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EVALUATION OF BIOPRODUCTIVITY AND BIODIESEL PRODUCTION STUDIES OF BIOFUEL SPECIES UNDER SEMI-ARID REGION OF NORTH-KARNATAKA

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ABSTRACT
Depletion of agricultural land due to salinization of soil and decrease in fresh water resources plays major problem throughout the world on crop productivity. An idea of using the plant feedstock for biodiesel production which only be realized, if we identified alternate species that can be grown on the saline lands and therefore, would not compete for the resources required for the conventional agriculture. Meeting energy demand needs for the rapid growth in the economics of the country. Biodiesel emerged has one of the promising alternatives for fossil fuels, and is now gaining vital importance. The use of non-arable land for production of tree-borne oil seeds can fulfils the twin objectives that is restoring of the natural land resource and getting the energy. The investigation was carried from every 6 months to 4 years by comparative and evaluation studies in biofuel species on bioproductivity and biodiesel quality parameters. The variations in the bioproductivity parameters such as plant height (PH), collar diameter (CD), number of branches per plant-NBP, number of leafs per branch-NLB, number of flowers per bunch-NFB and number of seeds per branch-NSB studies on *A. indica* (Neem) traits have shown better results followed by *Pongamia* and *Simarouba*. The *A. indica* shown better results in germination percentage (GP-94.6 ±0.50%) followed by *P. pinnata* (GP-94 ±0.44%) and *S. glauca* (GP-92.8 ±0.58%). The 100 seeds weight under fertile land and saline land shows maximum in *P. pinnata* 175.26 ±2.58g and 169.26 ±1.41g followed by *S. glauca* kernels (43.63 ±1.47g and 41.2 ±1.27g) *and A. indica* kernels (41.2 ±1.97g and 39.1 ±1.39g) respectively. The maximum oil content was observed *in A. indica* kernels (48.25 ±1.45%) followed by *S. glauca* (39.82 ±1.38%) *and P. Pinnata* (36.72 ±1.62%) when compared with the fertile land plant seeds. The quality of the biodiesel produced from the *A. indica, S. glauca*, and *P. Pinnata* meets the American Society for Testing and Materials (ASTM), Bureau of Indian standards (BIS) and European National (EN) standards. The byproduct seed cake obtained used as manure for agricultural crops and crude glycerol for pharmaceutical, medical and cosmetic industries. The biomass obtained from these trees used as organic manure, biogas production and bio-ethanol production.

KEY WORDS: Bioproductivity, transesterification, *Simarouba*, Neem, *Pongamia*, biodiesel.

INTRODUCTION
The significance of biofuel species cultivation with the use of non-arable land have extensively highlighted in the recent years. Energy demands in India and all over the world plays vital role in the country's economic growth. Biofuel is an alternative has gained much importance over the past years due to the gradual depletion of conventional fuels. In India, around 100 non-edible tree born oil (TBO) species are available and their preliminary studies on evaluation on the growth, bioproductivity and adaptability shows that *Jatropha curcas*, *Pongamia pinnata*, *Azardiractha indica*, *Madhuca indica*, *Callophyllum innophyllum* and *Aphanamixis polystachya* etc., are promising according to the agro climatic regions. Among the many biofuel species *Simarouba glauca* (*S. glauca*), *Pongamia pinnata (P. pinnata), Azadirachta indica (A. indica)* and *Madhuca indica* (*M. indica)* are very much suitable species in the semi-arid regions and these tree born oil species have multiple uses such as pharmaceutical, pesticides, commercial (Neelakantan, 2004, Hegde, *et al.*, 2005 and Sureshbabu *et al.,* 2017). In Karnataka, availability of non-arable land is estimated of about 8,093 ha out of 19,179 ha (Trivedi, 2010). The productivity of agricultural crops in the arid and semi-arid regions of the world is very less because of abiotic stresses mainly the salt stress (Shrivastava and Kumar, 2015). But, the biofuel species are easily adoptable to the waste lands, relatively very well in marginal areas compared to other traditional crops; it helps to reclaim degraded land and protecting the soil from soil erosion. Biofuel species like *Pongamia pinnata, Azardiractha indica* and S*imarouba glauca* have been recognized as energy crops and renewable energy source with many other promising benefits such as reducing of environmental pollution, global warming and also helps in reducing the accumulation of carbon dioxide.

The global interest on biofuel emphasises for developing a mechanism to sustain the energy producing potentiality of the fast growing industries and elevating benefits from the growing international trade in biofuel. To meet the demand of biodiesel, identification of candidate plus trees non-edible oil yielding plants are very important. On an average, each elite *Pongamia pinnata*, Neem and *Simarouba glauca* tree produces about 15-115 kgs, 10-120 kgs and 15-40 kgs of seeds per year respectively when cultivated under favourable conditions (Environmental Management & Policy Research Institute, 2003; Kureel *et al.,* 2008 and 2009). North-Karnataka region in India is a house of huge non-arable land is available. The present investigation was conducted with an objective to study bioproductivity and biodiesel production from the locally available candidate plus trees viz., *Simarouba glauca, Pongamia pinnata* and *Azadirachta indica* under saline and fertile land.

MATERIALS & METHODS

Study site

The experiments were conducted at the University of Agricultural Sciences campus, Raichur district-North Karnataka, India between N 15^o 09′ and 16^o 34′ latitude and E 75^o 46′ and 77^o 35′ longitudes and a height of 1335ft from the sea level, an annual average temperature between 23-44^o C and an average rainfall 713 mm per year.

Soil salinity

The soil study was done wherever the crop cultivation cannot be carried out and soil salinity was estimated by measuring the electrical conductivity (EC) of soil sample. The soil conductance was measured by the conductometer (Systronics) as per the method of Gireesh Babu *et al.,* (2013). 10 g soil sample was dissolved in the 100ml of deionized water mixed well and kept undisturbed for overnight. The clear solution above the soil layer was used for the conductance analysis. The soil EC was estimated before plantation and after harvesting first yield period.

Selection of biofuel species material

The survey and investigation were conducted for identification of locally available high oil yielding *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* candidate plus tress (CPTs) in the North- Karnataka region of India. The fruits were collected during the respective harvesting season (*Pongamia pinnata*: March-May, *Simarouba glauca*: February-May, and *Azardiractha indica*: June-August). The good quality seeds were used for production of the seedlings under nursery practice using raised bed method with red soil: sand: FYM (2:1:1) with three replications according to the Mbora *et al.,* (2008) and Mathiyazhagan *et al.* (2011). The raised seedlings were transferred to both saline and fertile land (based on the electrical conductivity-EC of the soil) having 40×40×40cm pits and 5×5m spacing between the plants of *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica.*

Bioproductivity studies

The 50 seedlings of *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* were evaluated randomly for bioproductivity studies from seedling stage to yielding stage under selected fertile land and saline land. The bioproductivity parameters *viz*., germination percentage (GP), plant height (PH), collar diameter (CD), canopy growth (CG), number of branches per plant (NBP), number of leaves per branch (NLB), number of flowers per bunch (NFB) and number of seeds per bunch (NSB) were recorded as per the Manavalan (1990)**,** Kumaran (1991) and Divakara (2011) and descriptions of the methodology was mentioned in the table 1. The 100 seed weight was recorded by using the electronic weighing balance.

TABLE 1: Methodology followed for Bioproductivity studies

Sl. No	Parameters	Methodology
1	GP	The germination was started after the 5 days and continued for 25 days of the experiment. The appearance of the plumule and radical about 1mm considered to have germinated. The germination percentage of *Pongamia pinnata*, *Simarouba glauca* and *Azadirachta indica* were recorded.
2	PH	The height of the *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* plants were measured from the collar to the tip of the apical bud using a scale and tape which was calibrated in meters. The data measured was recorded in centimetres.
3	CG	The canopy growth of *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* plants were measured by the measure of crown spread of the plants from north to south and east to west and then average of it was considered as canopy growth.
4	CD	The collar diameter of the *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* plants was measured by the diameter of the plants at the ground level (in centimetres).
5	NBP	The total number of branches produced from the plants was considered as the number of branches per plants.
6	NLB	The total number of leaves produced by the branch was considered as the number of leaves per branch.
7	NFB	The total number of flowers produced per inflorescence is the number of flowers per bunch.
8	NSB	The total number of seeds per bunch is the number of seeds per bunch.
9	100 seed weight (g)	Randomly 100 clean and healthy seeds collected randomly and its weight was measured using the electronic weighing balance.

Note: Where, GP-germination percentage, PH- Plant Height (cm), CG- Canopy growth (cm), CD- collar diameter (cm), NBP- number of branches per plant, NLB- number of leaves per branch, NFB- number of flowers per bunch, NSB- number of seeds per bunch.

Reduction percentage

The percentage of reduction in the bioproductivity under saline land and fertile land for *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* were calculated and recorded according to the Berhanu *et al.* (2014).

Oil extraction and its analysis

Seeds collection and Oil extraction

The *Simarouba glauca, Pongamia pinnata* and *Azadirachta indica* seeds were collected from the University of agricultural Sciences campus, Raichur in respective seasons and were shade dried for 3-4 days to remove moisture content to avoid fungal growth and were used for oil content analysis.

Oil content

Oil extraction by soxhlet extraction

The total oil content in seeds or kernels was extracted by using the petroleum ether as solvent in a soxhlet apparatus which was designed by German chemist Fran on Soxhlet in 1879. The oil from plant seeds of the *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* were washed repeatedly with petroleum ether at 85 ^{o}C for 6 hrs. The extraction process was repeated until the sufficient oil in transesterification process. The percent of oil and seed cake obtained was calculated and recorded.

$$\text{Oil content (\%)} = \frac{\text{Amount of oil obtained in g}}{\text{amount of seeds used in g}} \times 100$$

Oil extraction by mechanical expeller

The oil was extracted from the mechanical expeller (Malnad Extraction, Shivamogga-Karnataka-India) and later oil was filtered by micro filters. The oil was used for large scale production of biodiesel production (40 litres per batch per 2 hrs).

Oil analysis

Specific gravity of oil (AOAC, 2000)

The specific gravity is the ratio of density of sample or fluids to the density of reference fluid or material. The oil sample was filled in a 500 ml measuring cylinder. The hydrometer was used to measure specific gravity of the oil sample at 30 ^{o}C. The specific gravity of the oil is determined using the formula:

$$\text{Specific gravity} = \frac{\text{Density of Oil sample}}{\text{Density of Water}}$$

Oil composition analysis:

Free Fatty Acids (FFA):

FFA is the measure of the amount of NaOH or KOH (in milligrams) required to neutralize the free fatty acids in the oil. Initially, 25 ml of isopropanol is neutralized with a few drops of N/10 NaOH and a few drops of phenolphthalein indicator. 10 ml of oil to the titrated solution heat and cool; titrate it against 0.1 N NaOH using phenolphthalein indicator. The mean burette Reading (MBR) was recorded by replicating thrice and the FFA calculated for biodiesel production by using the following formula:

$$\text{FFA} = \frac{\text{28.2 X 0.1 N NaOH X MBR}}{\text{W}}$$

Where, 28.2 is the molecular weight of oleic acid; N is the normality of NaOH; MBR is the titration mean burette reading and W is weight of oil sample taken For each FFA the amount of NaOH to be used is 4.5 gm (table 2) and methanol is 30% for biodiesel production through alkali-catalysed reaction.

TABLE 2: Sodium hydroxide (NaOH) calculation for biodiesel production through trans-esterification

Sl. No	FFA (%)	NaOH in g
1	0	3.5
2	1.0	4.5
3	2.0	5.5
4	3.0	6.5
5	4.0	7.5
6	5.0	8.5

Biodiesel production and its quality analysis

The amount of biodiesel (methyl ester) produced by transesterification of oil (filter oil) in the presece of alcohol (methanol or ethanol) and catalyst (NaOH or KOH) where the triglycerides are converted into diglycerides in a first step and followed by the monoglycerides and a byproduct glycerol.

Biodiesel production through transesterification process

The transesterification process was performed in a 3 neck flask and the reaction was achieved using methanol and oil in the ration of 1:3 in the presence of the NaOH catalyst based on the FFA content, the reaction was a base-catalysed transesterification process was done according to the method of Vivek, 2004 (acid based) and alkali based by Savitha *et al.,* (2010) with necessary modifications.

Base catalysed transesterification process

The base catalysed transesterification reaction was carried out in 3-neck flask by adding 1 L oil sample in the flask and heated to 70 ^{o}C with constant stirring with the magnetic stirrer; Simultaneously, methoxide mixture (for each FFA 4.5 g of NaOH and 30% methanol) was prepared based on the FFA content was added slowly to the reaction flask with oil and 65 ^{o}C temperature was maintained for 90 minutes. After 90 minutes the reaction was terminated and the whole mixture transferred to separating funnel to allow the biodiesel and glycerine to settle for about 6 hours without disturbance. The upper layer was methyl ester (biodiesel) and the lower layer was the glycerine. The glycerine obtained was measured and was stored in containers; the upper layer biodiesel was transferred to washing funnel to remove the un-reacted NaOH or methanol during the process. After the washing process, the methyl ester (biodiesel) transferred to 1 L beaker and then was heated to 110 ^{o}C to remove the moisture content and allowed to cool. The quantity of the methyl ester obtained from the base catalysed transesterification process was calculated from the following formula:

Yield of Biodiesel = (Methyl ester produced / oil sample used in the reaction) × 100

Biodiesel (Methyl ester) quality analysis

Viscosity test: ASTM D445

Viscosity is a measure of the resistance to the rate of flow of fluids or liquids. The biodiesel viscosity was determined by using Canon-Fenske Viscometer Tube No. 4, the oil samples were kept in Tube No. 4 up to the mark on the bulb and later kept in the water bath at 40 ^{o}C for a period of 30 minutes. After 30 minutes, biodiesel was allowed to flow through the upper meniscus by rubber bulb and leave it to reach the lower meniscus on the tube; a stopwatch was used to maintain the time required for the biodiesel to flow down on the mark. The time was noted and converted into seconds and then viscosity was calculated using the following formula:

$$\text{Kinetics viscosity (in cst)} = \text{time in seconds} + \text{std. factor of tube no. 4}$$

Flash point test: ASTM D93

Flash point temperature is the measure of tendency of the test sample to form a combustible mixture with air under the controlled laboratory conditions. The flash point is the measure that describes the fire risk and response to the heat of materials and assemblies. The flash point of biodiesel was determined by using the Pensky Martens open (closed cup apparatus). The cup was filled with the biodiesel up to the mark (approximately 75 ml) and the closed cup was heated at the rate of 5.6 ºC per minute with the continuous stirring. The fires spark was checked by applying the external flame was passed over the surface of the biodiesel. The temperature for the flash point is always within the 10 ^{o}C of the probable temperature. When the flash temperature was reached, the surface of the biodiesel catches sparks, and then the temperature at the moment was recorded and reported as the flash point temperature.

Copper strip corrosion test: ASTM D130

The biodiesel sample was taken in the copper strip test bomb up to the mark and polished copper strip was immersed in the test bomb. The copper strip corrosion test bomb apparatus was kept in a vertical water bath apparatus and the temperature is set at 50.0 ±1.0^{o}C for 3 hrs. Finally, the copper corrosion strip was compared with standard copper strip for any colour change as per the method of ASTM D130 standards.

Statistical analysis

The statistical analysis of data recorded were analysed by using the one way analysis of variance (ANOVA) by using Graph Pad Instat 3.0 version, Inc 2000 and online software (http://www.physics.csbsju.edu/stats/anova.html) for bioproductivity and biodiesel production parameters were compared by using Tukey's multiple comparison test.

RESULTS

Bioproductivity studies of biofuel species in saline soil in North-Karnataka region

Soil salinity

The soil salinity was recorded by measuring the electrical conductivity of the soil before plantation, after harvesting first yield and the recorded data are represented in the table 3.

TABLE 3: Soil salinity analysis during the bioproductivity studies

Soil salinity	Wasteland		Fertile land	
	Before plantation	After 48 months of plantation	Before plantation	After 48 months of plantation
EC (ds/m)	10.47±0.12	4.59±0.26	3.17±0.13	2.98 ±0.15

Where, ds/m is deciSiemens per meter

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at p≤0. 05 or p≤0. 01.

Bioproductivity studies

The ANOVA (Analysis of variance) of all bioproductivity parameters of biofuel species *Azardiractha indica, Pongamia pinnata* and *Simarouba glauca* revealed that the significant variations between and within the biofuel species which were cultivated in both saline land and fertile land. The bioproductivity data recorded during every six months under saline land and fertile land study showed a gradual increase in the plant height, collar diameter, canopy growth, number of branches per plant, number of leaves per plant, number of flowers per plant, number of seeds per plant and 100 seed weight. The descriptive statistical analysis by ANOVA with respect to bioproductivity studies are represented in the table 4-6; Figure 1 & 2.

Plant height (PH)

The maximum plant height was observed during initial stage (six months) and fruiting stage (48 months) in the *Azadirachta indica* (25.54cm and 283.6cm) followed by *Pongamia pinnata* (25.12cm and 270.7cm) and *Simarouba glauca* (21.78cm and 253.6cm) respectively under fertile land. However, under saline land significantly less growth reduction in plant height was observed during six and 48 months in the *Azadirachta indica* (19.43cm and 260.5cm) followed by *Pongamia pinnata* (17.78cm and 243.3cm) and *Simarouba glauca* (14.56cm and 224.2cm) respectively. From the results it is revealed that the *Azadirachta indica* has shown good plant height performances in both fertile and saline land when compared with the other two species *Pongamia pinnata* and *Simarouba glauca.*

Collar diameter (CD)

The maximum growth in the collar diameter was observed during initial stage (six months) and fruiting stage (48 months) in the *Azadirachta indica* (0.62cm and 12.39cm) followed by *Pongamia pinnata* (0.53cm and 9.82cm) and *Simarouba glauca* (0.39cm and 8.57cm) respectively under fertile land. However, under saline land significantly less growth reduction in collar diameter was observed during six and 48 months in the *Azadirachta indica* (0.37cm and 11.37cm) followed by *Pongamia pinnata* (0.31cm and 8.2cm) and *Simarouba glauca* (0.22cm and 6.63cm) respectively. From the results, it is observed that the *Azadirachta indica* has shown good collar diameter performances in both fertile and saline land followed by *Pongamia pinnata* and *Simarouba glauca.*

Canopy growth (CG)

The maximum canopy growth was observed during 48 months in the *Azadirachta indica* (191.72cm), for *Pongamia pinnata* during 42 months (169.22cm) and for *Simarouba glauca* during 42 months (157.52cm) respectively under fertile land. However, under saline land significantly less canopy growth was observed during 48 months in the *Azadirachta indica* (187.69cm) for *Pongamia pinnata* during 42 months (165.19cm) and for *Simarouba glauca* during 42 months (153.47cm) respectively. From the results it is revealed that the

Azadirachta indica has shown good canopy growth in both fertile and saline land followed by *Pongamia pinnata* and *Simarouba glauca.* The *Azadirachta indica* has shown decreased canopy growth during the 42 months under both fertile (124.7cm) and saline land (120.67cm) respectively because of the fruiting season. However, in *Pongamia pinnata* and *Simarouba glauca* decreased canopy growth was observed during 48 months under both fertile land (117.66) and saline land (115.29) respectively.

FIGURE 1: Bioproductivity studies of biofuel species *Pongamia pinnata, Azadirachta indica* and *Simarouba glauca*

FIGURE 2: Fruits and seeds *of Pongamia pinnata, Azadirachta indica, Simarouba glauca*

Number of branches per plant (NBP)

The more NBP was observed during initial stage (six months) and fruiting stage (48 months) in the *Azadirachta indica* (4.1 and 41.6) followed by *Pongamia pinnata* (3.8 and 38.8) and *Simarouba glauca* (3.4 and 29.4) respectively under fertile land. However, under saline land significantly very less growth reduction in plant height was observed during six and 48 months in the *Azadirachta indica* (3.5 and 39.4) followed by *Pongamia pinnata* (2.9 and 32.7) and *Simarouba glauca* (2.4 and 23.2) respectively. From the results it is revealed that the *Azadirachta indica* has shown a maximum number of branches per plant in both fertile and saline land when compared with the other two species *Pongamia pinnata* and *Simarouba glauca.*

Number of leaves per branch (NLB)

The maximum number of leaves per branch for *Azadirachta indica* was observed during 36 months (269.6) and for *Pongamia pinnata* (332.8) and *Simarouba glauca* (303.8) during 42 months respectively. The results revealed that variations in the number of leaves per plant for *Azadirachta indica*, *Pongamia pinnata* and *Simarouba glauca* have observed that is because of the fruiting period of the species. However, under saline land also the numbers of leaves per branch have varied in *A. indica, P. pinnata* and *S. glauca*. The results revealed that the number of leaves per branch has varied from one species to another species.

Number of flowers per bunch (NFB)

The number of flowers per bunch was observed during 36 and 48 months for *Azadirachta indica* (38.04 and 52) and for *Pongamia pinnata* and *Simarouba glauca* number of flowers per bunch was observed during 42 months (27.8 and 23.2) respectively under fertile land. However, under saline land also the number of flowers per bunch for *Azadirachta indica* was observed during 36 and 48 months (33.3 and 43) and for *Pongamia pinnata* and *Simarouba glauca* the number of flowers per bunch was observed during the 42 months (22.8 and 19.1) respectively. The results revealed that the number of flowers per bunch has varied from one species to another species.

Number of seeds per branch (NSB)

In *Azadirachta indica* the number of seeds per branch was observed at 42 months (32.2), in *Pongamia pinnata* and *Simarouba glauca* NSB was observed at 48 months (22.8 and19) under fertile land respectively. However, under saline land the seeds were observed during 42 months for *Azadirachta indica* (43) and at 48 months for *Pongamia pinnata* (20.6) and *Simarouba glauca* (17.4) respectively. The results revealed that the *Azadirachta indica* has shown significant in a number of seeds per branch followed by the *Pongamia pinnata* and *Simarouba glauca* respectively.

Percentage of Reduction

The reduction percentage of bioproductivity studies under saline land and fertile land for *Pongamia pinnata, Simarouba glauca* and *Azadirachta indica* were analysed statistically. From the data it was clear that, after 48 months the *Azadirachta indica* showed less average reduction percentage followed by *Pongamia pinnata* and *Simarouba glauca* and their values were 5.63%, 10.29% and 14.18% respectively. The average reduction percentage of *Azadirachta indica* was more during six months when compared with 48 months old plants followed by *Pongamia pinnata* and *Simarouba glauca* and their values were 22.02%, 27.11% and 28.80% respectively (Table 7 and Figure 3).

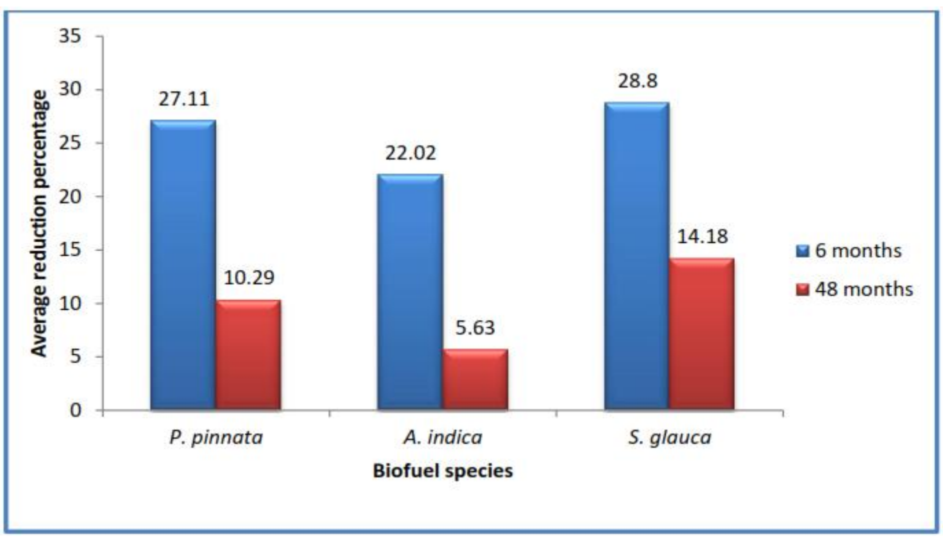

FIGURE 3: Reduction percentage of Biofuel species during 6 and 48 months old plants

Seed weight

The maximum value for 100 seed weight was observed in the *Pongamia pinnata* (175.26±2.58) followed by *Simarouba glauca* (43.63±1.47) and *Azadirachta indica* (41.2±1.95) respectively when compared to the seeds collected from the fertile land plants (Table 8).

Oil extraction and its analysis

Seed collection and Oil extraction

The oil was extracted from the collected seeds as mentioned in the materials and methods chapter. The data were analysed statistically and was represented in the table 9.

Oil content

Soxhlet extraction method

The oil extracted from soxhlet extraction method was analysed statistically and the data are represented in the table 9. From the data, the maximum oil content was observed in *Azadirachta indica* (48.25±1.45) followed by *Pongamia pinnata* (39.82±1.37) *and Simarouba glauca* (36.72±1.62) respectively under saline land grown plants.

Oil analysis

Specific gravity of Oil (AOAC, 2000)

The specific gravity of the oil was measured as per the methodology of AOAC, 2000 and was noted to be about for *Azadirachta indica* (885.8±1.73 kg/m^3), Pongamia pinnata (875.43±1.69 kg/m^3) and *Simarouba glauca* (880.24±2.68 kg/m^3). The specific gravity of the oil meets the major specification of AOAC (Association of Official Analytical Chemists) standards. The very less differences have observed in the specific gravity of the oil, which obtained from the saline and fertile land plant seeds.

DISCUSSION

Bio-productivity studies of biofuel species on saline soil in North-Karnataka region

The results depicted that *Azadirachta indica* plants show a gradual increase in the all bioproductivity parameters from six to 48 months, followed by *Pongamia pinnata* and *Simarouba glauca* under fertile land. However, under the saline land there is a gradual decrease in the bioproductivity parameters in *Azadirachta indica* when compared with the fertile land plants followed by the *Pongamia pinnata* and *Simarouba glauca.* The high germination percentage has observed in *Azadirachta indica* followed by the *Pongamia pinnata* and *Simarouba glauca.* The bioproductivity parameters such as plant height (PH), collar diameter (CD), canopy growth (CG), number of branches per plant (NBS), number of leaves per branch (NLB), number of flowers per bunch (NFB) and number of seeds per bunch (NSB) has shown a gradual increase in the fertile land when compared with the saline land bioproductivity parameters. In Azadirachta indica, the flowering and fruiting was observed during 36 months and 42 months respectively in both fertile and saline lands. Girish and Bhat., 2008 has reported that the neem tree usually starts fruiting after 3-5 years and after 10 years it becomes fully productive. However, in *Pongamia pinnata* and *Simarouba glauca* flowering and fruiting was observed during the 42 months and 48 months respectively. The 100 seed weight was observed more in *Pongamia pinnata* followed by *Simarouba glauca* and *Azadirachta indica* under both fertile and saline land. However, there was a little decrease in weight of 100 seeds was observed under saline land plants. The data recorded were analysed by ANOVA shown that there was a significant variation within the species and between the species of *Pongamia pinnata*, *Azadirachta indica* and *Simarouba glauca* among all the bioproductivity parameters during their growth under both fertile and saline land.

In the present investigation the reduction percentage was also calculated for bioproductivity parameters during the six months and 48 months for biofuel species. The *Azadirachta indica* shown very less reduction percentage in 6 months and 48 months for Plant height-PH (22.02%, 8.14%), collar diameter-CD (40.32%, 8.23%), canopy growth-CG (23.39%, 2.1%), number of branches per plant-NBP (14.63%, 5.28%) and number of leaves per bunch NLB (7.84%, 4.41%) followed by *Pongamia pinnata* (PH-29.21%, 10.12%; CD-41.5%, 16.49%; CG-26.9%, 3.31%; NBP-23.68%, 15.72% and NLB-14.28%, 5.84%) and *Simarouba glauca* (PH-33.14%, 11.59%; CD-40.32%, 22.63%; CG-34.9%, 3.36%; NBP-29.41%, 21.08% and NLB-6.25%, 12.28%) respectively.

The Azadirachta indica has shown maximum oil content in seeds (45.5%) by the soxhlet extraction method followed by *Pongamia pinnata* (36.54%) and *Simarouba glauca* (35.3%) under fertile land. However, the oil content under saline plant seeds also shows maximum in *Azadirachta indica (*48.15%) followed by *Pongamia pinnata* (39.82%) and *Simarouba glauca* (36.72) when compared with the fertile land. Similarly the oil extracted from the mechanical expeller was less compared with the soxhlet extraction method. The seed oil content in plant identification plays a vital role and is the main step in the biofuel crop improvement approaches (Sunil *et al.,* 2008).

The present investigation will provide necessary and valuable information for supporting and establishing large scale plantations under saline lands utilization programs. India is an agricultural based country and provides a great promise as a producer of excess raw materials for the production of biodiesel. There is large area of degraded forest land fallow land and unutilized public land, so this type of land can be used for the cultivation of *Pongamia pinnata*, *Azadirachta indica* and *Simarouba glauca* plants. Local marketing and production of biodiesel will also save the exchanging of money and also generates the rural employment opportunities.

Oil composition analysis

Free Fatty Acids (FFA)

The FFA content in oil samples was represented in the table 10. From the results, *Azadirachta indica* has shown less FFA content under saline land followed by the *Pongamia pinnata* and *Simarouba glauca when compared* with the fertile land.

Biodiesel production and its quality analysis

Biodiesel production through transesterification process

The biodiesel and glycerin content of the samples was represented in the table 11. From the results, the *Azadirachta indica* methyl ester (biodiesel) obtained through the transesterification process is 898.7±1.49 ml/l and glycerine yield is 202.7±2.41 ml/l; the *Pongamia pinnata* biodiesel yield is 896.3±1.59 ml/l and glycerine yield is 198.8±2.78 ml/l from the fertile land plant seed oil (Figure 4). However, there no much difference has observed in the biodiesel and glycerine content which obtained from the saline and fertile land plant seeds.

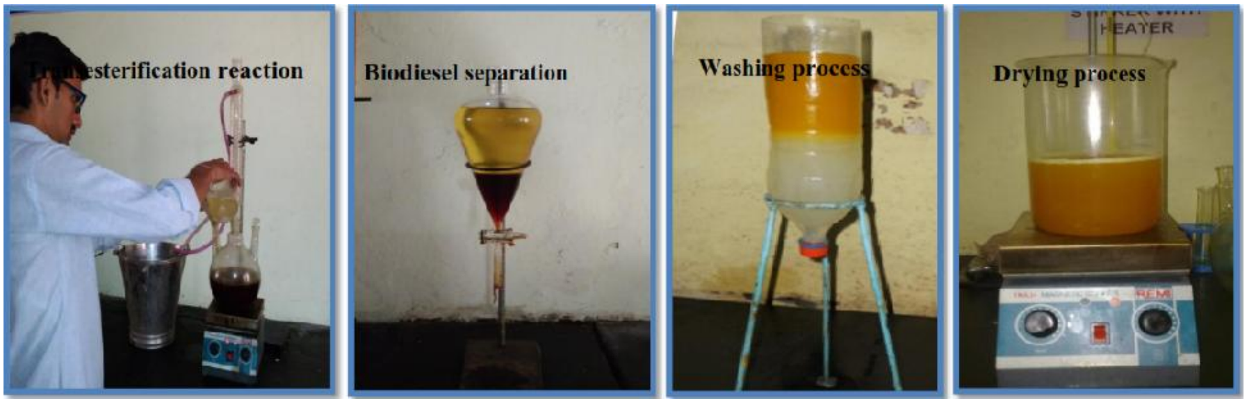

FIGURE 4: Biodiesel production through alkali base transesterification process and its quality analysis

Biodiesel quality analysis

The quality test for methyl ester was represented in the table 12 and figure 5. All the quality tests, namely Viscosity (ASTM D445), Flash point (ASTM D93) and Copper corrosion (ASTM D130) tests were achieved by the ASTM standard methods and confirmed to be good in the quality wise according to ASTM D6751, BIS and EN standards.

FIGURE 5: Biodiesel quality analysis test

Citation by: Patil RS et al., 2018

Viscosity Test-ASTM D445

The biodiesel viscosity was measured as per the ASTM D445 method and was calculated and found to be 4.43, 4.66 and 5.23 for *Azadirachta indica*, *Pongamia pinnata* and *Simarouba glauca* respectively. Similarly, the quality of the biodiesel obtained from the saline land plants also meets the ASTM standards.

Flash Point Test-ASTM D93

The biodiesel flash point was measured as per the ASTM D93 method and flash point is the assessing of the sample to form the flammable combination at a particular temperature under controlled conditions. The flash point was found to be 161.33 ^{o}C, 160.2 ^{o}C and 158.9 ^{o}C for *Azadirachta indica, Pongamia pinnata* and *Simarouba glauca* respectively. Similarly, the flash point biodiesel obtained from the saline land plants also meets the ASTM standards.

Copper Strip corrosion Test-ASTM D130

The copper strip corrosion test (ASTM D130) covers the corrosiveness of the fuel on copper strip. This is based on the effect of the test sample on a polished copper strip. The copper strip corrosion strip test for all biodiesel samples was observed as per the ASTM D130 method and found to be no corrosion on the copper strip. Similarly, the copper test quality of biodiesel obtained from the saline land plants also meets the ASTM standards.

TABLE 4: The bioproductivity studies of *Pongamia pinnata*-biofuel species in North-Karnataka region of India under fertile and saline land

Productivity parameters/ Duration	Land type	06 months	12 months	18 months	24 months	30 months	36 months	42 months	48 months
PH	Fertile land	25.12±1.56	40.08±2.42	74.5±2.85	129.16±2.45	157.72±4.19	184.66±4.29	233.78±4.41	270.7±4.62
	Saline land	17.78±1.51	35.09±1.39	67.5±2.49	123.5±2.16	151.71±3.16	179.21±3.17	219.27±4.56	243.3±4.63
CD	Fertile land	0.53±0.14	1.46±0.16	2.54±0.19	3.08±0.22	4.62±0.26	7.93±0.27	8.66±0.3	9.82±0.34
	Saline land	0.31±0.17	1.28±0.19	2.26±0.21	2.81±0.23	3.6±0.26	6.65±0.23	7.42±0.29	8.2±0.31
CG	Fertile land	14.98±1.12	30.15±1.57	51.7±1.75	70.66±1.58	90.45±1.92	136.18±1.78	169.22±1.28	121.7±1.46
	Saline land	10.95±1.27	26.13±1.51	47.68±1.79	66.63±1.61	86.43±1.92	132.15±1.88	165.19±1.27	117.66±1.51
NBP	Fertile land	3.8±0.37	6.2±0.41	10.8±0.47	19.2±0.52	23±0.59	26±0.63	32.4±0.69	38.8±0.73
	Saline land	2.9±0.36	5.1±0.38	9.6±0.46	16.3±0.51	20.3±0.49	21.2±0.59	27.4±0.62	32.7±0.72
NLB	Fertile land	8.4±0.67	15.2±1.12	37±2.3	105.4±3.12	233.6±3.19	268±3.29	332.8±4.12	236.2±4.27
	Saline land	7.2±0.71	13.5±1.18	35.2±2.19	95.42±3.19	200.5±3.28	228.4±3.17	302.7±4.02	222.4±4.37
NFB	Fertile land	0	0	0	0	0	0	27.8±1.06	0
	Saline land	0	0	0	0	0	0	22.8±1.18	0
NSB	Fertile land	0	0	0	0	0	0	0	22.8±0.81
	Saline land	0	0	0	0	0	0	0	20.6±0.91

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Turkey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

where, PH- Plant Height, CD- collar diameter, CG- Canopy growth, NBP- number of branches per plant, NLB- number of leaves per branch, NFB- number of flowers per bunch, NSB- number of seeds per branch.

TABLE 5: The bioproductivity studies of *Azadirachta indica*-biofuel species in North-Karnataka region of India under fertile and saline land

Productivity parameters / Duration	Land type	06 months	12 months	18 months	24 months	30 months	36 months	42 months	48 months
PH	Fertile land	25.54±1.12	49.15±2.51	77.53±3.12	133.44±2.88	170.42±4.38	185.94±4.35	250.06±4.39	283.6±4.46
	Saline Land	19.43±1.11	43.16±1.48	70.14±2.76	128.43±2.27	165.21±3.56	180.23±3.66	235.12±4.87	260.5±4.78
CD	Fertile land	0.62±0.12	1.55± 0.14	2.55± 0.17	4.32± 0.21	6.13±0.23	9.2±0.26	11.46±0.29	12.39±0.33
	Saline Land	0.37±0.13	1.4±0.16	2.27±0.18	4.03±0.24	5.86±0.22	6.5±0.26	8.46±0.24	11.37±0.32
CG	Fertile land	17.18±1.03	32.91±1.31	54.72±1.47	74.7±1.34	93.76±1.75	141.5±1.56	124.7±1.1	191.72±1.29
	Saline Land	13.16±1.14	28.89±1.42	50.7±1.68	70.68±1.72	89.74±1.75	137.47±1.71	120.67±1.49	187.69±1.46
NBP	Fertile land	4.1±0.28	6.4±0.35	14.4±0.39	20.6±0.43	25.4±0.47	31.8±0.54	37.6±0.57	41.6±0.65
	Saline Land	3.5±0.29	5.9±0.33	12.2±0.41	17.3±0.46	23.7±0.42	27±0.52	32.5±0.59	39.4±0.69
NLB	Fertile land	10.2±0.76	16.2±1.18	48.4±2.26	123.8±3.71	234±3.27	269.6±3.28	170±4.28	228.6±4.45
	Saline Land	9.4±0.69	14.5±1.02	46.6±2.02	112.7±3.28	208.9±3.06	231.6±3.25	152.4±2.18	218.5±4.42
NFB	Fertile land	0	0	0	0	0	38.4±1.02	0	52±1.21
	Saline Land	0	0	0	0	0	33.3±1.12	0	43±1.37
NSB	Fertile land	0	0	0	0	0	0	32.2±0.75	0
	Saline Land	0	0	0	0	0	0	31.7±0.89	0

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

where, PH- Plant Height, CD- collar diameter, CG- Canopy growth, NBP- number of branches per plant, NLB- number of leaves per branch, NFB- number of flowers per bunch, NSB- number of seeds per branch.

TABLE 6: The bioproductivity studies of *Simarouba glauca*-biofuel species in North-Karnataka region of India under fertile and saline land

Productivity parameters / Duration	Land Type	06 months	12 months	18 months	24 months	30 months	36 months	42 months	48 months
PH	Fertile land	21.78±1.77	39.4±2.12	74±2.93	112.34±3.61	143.64±4.24	168.96±4.65	216.22±4.72	253.6±4.85
	Saline land	14.56±1.63	29.4±2.19	67.2±2.11	108.32±2.08	137.5±3.42	163.2±3.07	201.65±4.34	224.2±4.52
CD	Fertile land	0.39±0.15	0.84±0.17	1.27±0.2	2.54±0.23	3.39±0.27	5.79±0.28	7.39±0.31	8.57±0.35
	Saline land	0.22±0.14	0.56±0.16	0.98±0.21	2.25±0.19	3.16±0.25	4.56±0.27	5.72±0.31	6.63±0.34
CG	Fertile land	14.04±1.56	26.55±1.97	46.68±1.87	59.14±1.78	83.42±1.99	128.29±1.87	157.52±1.37	119.3±1.78
	Saline land	9.14±1.65	22.15±1.59	42.38±1.91	55.37±1.98	79.19±1.95	124.16±1.92	153.47±1.74	115.29±1.82
NBP	Fertile land	3.4±0.36	6±0.43	9.8±0.49	13.4±0.56	20.2±0.61	25.2±0.68	26.6±0.74	29.4±0.79
	Saline land	2.4±0.37	4.6±0.41	8.6±0.38	10.1±0.52	17.3±0.55	20.4±0.57	21.5±0.64	23.2±0.71
NLB	Fertile land	8±0.58	12.4±1.21	35±2.17	99.8±3.18	197.6±3.35	243.4±3.48	303.8±4.19	190.2±4.39
	Saline land	7.5±0.73	10.2±1.21	33.3±2.26	88.3±3.37	177.5±3.47	201.4±3.39	278.9±4.14	165.8±4.49
NFB	Fertile land	0	0	0	0	0	0	23.2±1.13	0
	Saline land	0	0	0	0	0	0	19.1±1.28	0
NSB	Fertile land	0	0	0	0	0	0	0	19±0.83
	Saline land	0	0	0	0	0	0	0	17.4±0.97

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

where, PH- Plant Height, CD- collar diameter, CG- Canopy growth, NBP- number of branches per plant, NLB- number of leaves per branch, NFB- number of flowers per bunch, NSB- number of seeds per branch.

TABLE 7: Comparative analysis of reduction percentage in biofuel species productivity parameters during initial stage (6 months) and fruiting stage (after 42 months)

Biofuel species / Reduction percentage	Pongamia		Neem		Simarouba	
	6 months	48 months	6 months	48 months	6 months	48 months
PH	29.21	10.12	23.92	8.14	33.14	11.59
CD	41.5	16.49	40.32	8.23	40.32	22.63
CG	26.9	3.31	23.39	2.1	34.9	3.36
NBP	23.68	15.72	14.63	5.28	29.41	21.08
NLB	14.28	5.84	7.84	4.41	6.25	12.28
Average	27.11	10.29	22.02	5.63	28.80	14.18

where, PH- Plant Height (cm), CG- Canopy growth (cm), CD- collar diameter (cm), NBP- number of branches per plant, NLB- number of leaves per branch

TABLE 8: Comparative analysis of 100 seed weight of biofuel plants grown on fertile and saline land

Biofuel species	100 seeds weight	
	Fertile land plants	Saline land plants
Pongamia pinnata	175.26±2.58	169.26±1.41
Azadirachta indica	41.2±1.95	39.1±1.39
Simarouba glauca	43.63±1.47	41.2±1.27

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

TABLE 9: Comparative analysis of oil content and seed cake for biofuel plants grown on fertile and saline land

Biofuel species / Oil content (%)	Oil content (%) by Soxhlet extraction		Seed cake obtained per kg of seeds (in kg)	
	Fertile land plants	Saline land plants	Fertile land plants	Saline land plants
Pongamia pinnata	36.54±1.38	39.82±1.37	0.47±0.12	0.42±0.13
Azadirachta indica	45.5±1.94	48.25±1.45	0.43±0.11	0.39±0.16
Simarouba glauca	35.3±1.17	36.72±1.62	0.49±0.14	0.45±0.18

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

TABLE 10: Comparative analysis of FFA content in the oil samples

Biofuel species	FFA (%)	
	Fertile land	Saline land
Pongamia pinnata	2.92±0.05	2.61±0.16
Azadirachta indica	2.19±0.03	2.05±0.12
Simarouba glauca	3.89±0.05	2.93±0.11

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

TABLE 11: Comparative analysis of biodiesel and glycerin content obtained from the fertile and saline land plant seed oil

Biofuel species	Biodiesel obtained (ml/l)		Glycerine obtained (ml/l)	
	Fertile land	Saline land	Fertile land	Saline land
Pongamia pinnata	896.3±1.59	894.3±1.37	204.3±2.07	198.8±2.78
Azadirachta indica	898.7±1.49	896.2±1.38	202.7±2.41	196.4±2.59
Simarouba glauca	871.2±1.86	870.4±1.16	213.1±2.56	201.5±2.48

Each value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

TABLE 12: Comparative analysis of biodiesel quality from the different biofuel species grown under fertile and saline soil

Sl. No.	Biodiesel quality parameters	ASTM standards	BIS standards	EN 14214:2012 standards	*Pongamia pinnata*		*Azadirachta indica*		*Simarouba glauca*	
					Fertile land	Saline land	Fertile land	Saline land	Fertile land	Saline land
1	Colour	Golden yellow	Golden yellow	---	Golden yellow	Golden yellow	Golden yellow	Golden yellow	Light greenish yellow	Light greenish yellow
2	Density at 15 °C	860-900 kg/m^3	0.87-0.90 gm/ml	860-900 kg/m^3	859.33 ±5.89	853.33 ±5.12	857.33 ±6.96	854.33 ±5.29	861.33 ±5.23	858.33 ±5.17
3	Kinematic Viscosity @ 40 °C	1.9-6.0	2.5-6.0 cst	3.5-5.0 mm^2/s	4.66 ±0.24	4.32 ±0.18	4.43 ±0.19	4.21 ±0.16	5.23 ±0.22	5.17 ±0.13
4	Copper strip corrosion test	No. 1-3 max	---	Class I	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1
5	Flash Point	100-170 °C	>120	101° C	158.33 ±2.72	160.2 ±2.62	161.33 ±2.33	161.7 ±2.18	156.66 ±2.85	158.9 ±1.56

Each Value represents the mean of the three replications and the vertical bars indicates ± SEM (standard error of the mean) within each column were significantly different from each other according to the Tukey's one way ANOVA test at $p \leq 0.05$ or $p \leq 0.01$.

Biodiesel production and quality analysis

Free fatty acids (FFA) content

The non-edible oils used for the biodiesel production have extremely contaminants, which increases the FFA content and reduces the efficiency of the biodiesel production process. To determine the solvents (methanol or ethanol) and catalyst (NaOH or KOH) concentrations in the transesterification process the determination of FFA also plays a vital role (Ribeiro *et al.,* 2011 and Doddabasawa and Doddabasawa *et al.*, 2014). The FFA present is a major problem when using a base-catalysed transesterification process since FFA reacts with base catalyst to form soap, which leads to the loss of the catalyst and methyl ester product and also increases the processing and production costs (Lotero *et al.,* 2005). This reason creates necessary of the acid catalyst for the transesterification of triglycerides and FFA esterification. The strong acid catalysts are less sensitive to the FFA and which can conduct the esterification and transesterification simultaneously (Serio *et al.*, and Encinar *et al.*, 2005).

In the present investigation, free fatty acid (FFA) content of the oil has determined and observed that Azadirachta indica shown less FFA content followed by the *Pongamia pinnata* and *Simarouba glauca* when compared with the control plant oils and their percentage were 2.05%, 2.61% and 2.93% respectively.

Biodiesel production through alkali based trans esterification and its quality analysis

In the present investigation, biodiesel production was carried out through alkali-based transesterification and data generated were analysed statistically, the highest yield of the biodiesel was observed in Azadirachta indica

(898.7ml/l) followed by *Pongamia pinnata* (896.3ml/l) and *Simarouba glauca* (871.2ml/l) and less glycerine yield in Azadirachta indica (202.7 ml/l) followed by *Pongamia pinnata* (204.3 ml/l) and *Simarouba glauca* (213.1 ml/l). Similarly, many reports suggested that starting with the clean and good oil helps for biodiesel that is easier to produce and saves time during processing. The waste grease and animal-based biodiesel have the additional requirements at the end of transesterification to ensure the acceptable properties of the biodiesel as per standards (Kinast, 2003). The biodiesel and glycerin yield was reported in *Jatropha curcas* (Savitha *et al.,* 2011), *Simarouba glauca* (Mishra *et al.*, 2012 and Dash *et al.*, 2008) in *Annona squamosa* (Doddabaswa *et al.*, 2014).

The present investigation was carried out on biodiesel quality analysis and achieved better results for viscosity, copper corrosion strip and flash point tests. The *Azadirachta indica*, *Pongamia pinnata* and *Simarouba glauca* biodiesel produced from both fertile and stress land plants are also meeting the ASTM standards and BIS standards. The viscosity of the *Azadirachta indica* biodiesel was 4.43±0.19 mm^2/s at 40 ^oC followed by *Pongamia pinnata* (4.66±0.24mm^2/s at 40 ^oC) and *Simarouba glauca* (5.23±0.22 mm^2/s at 40 ^oC) as. The copper strip corrosion test was carried and observed that biodiesel samples shown good results as per the ASTM D130 method. The flash point of *Azadirachta indica* biodiesel observed was 161.33±2.33 ^oC followed by *Pongamia pinnata* (158.33±2.72 ^oC) and *Simarouba glauca* (156.66±2.85 ^oC). Similarly, the quality of the biodiesel reported as per ASTM standards in *Pongamia pinnata* (Dash *et al.,* 2008; Arpiwi *et al.,* 2012), in *Jatropha curcas* (Savitha *et al.,* 2011), *Simarouba glauca* (Mishra *et al.,* 2012) in *Annona squamosa* (Doddabaswa *et al.,* 2014).

CONCLUSION

In this study the locally available candidate plus trees (CPTs) such as *S. glauca*, *P. pinnata* and *A. Indica w*ere studied in the North-Karnataka region. Based on the bioproductivity and bioproductivity studies, it is concluded that the biofuel plants play a vital role in conferring the salt tolerance. Further, by considering the bioproductivity parameters, plants were shown better results in *A. indica* fallowed by *Pongamia pinnata* and *Simarouba glauca*. Considering the oil and FFA content, the *P. pinnata* have good results followed by *S. glauca* and *A. indica*. These CPTs yields the seeds or kernels one after the other seasons. So, it is very much useful for extraction of oil and its conversion to biodiesel throughout the year. After the completion of trans-esterification process, the biodiesel washing is necessary to remove the unutilised NaOH and methanol, it requires the huge amount of water for processing and it leads to the environmental damage. So, the optimisation of the trans-esterification process is very essential to avoid the wastage of water and time consumption.

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