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DECLINATION IN PHYSICO-CHEMICAL PROPERTIES OF BIO-DIESEL EXTRACTED FROM FUNGAL INFESTED SEEDS OF *JATROPHA CURCAS* L.

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ABSTRACT:

Bio-fuel has been regarded as potential alternative fuel for partial substitution of petro-diesel. The aim of the present study is to estimate the changes occur in physico-chemical properties of *Jatropha curcas* oil extracted from infested *Jatropha* seeds with six dominant fungi viz. *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium chlamydosporum* and *Penicillium glabrum* as well as fresh seeds. Drastic changes were observed in the physico-chemical properties of *Jatropha* oil

due to fungal infestation which affects the quality of bio-diesel. UV-visible absorbance spectra revealed that oil from fresh *Jatropha* seeds showed weak absorbance i.e. 0.22-0.51 with compare to fungal infested seeds ranged between 0.25-0.84 while oil from *A. niger* infested seeds not showed the promising absorbance. The oil colours from infested seeds were observed yellowish brown to dark brown. The acid value, saponification value and cetane values of oil from fungal infested seeds showed slight increment with compare to control. The higher cetane index of biodiesel compared to petro-diesel indicated that, it will be the high potential for engine performance. The viscosity of oil extracted from fungal infested seeds showed a decrease in viscosity which is good for biodiesel purpose while iodine values of fungal infested *Jatropha* seeds showed decrement in compare to control.

KEYWORDS: *Bio-diesel, Fungi, Jatropha Curcas, Physico-chemical properties, Seeds.*

INTRODUCTION:

Seed oils are important sources of nutritional oils, industrial raw materials and nutraceuticals (Basawa and Kumar 2014). *Jatropha* bush and have multiple uses it well to produce outstanding biodiesel as fuel and due to fires without emissions that pollute the environment, so-called oil friend of the environments is also used for lighting and several other industrial purposes (Gupta *et al.* 2011). Biodiesel has therefore attracted extensive attention as a renewable, biodegradable and non-toxic fuel since the past decade (Stavarache *et al.* 2007; Sarin *et al.* 2007 and Srivastava *et al.* 2013a).

Several examples exist in agriculture literature for the spread of plant diseases as a result of the seeds infected or contaminated with fungal pathogens (Agarwal and Sinclair, 1996). Seed fungal mycoflora are of considerable importance due to their influence on the overall health, germination, vigour, oil yield and final survival percentage of the plantations. A seed-borne pathogen may be present externally or internally or associated with the seeds as contaminant. Some of the seed-borne fungi like *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium chlamydosporum*, *Penicillium glabrum* were found to be very destructive, caused seed rot, and decreased seeds germination and also cause pre and post germination death (Tiwari *et al.* 2012) in different host species which affect the quality of *Jatropha* seeds during storage, thus making the seed unfit for extraction of good quality of oil and also affect the quality of bio-diesel.

The potential use of extracted oil from *Jatropha curcas* as transesterified oil (biodiesel), or as a blend with diesel have been studied (Narayana Reddy and Ramesh 2006; Sirisomboon

et al. 2007; Savitha and Naik 2011). The characteristics of *Jatropha* oils depend mainly on their compositions; no oil from infected seeds can be suitable for biodiesel production thus the physic-chemical study of their constituents is very important. The accurate determination of crude oil physical and chemical properties is critical not only to characterize and produce a reservoir, but also to design well completions, subsea tiebacks and topside facilities (Srivastava *et al.* 2015). To measure these properties, reservoir crude oil samples are frequently evaluated by UV/visible absorption (UVVA) spectroscopy (Mullins 1999). Higher iodine value indicated that higher unsaturation of fats and oils. The iodine value of *Jatropha* oil is reported higher (196-200) than other oil (Crabbe *et al.* 2001). The calorific value and cetane number of *J. curcas* oil are comparable to diesel, but the density and viscosity are much higher (Namasivayam *et al.* 2007). The extracted oil could not be used directly in diesel engines because of its high viscosity. High viscosity of pure vegetable oils would reduce the fuel atomization and increase fuel spray penetration, which would be responsible for high engine deposits and thickening of lubricating oil. The use of chemically altered or transesterified vegetable oil called biodiesel does not require modification in engine or injection system or fuel lines and is directly possible in any diesel engine (Kyne and Oo 2009).

On the basis of above aspects, the objective of the present study was to estimate the changes occur in physic-chemical properties of bio-diesel extracted from fungal infested *Jatropha* seeds in comparison to oil extracted from fresh seeds.

MATERIALS AND METHODS:

Seed Treatment and Oil extraction

Seeds were infested with six dominant fungi *viz.* *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium chlamydosporum* and *Penicillium glabrum*. *Jatropha* seeds as well as fresh seeds were used for the extraction of *Jatropha* oil. After extraction of oil, physicochemical properties of oil *viz.* acid value, iodine value, saponification value, UV spectroscopy, cetane value, refractive index, viscosity were estimated.

Colour (UV- Visible Spectroscopy):

UV-visible spectra of *Jatropha* oil in petroleum ether medium was recorded by Perkin-Elmer Lambda-25 operated at a resolution of 1 nm as a function of reaction time;

absorption experiment was performed at room temperature. Origin 6.1 software was used to plot the graph.

Acid Value:

Acid value was estimated by the method demonstrated by Cox and Pearson (1962). 2-3 grams of the oil sample in a previously weighed small beaker was taken, 10ml of methanol added and shake the contents of the beaker to dissolve the free fatty acids. a few drops of phenolphthalein indicator were added and titrated the solution against KOH using burette till a permanent light pink colour appears, the volume of KOH required was recorded. 10ml of the standard oxalic acid solution in a conical flask was taken and after adding a few drops of phenolphthalein it was titrated against the KOH solution till a permanent pink colour appears. Volume of KOH used was recorded and acid values of oils were calculated using following formulae.

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample (g)}}$$

The free fatty acid is calculated as oleic acid using the equation 1 ml N/10 KOH = 0.028 g oleic acid.

Iodine Value:

Iodine value was of all oil samples against blank estimated by the method demonstrated by Horowitz (1975). 0.5 g of oil weighed into an iodine flask and dissolved in 10 mL of chloroform. 25 mL of Hanus iodine solution was added using a pipette, draining it in a time. Mixed well and allowed to standing in dark for exactly 30 min with occasional shaking. After then 10 mL of 15% KI was added, shake thoroughly and added 100 mL of freshly boiled and cooled water, washing down any free iodine on the stopper. It was titrated against 0.1 N sodium thiosulphate until yellow solution turns almost colourless. Few drop of starch were added as indicator and titrated until the blue colour completely disappears. Towards the end of titration, stopper the flask and shake vigorously so that any iodine remaining in solution in CHCl₃ is taken up by potassium iodine solution. The quantity of thiosulphate required for blank minus the quantity required for sample gives thiosulphate equivalent of iodine absorbed by the fat or oil taken for analyses using following formulae.

$$\text{Iodine number} = \frac{(B - S) \times N \times 12.69}{\text{g sample}}$$

Where, B = mL thiosulphate for blank.

S = mL thiosulphate for sample.

N = normality of thiosulphate solution.

Amount of fat/oil taken should be adjusted such that the excess iodine in the added 25 mL of Hanus iodine solution has about 60% of excess iodine of the amount added, i.e., if (B-S) is greater than B/2, repeat with smaller amount of sample.

Saponification Value:

Saponification value was estimated by the method of Horowitz (1975). Oil samples melted and filter through paper to remove impurities and the last traces of moisture. 4-5 g sample weighed into a flask and 50 mL of alcoholic KOH added through burette by allowing it to drain for a definite period of time. A blank also prepared by taking only 50 mL of alcoholic KOH allowing it to drain at the same duration of time. Air condenser connected to the flasks and boiled them gently for 1 h. After cooling of flask and condenser, rinse down the inside of the condenser with a little distilled H₂O and removed. Titrated against 0.5 N HCl after adding 1mL of indicator until the pink colour just disappears. Saponification values of oils were calculated using following formulae.

$$\text{Saponification value} = \frac{28.05 \times (\text{titre value of blank} - \text{titre value of sample})}{\text{Weight of sample (g)}}$$

Determination of Cetane Number (CN) (ASTM D613):

Cetane number is a measurement of the combustion quality of diesel fuel during compression ignition. It is a significant expression of diesel fuel quality among a number of other measurements that determine overall diesel fuel quality (Sundar Raj and Sahayaraj 2010). Cetane number determination was done by using an empirical formula developed by Kalayasiri *et al.* (1996). They were able to create empirical approach for predicting the cetane number of biodiesel. The calculation is based on the results from Saponification number (SN) and Iodine value (IV) of oils. The CN was calculated with the help of the following formula:

$$\text{CN} = 46.3 + \frac{5458}{\text{SN}} - 0.225 \cdot \text{IV}$$

Refractive Index:

The prism minimum deviation technique can be used to measure the refractive index of (semi) transparent liquids by introducing the liquid into a hollow prism. The prism

minimum deviation method is a refractive technique measuring a transmitted light beam, thus requiring both the prism cell windows and the liquid to be (semi) transparent at the measured wavelength (French *et al.* 2004). The angle of deviation (δ) depends on the angle of incidence (i). At the minimum deviation δ_m , the refracted ray inside the prism becomes parallel to its base.

The refractive index of the prism is calculated by the formula:

$$n = \frac{\sin [(A + \delta_m)/2]}{\sin [A/2]}$$

Where, A = Angle of prism

δ_m = Angle of minimum deviation

The angles A and δ_m can be measured experimentally.

Viscosity:

Viscosity is considered to be the most important property of any lubricating oil. It is defined by the measure of fluid resistance to flow at corresponding temperature (Yunus *et al.* 2003). Viscosity of *Jatropha* oil was recorded by the Brookfield DV-III Ultra Programmable Rheometer. Viscosity is a measure of a fluid's resistance to flow. The principle of operation of the DV-III Ultra is to drive a spindle (which is immersed in the test fluid) through a calibrated spring. The viscous drag of the fluid against the spindle is measured by the spring deflection. Spring deflection is measured with a rotary transducer. The viscosity measurement range of the DV-III Ultra (in centipoise or cP) is determined by the rotational speed of the spindle, the size and shape of the spindle, the container the spindle is rotating in, and the full scale torque of the calibrated spring.

Statistical Analysis:

Mean value with standard error was calculated to check the variation in physico-chemical properties of *Jatropha curcas* L. oil. The term 'Standard Error' of any estimate is used for a measure of the average magnitude of the difference between the sample estimate and the population parameter taken over all possible samples of the same size, from the population (Chandel 2002).

$$S.E. = \frac{S}{\sqrt{n}}$$

Where, S = Standard Deviation of Sample

n = Sample size

All results obtained from the physic-chemical estimation of *Jatropha curcas* oil extracted from fresh, stored and infested *Jatropha* seeds were subjected to analysis (ANOVA) using statistical packaged for social sciences (SPSS). The (DMRT) Duncan multiple range tests at 5% level of probability was used to ascertain the significance between the different treatments used (Levesque 2007).

RESULTS:

The effect of selected seed mycoflora of *Jatropha curcas* L. on their physico-chemical properties viz., colour, acid-value, iodine-value, saponification-value, cetane value, refractive index and viscosity shown in Table-1.

The UV-visible absorbance spectra of oil extracted from fresh and infested *Jatropha* seeds shown in Fig. 1. Oil extracted from fresh (A) *Jatropha* seeds showed weak absorbance (0.22-0.51) in the range of wavelength 274-355nm. Oil extracted from *Alternaria alternata* infested *Jatropha* seeds show absorbance (0.25-0.27) in the range of wavelength 260-283nm. Oil extracted from *Aspergillus flavus* infested *Jatropha* seeds show absorbance (0.49-0.54) in the range of wavelength 269-295nm. Oil extracted from *Aspergillus fumigatus* infested *Jatropha* seeds show absorbance (0.45-0.51) in the range of wavelength 268-295nm. Fig.-27 reveals that oil extracted from *Aspergillus niger* infested *Jatropha* seeds not show the promising absorbance in the UV-visible range. Oil extracted from *Fusarium chlamydosporum* infested *Jatropha* seeds show absorbance (0.77-0.84) in the range of wavelength 271-293nm. Oil extracted from *Penicillium glabrum* infested *Jatropha* seeds show absorbance (0.59-0.65) in the range of wavelength 267-295nm. Oil extracted from infested *Jatropha* seeds is yellowish brown to dark brown in colour.

The acid value of oil extracted from fresh *Jatropha* seeds was 9.163mgKOH/g whereas the acid value of oil extracted from *A. alternata*, *A. flavus*, *A. niger* and *F. chlamydosporum* infested seeds show slight increment i.e., 10.594mgKOH/g, 11.298mgKOH/g, 11.794mgKOH/g and 11.191mgKOH/g, respectively. But in case of, oil extracted from *A. fumigatus* and *P. glabrum* infested *Jatropha* seeds showed decreased acid value i.e., 05.449mgKOH/g and 06.383mgKOH/g respectively.

The iodine value of oil extracted from fresh *Jatropha* seeds was 93.635g I₂/100g whereas the oil extracted from fungal infested *Jatropha* seeds showed decrement in their iodine values. The iodine value of oil extracted from infested *Jatropha* seeds by *A. alternate*, *A. flavus*, *A. fumigates*, *A. niger*, *F. chlamydosporus* and *P. glabrum* were 67.431g I₂/100g,

73.551g I₂/100g, 69.717g I₂/100g, 64.594g I₂/100g, 71.195g I₂/100g and 65.992g I₂/100g respectively.

The saponification value of oil extracted from fresh *Jatropha* seeds was 189.532mgKOH/g whereas the oil extracted from fungal infested *Jatropha* seeds showed increment in their saponification value which affects the quality of *Jatropha curcas* oil. The saponification value of infested *Jatropha* seeds by *A. alternate*, *A. flavus*, *A. fumigates*, *A. niger*, *F. chlamydosporus* and *P. glabrum* were 192.095mgKOH/g, 190.660mgKOH/g, 194.865mgKOH/g, 196.400mgKOH/g, 199.897mgKOH/g and 201.339mgKOH/g respectively. From the above data it was cleared that oil extracted from seeds infested by *P. glabrum* shows much increase in saponification value as compared to control.

The cetane value of oil extracted from fresh *Jatropha* seeds was 54.029 whereas the oil extracted from fungal infested seeds by *A. alternate*, *A. flavus*, *A. fumigates*, *A. niger*, *F. chlamydosporus* and *P. glabrum* showed slight increment in their cetane value. The cetane value of infested *Jatropha curcas* L. seeds were 59.605, 58.403, 58.562, 59.523, 57.581 and 58.644 respectively.

The refractive index (25°C) of oil extracted from fresh *Jatropha* seeds was 1.457 whereas the refractive index (25°C) of oil from infested seeds by *A. alternate*, *A. flavus*, *A. fumigates*, *A. niger*, *F. chlamydosporus* and *P. glabrum* were 1.497, 1.593, 1.513, 1.557, 1.563 and 1.587 respectively.

The viscosity (30°C) of oil extracted from fresh *Jatropha* seeds was 54.267cP (centipoise) whereas the viscosity (30°C) of oil extracted from fungal infested seeds show a slight decrease in viscosity which is good for biodiesel purpose. The viscosity of infested *Jatropha* seeds by *A. alternate*, *A. flavus*, *A. fumigates*, *A. niger*, *F. chlamydosporus* and *P. glabrum* were 38.590cP, 35.527cP, 35.317cP, 41.447cP, 35.973cP and 34.600cP respectively.

DISCUSSION:

According to Srivastava *et al.* (2013c) the decrease of seed quality was followed by the increase of free fatty acid value. The level of free fatty acid value gave an indication concerning the decrease of seed quality. The level of free fatty acid depends on fungal species infecting the seeds. Yee *et al.* (2011) were reported that the characterization results of *J. curcas* L. oil revealed that it contains 0.159 %w/w moisture content and acid value of 22.7 mKOH/m oil. The saponification value of *J. curcas* L. oil (194.7 mKOH/m) was found to be small, indicating high concentration of triglycerides and therefore *J. curcas* L. oil can be a

suitable feedstock for the production of biodiesel (Kumar 2014). Srivastava *et al.* (2013b) reported a decrease in oil, iodine value, soluble carbohydrates and protein contents in groundnut seed infested with *Aspergillus* spp. Ashraf and Basu Chaudhary (1986) reported that oil infested from *Fusarium* spp. shows increased saponification value but decreased iodine value.

Kyne and Oo (2009) reported that the Cetane Number of the biodiesel was considerably increased and well within the ASTM specified limit which indicates the better combustion quality of the fuel. The higher cetane index of biodiesel compared to petro-diesel was indicated that it will be the high potential for engine performance. Same results had been reported by Ashraf and Chaudhary 1986 that infested oil emitted mouldy odour and the refractive index increased. Hanny *et al.* 2008; Srivastava *et al.* (2014) has been reported that there is the possibility of lipid breakdown and release of free fatty acids (FFA) may occur during the growth of storage fungi in *Jatropha* seeds and oil seeds. The degradation of Free Fatty Acids (FFA) of oilseeds and oils also decreases the viscosity comparable with earlier studies (Abulude *et al.* 2007; Hanny *et al.* 2008).

From the above findings it was concluded that there have been drastic changes were observed in the physico-chemical properties of *Jatropha* oil due to fungal infestation which affects the quality of bio-diesel. So for the good bio-diesel production at industrial level the supplied *Jatropha* seeds should be fresh and contaminated free.

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Physico-chemical Property	Unit	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	Control
Colour (UV-Spectroscopy)	-	Dark Brown	Yellowish Brown	Yellowish Brown	Dark Brown	Yellowish Brown	Yellowish Brown	Olive Yellow
Acid Value	mg KOH/g	10.594±0.16 c*	11.298±0.23 b	05.449±0.09 f	11.794±0.17 a	11.191±0.05 b	06.383±0.16 e	9.163±0.13 d
Iodine Value	g I ₂ /100g	67.431±0.30 e	73.551±0.09 b	69.717±0.13d	64.594±0.22 g	71.195±0.23c	65.992±0.01f	93.635±0.55a
Saponification Value		192.095±0.08 e	190.660±0.14 f	194.865±0.14 d	196.400±0.09 c	199.897±0.07 b	201.339±0.14 a	189.532±0.39g
Cetane Value	-	59.605±0.06 a	58.403±0.08 c	58.562±0.05 c	59.523±0.02 a	57.581±0.07 d	58.644±0.12 b	54.029±0.17 e
Refractive Index(25°C)	-	1.497±0.01 d	1.593±0.01 a	1.513±0.04 c	1.557±0.01 b	1.563±0.02 a	1.587±0.04 a	1.457±0.02 d
Viscosity (30°C)	cP	38.590±0.26 c	35.527±0.32 e	35.317±0.50 e	41.447±0.27 b	35.973±0.02 d	34.600±0.33 f	54.267±0.36 a

*Means on the same column with same superscripts are not significantly different (P>0.05) & DMRT compare between the rows

T₁= Oil extracted from seeds infested with *Alternaria alternata*

T₂= Oil extracted from seeds infested with *Aspergillus flavus*

T₃= Oil extracted from seeds infested with *Aspergillus fumigatus*

T₄= Oil extracted from seeds infested with *Aspergillus niger*

T₅= Oil extracted from seeds infested with *Fusarium chlamydosporum*

T₆= Oil extracted from seeds infested with *Penicillium glabrum*

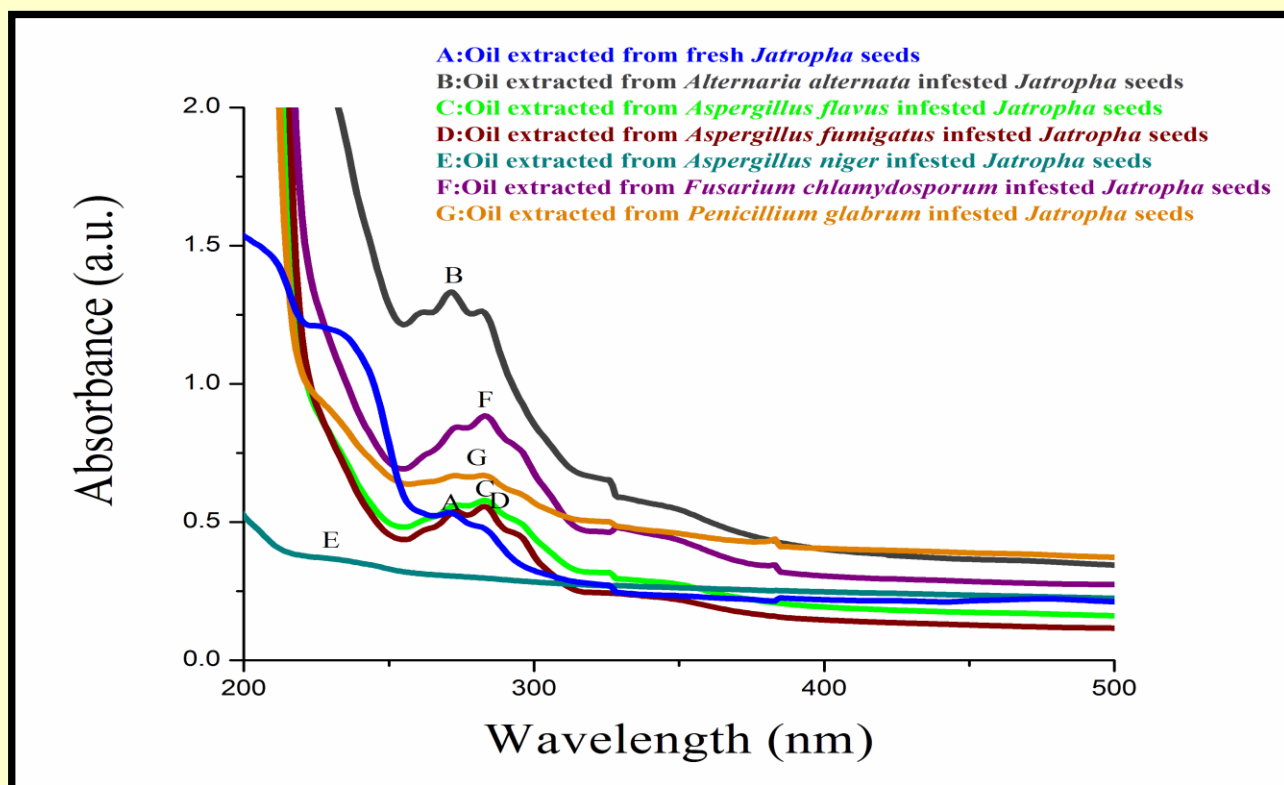


Fig. 1: UV-Spectroscopy of *Jatropha* oil extracted from fresh and infested *Jatropha* seeds