *Jatropha curcas* seed oil were caprylic acid,capric acid,myristic acid,palmitic acid and lauric acid;the main unsaturated fatty acids were palmitoleic acid and oleic acid . *Jatropha curcas* contained saturated and unsaturated fatty acids (66.2% and 33.8%)respectively. Micropropagated *Jatropha curcas* oil have highest saturated fatty acids.

Table 1: Physico-Chemical properties *Jatropha* Oil

Physico-chemical properties	Result (%)	Standard specification of *Jatropha* oil.
Sp.gravity	0.96	0.9186
Kinematic viscosity @40ºC,cSt	46.97cSt	50.73 cSt
Pour point	6ºc	8°C
Flash point	237ºC	240/110 °C
Carbon Residue	0.60%	0.64 %
Ash content	0.10	-
Calorific value	9449.05kcal/g	9470 kcal/kg
Sulphur content	0.083%	0.13%
Iodine value	96.56	90.8–112.5
Saponification	190	188–198
Copper strip corrosion	Slight tarnish almost the same as a freshly	-
Total acid no	21.32mg KOH/g	1.0–38.2 mg KOH/g
Oil content	40.39%	27-40 %

cSt - centistokes , kcal/g - kilocalorie/gm

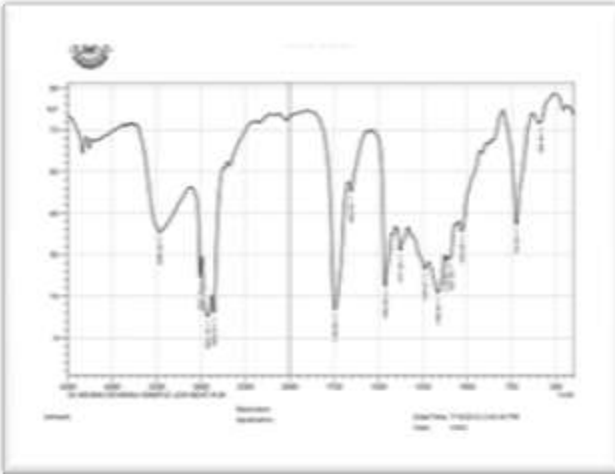

Fig 1 : FT-IR spectrum of *J. curcas* seed oil

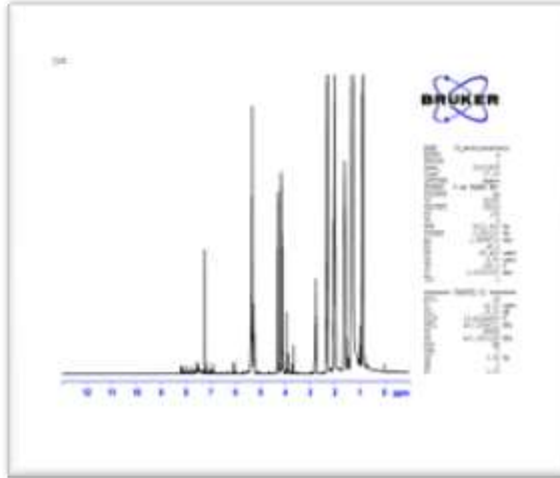

Fig 2: ^{1}H NMR results of *Jatropha* oil

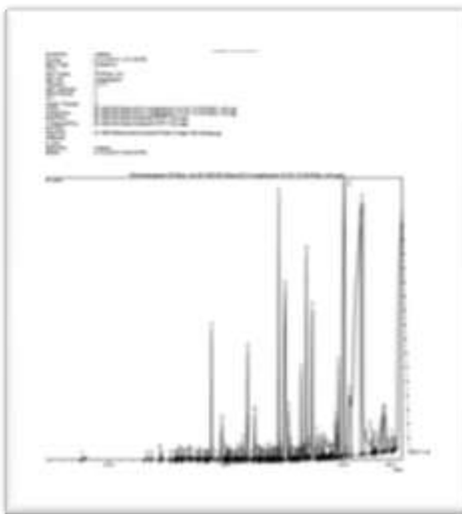

Fig.3 GC-MS results of Methyl esters of *Jatropha* oil

Table 2: The main wavelengths in the FT- IR spectrum of *Jatropha oil*

Sample	Wave number (cm^{-1})	Functional Groups
	3007.12	C=C bending vibration (aliphatic)
	2924.18 , 2852.81	C-H stretching vibration (aliphatic)
Jatropha Oil	1745.64	C=O stretching vibration (ester)
	1462.09	C-H scissoring and bending for methylene
	1377.22	CH_3

Discussion:

The results obtained revealed that the effect of fertilizer on micropropagation *Jatropha* plants. Plant height, seed yield, oil percentage and physico-chemical properties of *Jatropha* oil was statistically significant difference at P<0.05. The interaction of biofertilizer increased oil content of micropropagated *Jatropha* plants. Oil content of *Jatropha* kernel was found higher than linseed, soybean, and palm kernel [16].Significant increase of oil content were noted in micro propagated plants. High oil content of *Jatropha curcas* indicated that it was are suitable as non-edible vegetable oil feedstock in oleo chemical industries (biodiesel, fatty acids, soap, fatty nitrogenous derivatives, surfactants and detergents, etc). Currently, *Jatropha curcas* can produce 2000 liter/ha oil per annual [17].

Density and other gravities are important parameters for diesel fuel injection systems. The values must be maintained within tolerable limits to allow optimal air to fuel ratios for complete combustion. High-density biodiesel or its blend can lead to incomplete combustion and particulate matter emissions [18].In present study kinematic viscosity is 46.9 that fell was within the scope of both the American and EU biodiesel specification ranges. The viscosity of vegetable oil derived biodiesel can go to very high levels and hence it is important to control it within acceptable level to avoid negative impact on fuel injector system performance. Therefore viscosity specifications proposed are nearly same as that of the diesel fuel. It is further reduced with increase in petroleum diesel amount in the blend.

The iodine value is a measured of the unsaturation of fats and oils. Higher iodine value indicated that higher unsaturation of fats and oils [19,20]. The iodine value of *Jatropha* oil was determined at 96.56, 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 [21]. The iodine values (96.56) of *Jatropha curcas* place them in the non-drying oil group. Low iodine value of *Jatropha* are caused by low content of unsaturation fatty acid .Non drying oils contain mostly saturated and oleic acid glycerides with very little to no linoleic or linolenic acids. *Jatropha* oil seed oil consists of 33.8% unsaturated fatty acid . The iodine values of *Jatropha* oil seed of suggest their use in production of alkyl resin, shoe polish, varnishes etc. [22]. Oils with iodine value above 125 are classified as drying oils; those with iodine value 110–140 are classified as semidrying oils. Those with iodine value less than 110 are considered as nondrying oil.[23]. Upon exposure to air they remain liquid for long periods of time and have a low iodine number (less than 100).

Flash point of a fuel is the temperature at which it ignites when exposed to a flame or spark. The flash point of biodiesel is higher than the petro diesel, which is safe for transport purpose. Flash point is the temperature that indicates the overall flammability hazards in the presence of air; higher flash points make for safe handling and storage of biodiesel [24]. Flash points of biodiesel from *Jatropha oil* meet the required specification though neutralisation lowers flash points slightly, 237ºc noted in *Jatropha* biodiesel (Table 1). The impurities responsible for high flash points in crude oils are removed by neutralisation. Acid values of biodiesel from *Jatropha* oils are still very high (Table 10). Neutralisation improves the quality of the oil and makes it suitable for biodiesel production.. For non-edible based seeds oils flash point are higher than fossil diesel [25,26,27].

H igh saponification value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries. High saponification values indicates the presence of high percentage of fatty acids in the oil and therefore implies the possible tendency to soap formation and difficulties in separation of products if utilized for biodiesel production. This would also suggest that using the oils for biodiesel production would lead to very low yields in the methyl esters.

Ash content value of *Jatropha* oil increased with extraction temperature. The ash content in 0.10 in 40ºc. This suggests a better usage quality. Increase in ash level in *Jatropha caucas* kernels temperature increased could be attributed to oil degradation. Specific gravity of *Jatropha* oil was 0.96 , which is close to the standard range of 0.87–0.90 for biodiesel [28].

An acid value is indication of the age and quality of the oil or fat. The acid value was determined to be 21.32 mg KOH/g which implies high fatty acid content, however the results from this study was quite consistent with values obtained by previous researchers.[29,30].The Free fatty acid (FFA) and moisture have significant effects on the transesterification of glyceride with alcohol using catalyst[31]. High FFA (%wt) cause soap formation during alcoholysis process and lead to difficulties in separation of biodiesel from it's by product; as a result it reduced the biodiesel yield [32]. However, if the vegetable oil has a higher free fatty acid content or more water, acid-catalyzed transesterification is more preferable to the base transesterification. But the liquid acid catalyzed transesterification process does not enjoy the same popularity in commercial application as the base catalyzed process because it is about 4000 times slower[33]. The degree of tarnish on the corroded strip correlates to the overall corrosiveness of the fuel sample. The result depicted Slight tarnish almost the same as a freshly. The copper strip corrosion property of the investigated methyl esters was found to be within the specifications of ASTM and EU methods. Sulphur content of *Jatropha* oil noted that 0.0083%.Low sulphur content in *Jatropha* oil results in lower SO_X emissions.The most valuable result is the reduction of total sulphur contents in *Jatropha* oil that was result in reduction of Sox in exhaust gases which is one of the reason of acid rain. Sulfur content of petrodiesel is 20–50 times higher than biodiesels [34].

The GC-MS spectra were studied. Fatty acid composition determination was another important characteristic carried out on this study (Fig. 3). The properties of the triglyceride and the biodiesel fuel are determined by the amounts of each fatty acid that are present in the molecules. Chain length and number of double bonds determine the physical characteristics of both fatty acids and triglycerides [21]. Transesterification does not alter the fatty acid composition of the feedstocks and this composition plays an important role in some critical parameters of the biodiesel, as cetane number and cold flow properties [35]. There are three main types of fatty acids that can be present in a triglyceride which is saturated (Cn:0), monounsaturated (Cn:1) and polyunsaturated with two or three double bonds (Cn:2,3). Various vegetable oil is a potential feedstock for the production of a fatty acid methyl ester or biodiesel but the quality of the fuel will be effected by the oil composition. Ideally the vegetable oil should have low saturation and low poly unsaturation i.e be high in monounsaturated fatty acid [36]. *Jatropha* oil constituents include various fatty acids, namely, palmitic acid, stearic acid, oleic acid, and linoleic acid and confirmed the presence of fatty acid methylesters as the major components of the biodiesels. This confirms the conversion of fatty acids in *Jatropha* oil by transesterification reaction into their respective biodiesel, the fatty acid methyl esters. It should be noted that the compounds confirmed by the GC-MS result interpretations were those of 90% quality assurance by the instrument. The analysis of the fatty acids of the seed oil shows that the methyl esters and palmitic acid is the main. It is followed by the palmitoleic acid ,oleic acid and myristic acid represents an important part of the fatty acids as present in the figure 3. In our study various fatty acid were obtained, from the peaks conclude saturated and unsaturated fatty acids, Palmitic acid ,palmitoleic acid and oleic acid peak values are high. The fatty acid composition of the *Jatropha* seed oil was determined by GC-MS. Caprylic, myristic, palmitoleic, palmitic, oleic, stearic, linoleic, octonoic,lauraic acids were found. The major saturated fatty acids in *Jatropha curcas* seed oil were caprylic acid,capric acid,myristic acid,palmitic acid and lauric acid;the main unsaturated fatty acids were palmitoleic acid and oleic acid .*Jatropha curcas* contained saturated and unsaturated fatty acids (66.2% and 33.8%)respectively. The detected fatty acids are grouped in to two major categories: Saturated FA (SFA) containing caprylic acid (C8:0)capric acid(C10:),myristic acid (C14:0), palmiticacid(C16:0)and lauric acid (C12:0). Unsaturated fatty acids(USFA)containing palmitoleic acid(C16:1),oleic acid(C18:1) . *Jatropha* oil have highest saturated fatty acids. Highest saturated fatty acids depicts the oxidative stability and higher induction period of biodiesel.

In our present study *Jatropha* oil contains mainly saturated fatty acids methyl esters, so the oxidation stability is better than other fats and oils. Biodiesel made from saturated fats will typically have a better oxidative stability and fewer NOx emissions than one made from unsaturated fats. biodiesel made from highly unsaturated fats will require an oxidative stabilizer to be used safely as fuel. Unsaturated fatty acid alkyl ester-like linoleic and linolenic acid esters are more susceptible to oxidation than saturated fatty acid ester. As a result, biodiesel can become oxidized by the oxygen in the air during storage. The oxidation rate can be influenced by many factors such as temperature and chemical composition. Oxidative degradation is harmful and can deteriorate many physical properties of the biodiesel including viscosity, and acid and peroxide values.

Conclusion:

In the current investigation, it has confirmed that *Jatropha* oil may be used as resource to obtain biodiesel. The effect of biofertilizers on micropropagated plants shown significant increase of plant height, seed yield, oil content and physico-chemical properties of *Jatropha* oil. The chemical composition of *Jatropha* oil indicates, it is better to motor vechicles with out any modification of fuel engines. Increased oil content in micropropagated

Jatropha plants influenced by growth regulators(cytokinin).Chemical composition of *Jatropha* oil influenced by biofertilizer applications.

"Cite this article"

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