PHYSICO-CHEMICAL ANALYSIS FOR DETERMINATION OF FATTY ACID METHYL ESTERS (FAME) CONTENTS IN MUSTARD OIL BIODIESEL: A RENEWABLE SOURCE OF ENERGY

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بسم الله الرحمن الرحيم

With the Name of Allah, the Most compassionate
And The Most Merciful!

PRAYER

In the name of ALLAH, The most beneficent, The most merciful.

Praise to ALLAH, Lord of worlds

The Beneficent, The Merciful

Master of the Day of Judgment

Thee we worship, Thee we ask for help

Show us the straight path

The path of those whom Thou worth favored

Neither of who earn Thins anger

Nor of those who go astray

(Ameen)

AL-QURAN
(SURRAH AL-FATIHA)

CERTIFICATE

This is to certify that this dissertation submitted by **Mr. Shakeel Ahmed** is accepted in its present form, by the Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the thesis requirements for the degree of Master of Philosophy (M.Phil.) in Biotechnology.

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DEDICATION

Dedicated to

HOLY PROPHET [PBUH]
The Greatest Social Reformer

To
SWEET BROTHERS
My beloved
MOTHER
And Affectionate
FATHER
Who taught me
The first word to speak,
The first alphabet to write,
And
The first step to take,

To
Those who live in my mind,
In my heart,
Throughout the whole span of my life,
And are
Nearest, Dearest and Deepest
To me

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SHAKEEL AHMED AWAN

ABSTRACT

Biodiesel has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. It is produced by transesterification in which, oil or fat is reacted with a monohydric alcohol in presence of a catalyst. The process of transesterification is affected by the mode of reaction condition, type and amount of catalysts, it is shown from the present work that by using different amount of catalyst produced variable yields of biodiesel. The technical tools and processes for monitoring the transesterification reactions like, GC-MS, ^{1}H NMR, ^{13}C NMR and FT-IR have also been summarized. Sample of plant Biodiesel and petro- diesel show different peaks. On the basis of these peaks determined the different types of fatty ester present in biodiesel. In addition, fuel properties and specifications provided by different countries are discussed. Important fuel properties of methyl esters of sunflower oil (Biodiesel) compare well with ASTM standards. The metals in plant parts and their by-products in contrast to petro–diesel were analyzed by atomic absorption spectrophotometer. 14 metals were analyzed in all samples. From the result in was concluded that the concentration of metal present in plant parts and their byproducts comparatively more than that present in petro-diesel. It has been found that Mustard plant *(Brassica campestres* L.*)* posses great potentials for biodiesel production.

INTRODUCTION

INTRODUCTION

1.1 BIODIESEL

Biodiesel is an alternative fuel produced by a chemical reaction between triglycerides present in vegetable oils and alcohol (Harun *et al.*, 2006). The reaction requires a catalyst, usually a strong base, such as sodium or potassium hydroxide, and produces new chemical compounds called methyl esters. These esters are known as biodiesel. (Gerpan, 2005)The first record of the use of vegetable oils as liquid fuels in internal combustion engines is from 1900 when Rudolf Diesel used peanut oil (Shay, 1993).

Biodiesel is produced from animal fat, plant oil or waste cooking oil, and that can be used as the basis for a clean substitute for fossil fuel without any modification to diesel engines, boilers or other combustion equipments (Canakci, 2007; Gerpen, 2005; Ma and Hanna, 1999; Meher *et al.*, 2006). Oil seeds have been one of the major biomass sources of fuel (Karaosmanoglu, 1999) A sustainable biofuel has two favorable properties which are its availability from renewable raw material and its lower negative environmental impact than that of fossil fuels. Various vegetable oil extraction and transesterification technologies are currently used in the production of biodiesel fuel. As an alternative fuel, vegetable oil is one of the renewable fuels. Vegetable oil is a potentially inexhaustible source of energy with an energetic content close to that of Diesel fuel. The vegetable oil fuels have not been acceptable because they were more expensive than petroleum fuels. The major problem associated with the use of pure vegetable oils as fuels, for Diesel engines, is caused by the high fuel viscosity in compression ignition. The direct use of vegetable oils as fuel for engines is problematic. Because of their high viscosity (about 10– 20 times higher than that of Diesel fuel) and low volatility, they do not burn completely and form deposits in the fuel injectors of Diesel engines. Different ways have been considered to reduce the high viscosity of vegetable oils: Dilution, Micro emulsions, Pyrolysis, Catalytic cracking and Transesterification. Among all these alternatives, transesterification seems to be the best choice, as the physical characteristics of fatty acid esters (biodiesels) are very close to those of Diesel fuel and the process is relatively simple. Furthermore, the methyl or ethyl esters of fatty acids can be burned directly in unmodified Diesel engines with very low deposit formation.

1.2 BIODIESEL: A RENEWABLE SOURCE

Renewable energy sources are developed worldwide, owing to high oil prices and to limit greenhouse gas emissions. The world energy demand continues to increase. The most feasible way to meet this growing demand is by utilizing alternative fuels. One such fuel that exhibits great potential is biofuel, in particular, biodiesel (Fernando *et al.*, 2006). The term biofuel is referred to as liquid or gaseous fuels for the transport sector that are predominantly produced from biomass (Demirbas, 2006). Biofuels are generally considered as offering many priorities, including sustainability, reduction of greenhouse gas emissions, regional development, social structure and agriculture and security of supply (Reijnders, 2006). In developed countries there is a growing trend towards employing modern technologies and efficient bioenergy conversion using a range of biofuels, which are becoming cost-wise competitive with fossil fuels (Puhan *et al.*, 2005). The scarcity of conventional fossil fuels, growing emissions of combustion generated pollutants and their increasing costs will make biomass sources more attractive (Sensoz *et al.*, 2000). On the other hand, biomass use, in which many people already have an interest, has the properties of being a biomass source and a carbon neutral source (Dowaki *et al.*, 2007).

Petroleum is the largest single source of energy consumed by the world's population, exceeding coal, natural gas, nuclear, hydro, and renewable. Limited crude petroleum reserves and other sources are on the verge of reaching their peak production. The depletion of known petroleum reserves will make renewable energy sources more attractive. Bio-diesel is a renewable fuel that is produced mainly from vegetable oils and animal fats. Named by the National Soy Diesel Development Board (presently National Bio-diesel Board) which has pioneered the commercialization of bio-diesel in the US during 1992 (Rhamdas *et al.,* 2004). Stringent environmental regulations created huge interest in bio-diesel as an alternative fuel aiming major reduction of vehicular emissions. Bio-diesel is safe, renewable, non-toxic, and biodegradable in water (98% biodegrades in just a few weeks), contains less sulfur compounds and has a high flash point (>130 _C). Table 1 shows the average bio-diesel emissions compared to conventional diesel (Kiss Anton *et al.,* 2006).

Because Biodiesesl's primary feedstock is a vegetable oil or animal fat, biodiesel is generally considered to be renewable. Since the carbon in the oil or fat originated mostly from carbon dioxide in the air, biodiesel is considered to contribute much less to global warming than fossil fuels. Diesel engines operated on biodiesel have lower emissions of carbon monoxide, unburned hydrocarbons, particulate matter, and air toxics than when operated on petroleum-based diesel fuel (Gerpan, 2005). Biodiesel is technically competitive with or others technical advantages compared to conventional petroleum Diesel fuel. Besides being a renewable and domestic resource, biodiesel reduces most emissions while engine performance and fuel economy are nearly identical to those of conventional fuels. Alternative fuels and propulsion systems have the potential to solve many of the current social problems and concerns, from air pollution and global warming to other environmental improvements and sustainability issues (Macleana and Laveb, 2003) (Rhamdas *et al.,* 2004).

1.3 SIGNIFICANCE/ BENEFITS OF BIODIESEL

Although biodiesel cannot entirely replace petroleum-based diesel fuel, there are some of its advantages that justify its development.

The advantage of biodiesel as diesel fuel are liquid nature portability, ready availability, renewability, higher combustion efficiency (Ma and Hanna, 1999; Knothe *et al.,* 2006), higher cetane number and higher biodegradability (Mudge and Pereira, 1999; Speidel *et al.,* 2000; Zhang *et al.,* 2003). Main advantages of biodiesel given in the literature include domestic origin, reducing the dependency on imported petroleum, biodegradability, high flash point and inherent lubricity in the neat form (Mittelbach and Remschmidt, 2004; Knothe *et al.*, 2005)

The exhaust emissions of carbon monoxide, unburned hydrocarbons, and particulate emissions from biodiesel are lower than with regular diesel fuel. Unfortunately, most emissions tests have shown a slight increase in oxides of nitrogen (NOx).When added to regular diesel fuel in an amount equal to 1–2%, it can convert fuel with poor lubricating properties, such as modern ultra-low-sulfur diesel fuel, into an acceptable fuel.(Gerpan, 2005). Biodiesel is renewable and does not contribute to global warming due to its closed carbon cycle. A life cycle analysis of biodiesel showed that overall CO2 emissions were reduced by 78% compared with petroleum-based diesel fuel (Sseehan *et al.*, 1998). Its additional advantages include outstanding lubricity, excellent biodegradability superior combustion efficiency among others (Gerpen, 2005; Holser and O'Kuru, 2006; Ramadhas *et al.*, 2005).

1.4 DISADVANTAGES

First of all, the direct use in internal combustion engines is problematic. Due to their high viscosity (about 11–17 times greater than diesel fuel) and low volatility, they do not burn completely and form deposits in the fuel injectors of diesel engine (Demirbas, 2003) (Rhamdas *et al.,* 2008).

An improvement on viscosity can be obtained with transesterification, which seems to be the process that assures best results in terms of lowering viscosity and improving other characteristics (Sharma *et al.*, 2008).

Besides these technical difficulties, there are some social problems to be considered, as the extensive use of vegetable oils may cause starvation in poor and developing countries.

1.5 BIOFUEL TECHNOLOGIES

Increased concern for the security of oil supply and the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions, has put pressure on society to find renewable alternatives (Midili, 2006). Bioenergy from renewable resources is already today a viable alternative to fossil fuels; however, to meet the increasing need for bioenergy several raw materials have to be considered. Lignocelluloses are the most abundant organic material on earth and is therefore a promising raw material for bioenergy production (Wyman, 1996).

1.6 TYPES OF BIOFUEL

1.6.1 BIODIESEL

Biodiesel is an alternative fuel consisting of fatty acid alkyl esters. Biodiesel can begenerated by chemical or biochemical processes from various types of biomass including vegetable oils, animal fats, and waste edible oil (Anon, 1990).

Transesterification is the primary biodiesel conversion process in Taiwan. (Nelson and Schrock, 2006) Furthermore, the biochemical processes are gradually developing in Taiwan. Biodiesel is relatively safe for use in diesel engines and storage in diesel container due to its high flash point. Biodiesel can be used alone as fuel, or mixed with fossil diesel in diesel engines without major adjustments. An engine using biodiesel has fuel consumption, torsion, and traction ratio similar to that of fossil diesel. Biodiesel offers similar performance and engine durability as fossil diesel and there is no limitation on the mixing ratio of biodiesel and fossil diesel. However, the mixing ratio will affect power output to some extent. A frequently-seen mixture is 20% of biodiesel blended with 80% of fossil diesel (B-20). Biodiesel actually has better lubricating qualities than fossil diesel, and this can prolong engine life and reduce the need for maintenance (Bozbas, 2005). It is estimated that a biodiesel blend of just 1% could increase lubrication by as much as 65% (Althoff *et al.*, 2003).

1.6.2 BIOGAS

Biogas consists mainly of methane and carbon dioxide, and is the product after anaerobic digestion of a wide range of biomass resource like e.g. organic fraction of municipal solid waste, sewage sludge, industrial effluents, fruit and vegetable solid waste, leaves, grasses, woods, terrestrial weeds, aquatic biomass(Gunaseelan, 1997).

Biogas can easily be produced from winter rye straw, oilseed rape straw and faba bean Straw (Peterson, 2007)

1.6.3 BIOETHANOL

Ethanol is a commodity that whether produced from biomass or fossil feedstock's has been produced and traded globally in large volumes for decades. The bioethanol market is well-developed, as is the logistical infrastructure in many countries (Rossilo & Walter, 2006). Bioethanol can be produced from e.g. sugars, starch and various lignocellulosic materials such as straw, wood and waste (Jacques *et al.*, 1999)

Ethanol has advantages over biogas e.g. since it is a liquid fuel that can readily be integrated into existing fuel supply systems and directly substitute fossil fuels in the transportation sector. In order to make an economically feasible process out of ethanol production, ethanol yields need to be as high as possible while production costs are kept as low as possible. In 2005, about 60% of ethanol production was based on sugar crops, 30% from grains, 7% corresponding to synthetic ethanol, and 3% from other raw materials (Walter *et al* ., 2008).

For decades, the yield of ethanol could be increased, compared with what was found in this study, with optimized pretreatment conditions and if the micro- organism used could convert the pentoses originating from the hemicellulose fraction into ethanol. From the raw material analysis, it can be concluded that the maximum theoretical ethanol yield based on all available sugars in e.g. winter rye is 38.20 g (100 g) 1 DW. Another fact that will contribute to the ethanol production economy is that lignin is not affected in the process, but can be burned and the energy used in the process. (Peterson *et al.*, 2007)

1.6.4 BIOMASS

Biomass has been a marginal source of energy in industry and district heating. However, in countries such as Sweden, Finland, and Austria, which have a large forestry sector, forest-based biomass has a remarkable importance. For example, in Finland, renewable energy sources account for 24% of the total primary energy consumption, and 85% of renewable energy is was derived from wood (Statistics Finland. Energy statistics. Yearbook, 2007) (Helsinki, 2007). Forest biomass is the major raw material of the forest industry and has an important role as a source of bioenergy.Forest covers 30% of the earth's land area, of which about 96% are natural forests and 4% are plantations (FAO. Global forest resource assessment – progress towards sustainable forest management; 2006) (FAO. State of theWorld's forests, 2007)

1.7 ENEREGY CRISIS

The major share of all energy consumed worldwide comes from fossil sources (petroleum, coal and natural gas). However these sources are limited, and will be exhausted by the near future. Thus looking for alternative sources of new and renewable energy such as hydro, biomass, wind, solar, geothermal, hydrogen and nuclear is of vital importance. Alternative new and renewable fuels have the potential to solve many of the current social problems and concerns, from air pollution to global warming to other environmental improvements and sustainability issues (MacLeana and Laveb, 2003). Its low cost and easy availability, petroleum became the dominant energy source and petroleum diesel was then developed as the primary fuel for diesel engines. None-theless, petroleum and its derivatives fuels have periodi-cally been through short supply and, consequently, the search for alternative energy sources has emerged (Parente, 2003; Schuchardt *et al.*, 1998; Zanin *etal.*, 2000). Thus, in the 1930s and 1940s, neat vegetable oils were used in diesel engines under an emergency situation (Ma and Hanna, 1999). At that time, the pyrolysis of different triglycerides was also used for liquid fuel supply in different countries. For example, hydrocarbons were produced in China by a tung oil pyrolysis batch system and used as liquid fuels (Chang and Wan, 1947). Another approach proposed at this time was the use of fatty acids' ethyl or methyl esters, obtained by transesterification or alcoholysis of vegetable oils (Chavanne, 1937, 1942) or esterification of fatty acids combined with transesterification of triglycerides (Keim, 1945).

Furthermore, the petroleum international crises in the 1970s and 1990s, as well as an increasing concern about the depletion of the world's non-renewable resources and environmental awareness, provided new enthusiasm in the search for renewable fuel sources (Hill, 2000; Parente, 2003; Schuchardt *et al.*, 2001).

In Brazil, this history was not different. During the 1940 decade occurred our first attempts of energy exploitation from oils and fats in internal combustion engines. Indeed, there are reports of many studies about the use of neat vegetable oils, such as babassu, coconut, castor seed and cotton seed (for instance, see Borges, 1944), or hydro- carbons produced by their thermal-catalytic cracking (Otto,1945). It is worth mentioning that during the Second World War the exportation of cottonseed oil, which was the main vegetable oil produced in Brazil at that time, was forbidden in order to force a drop in its price and, thus, to make possible its use as fuel in trains (Chemical & Metallurgical Engineering, 1943), which is probably the first govern-mental program concerning the use of biofuels

1.8 RESEARCH TRENDS IN BIODIESEL RESOURCES

Vegetable oils, such as sunflower, soybean, palm, coconut, rapeseed, etc., are being used as sources for production of biodiesel in European countries (Korbitz, 1999).

In India, use of such oils for biodiesel production is not feasible because of the big gap in demand and supply of edible oils. Increased pressure to augment production of edible oil has also put limitations on the use of edible oils for production of biodiesel. Under Indian conditions, only such plant sources can be considered for biodiesel, which produce non-edible oil and can be grown in large scale on non-cropped marginal and wastelands. The vegetation of Rajasthan, India provides a number of oil-bearing species, including Azadirachta indica, Jatropha curcas, Salvadora persica, and Pongamia pinnata (Rathore *et al.*, 1997). Fatty acid methyl esters (FAMEs) of some of the species, such as *A. indica, J. curcas, and P. pinnata*, are most suitable for use as biodiesel (Azam *et al.*, 2005), and the suitability of other lesser-known species are to be established. Prior to their use as source materials for production of biodiesel, it is essentially required to establish their biodiesel properties, which depend upon the fatty acid compositions, saponification number (SN), iodine values (IV), and the Cetane number (CN) of the esterified oil. In this article, 23 wildly grown oil-bearing plant species of Rajasthan, India have been included. The SN, IV, and CN of FAMEs of these oils were calculated empirically, and biodiesel suitability was discussed and compared with the specification of the biodiesel Standard of the USA, Germany, and the European Standard Organization.

In Italy diesel fuel consumption was about 26 million tons in 2007 (Anonymous, 2008). Considering that from every hectare of rape is possible to obtain around 1.1–1.2 tons of oil (Bona *et al.*, 1999), the possibility of total Substitution of diesel fuel with biodiesel is unlikely. However, vegetables oil can represent a small contribution, if biodiesel and diesel were blended together. In 2007 469,707 tons of biodiesel were produced in Italy, of which 202,035 tons were used in domestic market (Anonymous, 2008). Biodiesel is blended at 5% and up to 25% with diesel in some petrol stations, while it is used unblended for heating purposes. Fox Petroli S.p.A. is one of the most important Italian companies amongst those operating in the production of biodiesel. The plant is situated in Vasto, Central Italy, and in 2007 it produced around 130,000 tons of biodiesel (Anon, 2008).

1.9 RESEARCH TRENDS IN BIODIESEL IN PAKISTAN

In Pakistan and the world-over, the environmental damage by fossil fuel generation and combustion, as well as the depletion of fossil fuel resources are very major concerns that justify a shift to renewable fuels (Ahmad *et al.*, 2007).

The initial research on identification of biodiesel resources in Pakistan is complete. The potential oil resources identified include Pongamia pinnata, rapeseed and castor bean (Pakistan energy yearbook 2003, 2004 & 2005).

Ahmad *et al.* (2008) carried out methanolysis of cottonseed oil for biodiesel: as renewable source of energy. They concluded that maximum conversion of 90% biodiesel con be achieved by methanolysis of crude cottonseed oil at 60OC and the properties of cottonseed biodiesel such as specific gravity, cetane index, flash point, pour point and cloud point are close to the high speed diesel properties.

Ahmad *et al.* (2008) investigated base catalyzed transesterification of Brassica alba oil and its association with mineralogy to environment friendly biodiesel. They found that this species is potential source for biodiesel and recommended that this species must be cultivated in soil with calcite and quartz minerals on large scale for biodiesel production and implementation to overcome the energy crisis.

Ahmad *et al.* (2009) conducted optimization of base catalyzed transesterification of peanut oil biodiesel and viewed the production and physico-chemical characterization of peanut oil biodiesel (POB).

Ahmad *et al.* (2009) studied biodiesel from Pongamia pinnata L. oil: a promising alternative bioenergy source. They accessed that pongame biodiesel is a good option for renewable energy to reduce dependency on import of foreign oil.

Ahmad *et al.* (2010) analyzed environment-friendly renewable energy from sesame biodiesel and noted that all fuel properties meet the specification of ASTM and there is no obvious change in engine output even at 100% biodiesel.

Biodiesel would be extensively tested in the auto industry of Pakistan, and depending upon its favorable results, scope of its supply would be extended throughout the country as a standard practice. At PSO, after the production of bio-diesel from Jatropha oil, an in-house testing has already begun on one vehicle. However, results would be known later.

A PSO official involved in the project told Dawn that it would take some time to produce bio-diesel in Pakistan on such a large scale because it needs mass cultivation of Jatropha and other non-edible seeds for which commitment/concerted efforts of the government is required. He said a separate department, alternative energy and new projects, has been established within the company to identify and take initiatives in terms of cheaper renewable and alternative energy projects and to address the country's energy crisis and lessen the fuel import bill which would result in saving of precious foreign exchange. PSO has selected only non-edible plants/seeds species, such as castor (Arind), Pongame (Sukh Chain), Jojoba, Jatropha (Karanja), etc., for production of bio-diesel. However, the company is currently focusing on Jatropha plant/seed for its better qualities as a substitute of petroleum diesel (Aamir, 2008).

In renewable energy field, Pakistan is using hydropower for energy needs successfully. Biomass, a renewable energy source, is gaining interest for new researchers because it produces similar type of fuel extracted from crude oil. Energy from biomass depends only upon the availability of cheap raw material. (Khan, 2008)

1.10 IMPORTANCE OF METALS IN BIODIESEL

As the final product is the ultimately burnt, the quality of the fuel is much important, not only structural analysis is important (Holcapek *et al.*, 2001).but the inorganic components are also be analyzed; the raw oil is generally low in inorganic contaminant and in sulfur, but the final product need to be analyzed to check that catalyst are low or any other kind of contamination is present.

Different kinds of elements that are present in catalyst residues are important to analyze as they lead to the ash content in the engine and can also cause corrosion, subsequently affecting the performance of engine and its life. Sulfur is much important as engine legislation and less sulfur requirements of modern engines. Different catalyst poison elements such as lead and vanadium also to be checked.

Phosphorus has been shown to damage the ability of after-treatment systems to reduce exhaust emissions as intended. The influence of phosphorus is cumulative; and as a result, very low levels of contamination over the significant amount of fuel consumed by an engine may lead to unexpected deterioration of the after treatment system.

Sodium and potassium hydroxides are utilized as catalysts and magnesium and calcium as absorbents in the production of biodiesel and should be removed through the biodiesel production process. These residual metals can form deposits in fuel injection system components and poison emission control after-treatment systems.

Many poisons are very dangerous and highly active even they are present in low quantity and with the increasing use of modern and advanced catalysts in biodiesel production, the need to check such elements is very important. Different other organo-metallic compounds are used in the fuels to enhance their fuel properties, such as Si(anti-foaming agent), Mn (a burn enhancer), and different other like Cr, Fe, Ni and they need to be checked to ensure that best amount is used.

1.11 SOURCCES OF BIODIESEL PRODUCTION

Considerable research has been done on vegetable oils as diesel fuel. That research includes palm oil, soybean oil, sunfower oil, coconut oil, rapeseed oil and tung oil (Ma and Hanna *et al.*, 1999).Soya biodiesel is predominantly used in the United

States,while canola biodiesel is dominant in Europe (Kinast *et al*., 2003).Currently, the most common feedstock for biodiesel or fatty acid methyl esters (FAME) is edible oils such as soybean, rapeseed, canola, sunflower, palm, coconut and even corn oil (Mondala *et al*., 2008), National Biodiesel Board, 2009). However, this practice has raised objections from various organizations, claiming that biodiesel is competing resources with the food industry. Therefore, a possible solution to this issue is to produce biodiesel from non-edible oils such as Jatropha curcas L. seeds (Om Tapanese *et al.,* 2008), beef tallow (Nelson *et al*., 2006), waste cooking oil (Chetri *et al*., 2008) and Cerbera odollam (sea mango) (Kandeso *et al*., 2009).

The source for biodiesel production is chosen according to the availability in each region or country. Any fatty acid source may be used to prepare biodiesel, but in the scientific articles reviewed, transesterification reactions have been studied for many vegetable oils such as soybean (de Oliveira, 2005), rapeseed (Jeong and Park, 1996), sunflower (Vicente *et al.*, 2004), safflower (Meka *et al.*, 2007), canola (Singh *et al.*, 2006), palm (Darnoko and Cheryman, 2000; Cheng *et al.*, 2004) and fish oil (El Mashad *et al*., 2006). Since the prices of edible vegetable oils, e.g. soybean oil, are higher than that of diesel fuel, waste vegetable oils (Encinar *et al*., 2005; Felizardo *et al*., 2006; Dorado *et al*., 2002; Cetinkaya and Karaosmanolu, 2004) and non-edible crude vegetable oils as Jatropha, Pongamia pinnata (Karmee and Chadha, 2005) and tigernut oil (Ugheoke *et al*., 2007) have been intensively investigated as potential low priced biodiesel sources. Biodiesel made from these feedstocks was predicted to be more economical than the biodiesel produced from refined vegetable oil. Meanwhile, it has proved to perform similarly (Pinto *et al*., 2005).

Oil from Algae, Bacteria and Fungai also have been investigated (Shay, 1993). Microalgae have been examined as a source of methyl ester diesel fuel (Nagel and Lemke, 1990). Terpenes and Latexes also were studied as diesel fuels (Calvin, 1985)

In Pakistan, we have a lot of indigenous oil seed crops that can be successfully used for the production of biodiesel and we can grow one or the other easily at any time in a year. These plants that are feasible for the production of biodiesel are Sun flower, Line seed, Pongame tree, Castor beans, Cotton seeds, Olive tree, Hemp plant, Oat plant, Carthmus, Safflower, Soybean, Sesame plant and Rape seeds.

1.12 *BRASSICA COMPESTRISS* (MUSTARD PLANT)

In this research project Mustard plant oil is analyzed for biodiesel production. Brassica ia a annual with slender, not tuberous tap root, siliqua with beak ½ - 1/3 as long as the valves, seeds smaller 1.5 mm in diameter. Frequently found as weed in Europe and Central Asia. Rare as an escape from cultivation in our area. Many verities may be found cultivated for their oilseeds with in the area.

The plant is reported for its different fatty acid composition (Dorado *et al.*, 2006). Prior to this physico-chemical study with reference to GC.MS, NMR, FTIR is lacking. So in this research project an attempt has been made to analyze this oil for biodiesel production, in order to over come the energy crisis by searching an alternative fuel like Mustard oil biodoesel.

1.13 OBJECTIVES

The main objective of the research is to find out the potential oil yielding plant species that are commonly available in our country and also not costly for the production of biodiesel.

1. To compare the biodiesel from this plant with conventional diesel (HSD).
2. To check the chemical composition of biodiesel and its different byproducts.
3. To produce good quality biodiesel from this plant.
4. To compare fuel properties of Mustard oil biodiesel and HSD for practical implementation.

5. To identify composition of different elements (metals) in biodiesel.
6. To screen out physico-chemical characterization of crude oil and biodiesel using Analytical techniques like GC-MS, NMR and FT-IR.

MATERIALS

&

METHODS

This research work was conducted during 2009-2010 in Biofuel laboratory, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad. The research work was plan to conduct Biodiesel production, physico-chemical characterization and phytochemical analysis of *Brassica campestriss* oil biodiesel.

2.1 PLANT MATERIAL

The Mustard plant and seeds were collected from the different localities of Attock and Rawalpindi. The soil was kept in the plastic bags for elemental analysis.

2.2 CHEMICALS

2.2.1 Chemicals used in biodiesel production

Name	Formula	Company
Methanol	CH_3OH	Merck Germany
Sodium hydroxide	$NaOH$	do
Potassium hydroxide	KOH	do
Acetic acid	CH_3COOH	do
Isopropyl alcohol	$CH_3\text{-}CH(OH)\text{-}CH_3$	do
Phenolphthalein	$C_{20}H_{14}O_4$	do
Sodium sulphate	Na_2SO_4	do
Oxalic acid	$(COOH)_2H_2O$	do
Iodine	I_2	do
Distilled water	H_2O	do

2.2.2 Chemicals used in Phytochemical Analysis

Name	Formula	Company
Nitric acid	HNO_3	Merck Germany
Sulphuric acid	H_2SO_4	Do
Perchloric acid	$HClO_4$	Do
Ammonium hydroxide	NH_4OH	Do
Ammonium bicarbonate	NH_4HCO_3	Do
Chloroform	$CHCl_3$	Do
Metal salts	Salts of 14 metals	do

2.3 INSTRUMENTS

2.3.1 Instrument used in Biodiesel production

Hot plate (VWR, VELP-Scientifica Berlin Germany)

Magnetic stirrer (TEFLON)

Titration apparatus

Electrical Balance (AND, GF-3000)

Oven (Memert Germany)

2.3.2 Instruments used in Phytochemical Analysis

AAS (Atomic Absorption Spectrometer Shimadzu AA-670)

FT-IR (Fourier Transfer Infrared Spectroscopy)

NMR (Nuclear Magnetic Resonance)
GC-MS (Gas Chromatography & Mass Spectroscopy)
Electrical Balance (AND, GF-3000)
Hot plate (VWR, VELP-Scientifica Berlin Germany)

2.4 DETERMINATION OF FREE FATTY ACID NUMBER IN MUSTARD OIL BIODIESEL

BY AQUEOUS ACID-BASE TITRATION

Free fatty acid (FFA) number of Brassica compestriss oil was determined by aqueous acid-base titration method (Komers *et al.*, 1997). Two types of titrations were executed, blank titration and sample titration.

Blank Titration

In blank titration sodium hydroxide /potassium hydroxide, isopropyl alcohol and phenolphthalein (indicator) were taken in flask and oxalic acid was added from the burette. End point was change in the color.

Sample Titration

In sample titration oil, isopropyl alcohol and phenolphthalein (indicator) were taken in the flask and were titrated against sodium hydroxide /potassium hydroxide. End point was changed color.

Following formula was used to determine free fatty acid number of oil

$$\text{Acid Number} = \frac{(A - B) \times C}{V}$$

A= Volume Used In Actual Titration

B= Volume Used In Blank Titration

C= Mass of Catalyst in g/l

D= Volume of Oil Used

2.5 BIODIESEL PRODUCTION

Following steps were involved in the production of biodiesel.

Filtration

Mustard oil contained lots of impurties which could effect the quality, yield and process of transesterification. Filteration was done in order to remove wax and other impurities. The filtration of crude oil was done by whattmann filter paper NO: 42. The time taken for filtration depends on the quality of oil. If oil is thich it will take more time to b filtered out from the filter paper.

Heating

The filtered oil of Mustard oil was heated up to 120°C on hot plate. Heating is done in order to remove any water and also to break the fatty acid bonds in oil. It took 35-40 minutes.

Transesterification

This method is used for the production of methyl ester by using different alkali catalysts. (Ahmad *et al.*, 2010)

In this method a known amount of sodium hydroxide (NaOH) was dissolved in pure distilled methanol (CH_3OH). Stir the mixture with the help of magnetic stirrer. Sodium hydroxide took 30 minutes to be completely dissolved. This sodium methoxide which acts as a catalyst was added to the oil at 60°C at which the conversion of triglycerides into fatty acid methyl esters (FAME) was assumed to be maximum. The final mixture was stirred vigorously for 60min with the help of magnetic stirrer at 600rpm. It should continue for at least 1 hr.

Settling

After stirring, leave the solution overnight at room temperature to settle down. Three products were obtained, with fine thin layer of soap then major portion was biodiesel (fatty acid methyl ester) and the dense glycerin was settled at the bottom.

Separation

The thin layer of soap at the top was removed carefully with the help of simple handling apparatus. Then biodiesel was poured out into separate vessel and glycerin was also separated into separate vessel.

Washing

Washing is done in a seprate container. Pour water in double the quantity into biodiesel and also add few drops of acetic acid in order to protect it from contamination. Allow the mixture to settle down for 4 hours in order to remove impurities and suspended particles and to stop any sort of fungal activity. Three to four washings are enough for complete clearance of biodiesel.

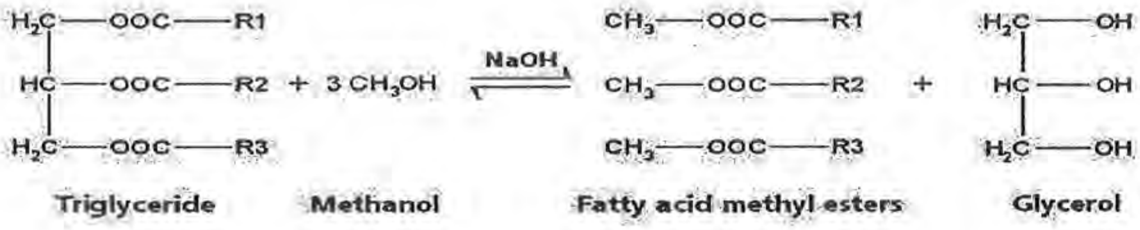
CHEMICAL CONVERSION

TRANSESTERIFICATION (ALCOHOLYSIS)

It reaction of a fat or oil with an alcohol to form esters and glycerol. The reaction is shown in Fig. A catalyst is usually used to improve the reaction rate and yield. Because the reaction is reversible, excess alcohol is used to shift the equilibrium to the products side.

The plant oils usually contain free fatty acids, phospolipids, sterols, water, odorants and other impurities. Because of these, the oil cannot be used as fuel directly. To overcome these problems the oil requires slight chemical modification (Canakci, 2007). There are three kinds of catalysts that can be used in transesterification reaction, a strong alkaline catalyst, a strong acid, and an enzyme. The main advantages of using a strong alkali as a catalyst are shorter reaction time and less amount of catalyst required in the manufacturing process of the transesterification reaction (Ma and Hanna, 1999; Meher *et al.*,2006).

Alcohols are primary and secondary monohydric aliphatic alcohols having 1±8 carbon atoms (Sprules and Price, 1950). Among the alcohols that can be used in the transesterification process are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used most frequently, especially methanol because of its low cost and its physical and chemical advantages (polar and shortest chain alcohol). It can quickly react with triglycerides and NaOH is easily dissolved in it. The reaction can be catalyzed by alkalis, acids, or enzymes. The alkalis include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide and sodium butoxide. Sulfuric acid, sulfonic acids and hydrochloric acid are usually used as acid catalysts. Lipases also can be used as biocatalysts. Alkali-catalyzed transesterification is much faster than acid-catalyzed transesterification and is most often used commercially. For an alkali-catalyzed transesterification, the glycerides and alcohol must be substantially anhydrous (Wright et al., 1944) because water makes the reaction partially change to saponification, which produces soap. The soap lowers the yield of esters and renders the separation of ester and glycerol and the water washing difficult. Low free fatty acid content in triglycerides is required for alkali-catalyzed transesterification. If more water and free fatty acids are in the triglycerides, acidcatalyzed transesterification can be used (Keim, 1945). The triglycerides can be purifieded by saponification (known as alkali treating) and then transesterified using an alkali catalyst.



General equation of Transesterification Reaction

2.6 BIODIESEL BLENDS AND THEIR FUEL PROPERTIES

The biodiesel of Mustard oil was blended with high speed diesel (HSD). Most common blends are B-10, B-20 and B-50. Pure biodiesel is some times called neat biodiesel and reffered to as B100.

2.7 PHYSICO-CHEMICAL CHARACTERIZATION

Analytical method was used to study chemical composition of biodiesel. Biodiesel was chemically analyzed by following spectra.

2.7.1 GC-MS Spectroscopy (Gas chromatography and Mass spectroscopy)

GC-MS detailed examination was performed using a GC-6890N directly coupled to MS5973MSD. 0.1μL biodiesel sample (B100) in chloroform ($CHCl_3$) was injected. The carrier was helium with column DB-5ms. The mass spectrometer was set to scan in the range of m/z 02-600 and ionization potential 70v.

2.7.2 FT-IR Spectroscopy (Fourier Transfer Infrared Spectroscopy)

The biodiesel was chemically analyzed by FT-IR Spectroscopy, using Bio-Rad Excalibur Model FTS3000MX in the range of $4000cm^{-1}$- $400cm^{-1}$

2.7.3 NMR Spectroscopy (Nuclear Magnetic Resonance)

NMR spectra (^1H and ^{13}C) were recorded in $CDCL_3$ as international reference by using a Bruker ARX-300MHz FT-NMR.

2.8 ELEMENTAL ANALYSIS

The Mustard seeds, oil, biodiesel , glycerin ,soap , soil and its different blends were analyzed for the presence of metals like K^+ ,Ni^{+3}, Li^+1, Mg^{+2}, Ca^{+2}, , Fe^{+2}, Co^{+3}, Mn^{+2}, Cu^{+3}, Cr^{+3}, Zn^{+2}, , Pb^{+4} , Cd^{+2} and Na^{+2} by using ammonium bicarbonate, diethylene triamine penta acetic acid using the method of Soltanpur and Schwab in 1977.

Preparation of sample solution

0.25gm sample was taken in a 100ml flask and added 6.5ml of acid solution. Acid solution was prepared by mixing Nitric acid ($HNO3$), Sulphuric acid ($H2SO4$) and Perchloric acid ($HClO4$). The sample was boiled in acid solution on hot plate until the sample was completely dissolved, indicated by white fumes coming out of the flask. After 2-4min added few drops of water and was allowed to cool at ambient temperature. Then these samples were poured into 50ml volumetric flask and the volume was raised up to 50ml by adding distilled water. Then filtered the sample solution and filtrate was collected in labeled bottles. This extract was used for the elemental analysis.

Procedure

Concentration of elements in the sample was analyzed by Shimadzu AA-670 Atomic Absorption Spectrophotometer by run along with the standard stock solution. (Anon, 1980)

Data Analysis

Following formula was used to calculate data and to prepare it for statistical analysis.

$$\text{Element cation in plant} = \frac{(\text{ppm in extract} - \text{blank}) \text{ x } A}{W \text{ x dilution factor}}$$

A = total volume of extract (ml)

W = weight of sample

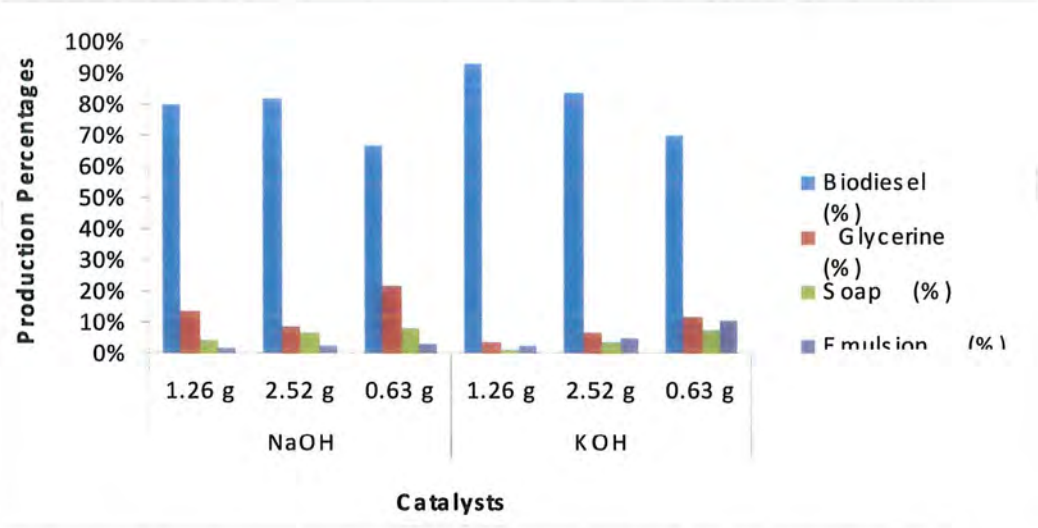


Fig.1 Production percentages of Biodiesel, Soap and Glycrine using different amount of KOH and NaOH

Fig. 2 **<u>BIODIESEL AND ITS BLENDS OF MUSTARD PLANT</u>**

Fig. 3 SEEDS OF MUSTARD

Fig. 4 OIL CAKE OF MUSTARD

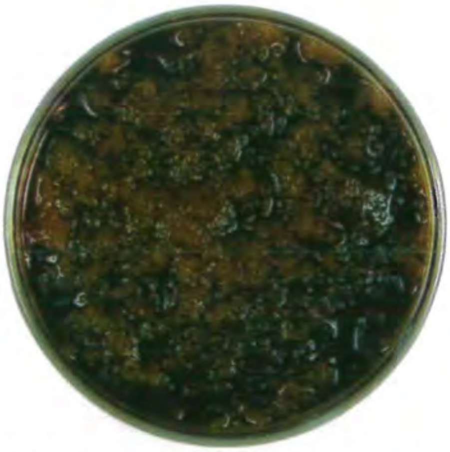


Fig. 5 GLYCERINE: A BYPRODUCT

Fig. 6 SOAP: A BYPRODUCT

3.3 Results of Concentration of Elemental (Metal) Analysis of Plant Parts (Brassica) & their By-products (Crude Oil, Biodiesel, Glycerin and Soap).

The concentration of metals and their distribution in Mustar plant and their by-products like crude oil, biodiesel, glycerin and soap and its different plant parts like stem, root, leaf and its soil was checked by atomic absorption spectrophotometer.

The concentration of 14 metals K^+, Mg^{+2}, Ca^{+2}, Na^{+2}, Fe^{+2}, Co^{+3}, Mn^{+2}, Cu^{+3}, Cr^{+3}, Zn^{+2}, Ni^{+3}, Li^+1, Pb^{+4} and Cd^+ was investigated in Mustard by-products. The amount of all the metals were taken in µg/g. the concentration of metals varied from maximum to very low value depends on sample to sample. In the investigated metals Potassium was the highest with (12211.6 ppm) found in the sample of Mustard plant stem and the minimum value of potassium was found in 10% biodiesel with (1024.2 ppm) while its amount in petro-diesel was 213.7ppm.

The highest concentration of Iron was calculated in oil cake (4338ppm) and the lowest was detected in leaves of the Mustad plant (245.8ppm). In petro-diesel its value is 224.1ppm. The amount of zinc determined in the flower of mustard plant was highest with (58ppm) and lowest was found in soap with (0.81ppm). Whereas the quantity of zinc detected in leaves, stem, crude oil, biodiesel and glycerin are 44ppm, 26ppm, 34ppm, 14ppm, and 18ppm respectively. The amount of zinc is 14ppm in Mustard biodiesel is higher in comparison with petro-diesel, which is 9.5ppm. Concentration of Chromium was high in biodiesel of Mustard plant (1.7 ppm) and the lowest value was found in glycerine 0.3ppm whereas its amount investigated in petro-diesel was 2.6ppm.

The quantity of Magnesium in flower (483.2ppm) found highest and the lowest was in 50% biodiesel (10.2ppm). The highest value of Calcium (6922ppm) was detected in leaves and the minimum was (501.6ppm) in 20% biodiesel. The concentration of Sodium (356ppm) was the maximum in glycerin and minimum amount (0.6ppm) was investigated in rice stem of Mustard plant. Concentration of Cobalt in high rank was found in petro-diesel (21.1ppm) crude oil (43.6ppm) and in low rank (3ppm) observed in soil of Mustard plant.

The amount of Manganese found in leaves (58.4ppm) which is highest and the lowest was found in 50% biodiesel (6ppm). The maximum amount of Copper was (466 ppm) found in soap and the minimum was in Mustard biodiesel (400ppm) whereas the amount of copper in petro-diesel is higher (98.4ppm) comparative to biodiesel.

The concentration of Nickel was highest in 50% biodiesel (36ppm) and lowest (0 ppm) present in soap, glycerine and soil of the Brassica compestriss. The amount of Lead (120ppm) was investigated maximum in 50% biodiesel whereas its lowest amount (26.2ppm) was found in Mustard stem. The high quantity of Lithium was found in glycerin (44ppm) whereas its low quantity is present in 10% biodiesel (0.7ppm). The presence of Cadmium was highest (137ppm) in seeds and its lowest presence (9.2ppm) was found in stem.

Table:1. Concentration of Metals in *Brassica compestriss* L. oil Biodiesel and By-products

Sample	Ca^{+2}	Li^{+}	Cr^{+3}	Fe^{+2}	Mn^{+2}	Cu^{+3}	Co^{+3}	K+	Na^{+2}	Mg+2	Ni^{+3}	Pb^{+4}	Zn^{+2}	Cd^{+2}
SEED	587	14	1.1	1698	63.2	406	22.4	11795.2	63.54	347.8	32	52	8	137.2
LEAVES	6922	28	0.8	245.28	58.4	424	21.2	12152	10	308.4	28	70	44	15.8
STEM	2210	4	0.6	1868.6	18	416	4.8	12211.6	0.6	202	19	26.2	26	9.2
FLOWER	2204	16	0.2	2394	26.6	418	10.02	11192	77	483.2	31.2	108	58	24.2
SOIL	945.2	6	0.9	1652	32.2	414	3	1676	28	201.4	0	62	24	13.2
OIL CAKE	1295.2	2	0.2	4338	36	422	23.8	11589	31.79	452.6	1.2	100	52	12.4
CRUDE OIL	1187.4	2	0.4	1568	58	410	43.6	1334	72	166.2	11.2	84	34	32.4
BIODIESEL	1043.6	32	1.7	1508	35.2	400	28.6	2238.6	57	141.4	11.6	36	14	26.2
10% B.D	502	7	0.2	1994	48.4	410	7.6	1024.2	144	115.4	14	112	12	27.6
20% B.D	501.6	8	0.3	1684	10.4	416	11.4	1097.8	307	84.6	7.4	56	6	17.6
50% B.D	556	10	0.8	2156	6	412	17	2456.5	23	10.2	36	120	32	27.2
SOAP	610	4	1.0	290	28.6	466	11	3522	40.21	89.6	0	30	0.81	9.2
GLYCERINE	817	44	1.3	1526	20.6	418	19.6	3086.2	356	45	0	106	18	36.6
PETRO-DIESEL	546	1.1	2.6	224.1	1.4	98.4	21.1	213.7	2998.44	35.4	12.1	114.1	9.5	5.1

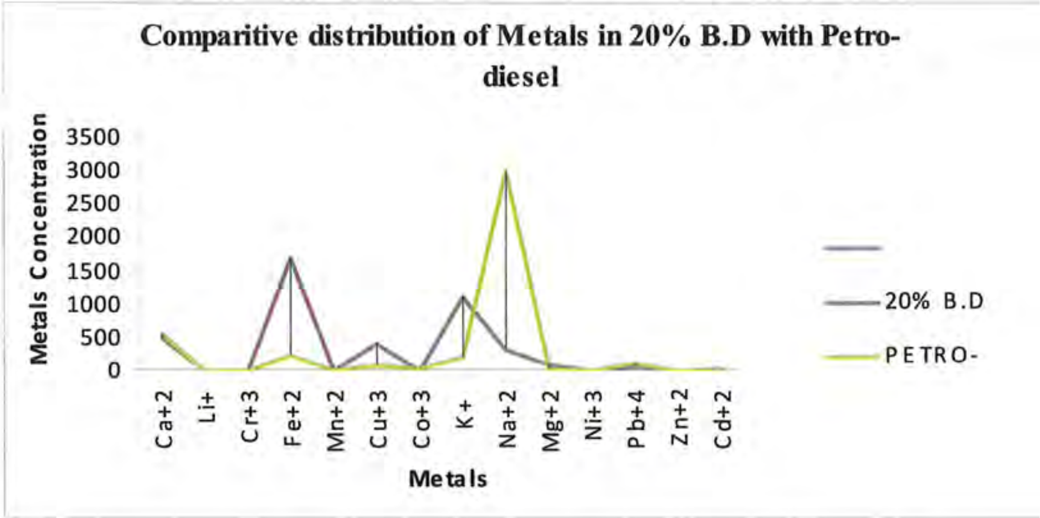


Fig.7. Comparative distribution of metals in 20% B.D with petro-diesel

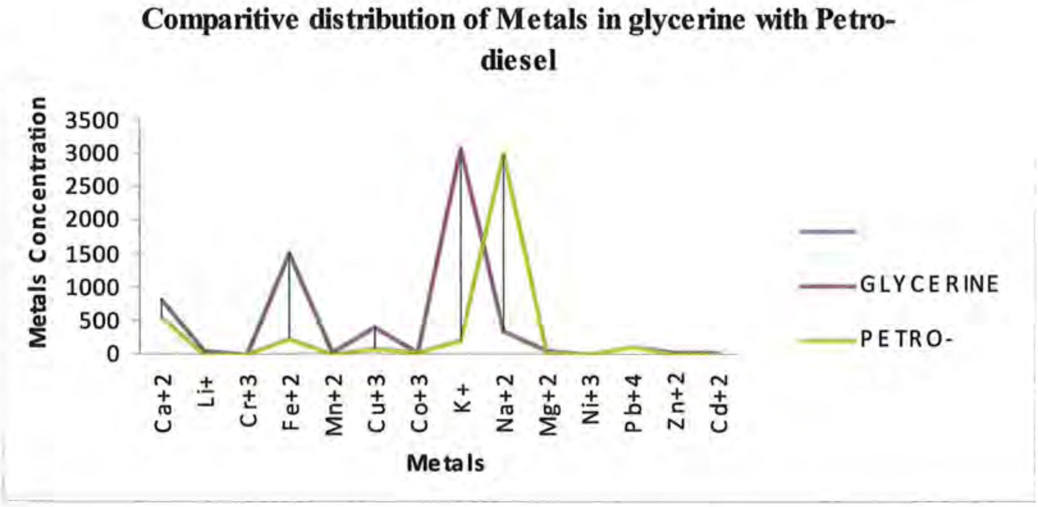


Fig.8. Comparative distribution of metals in glycerine with petro-diesel

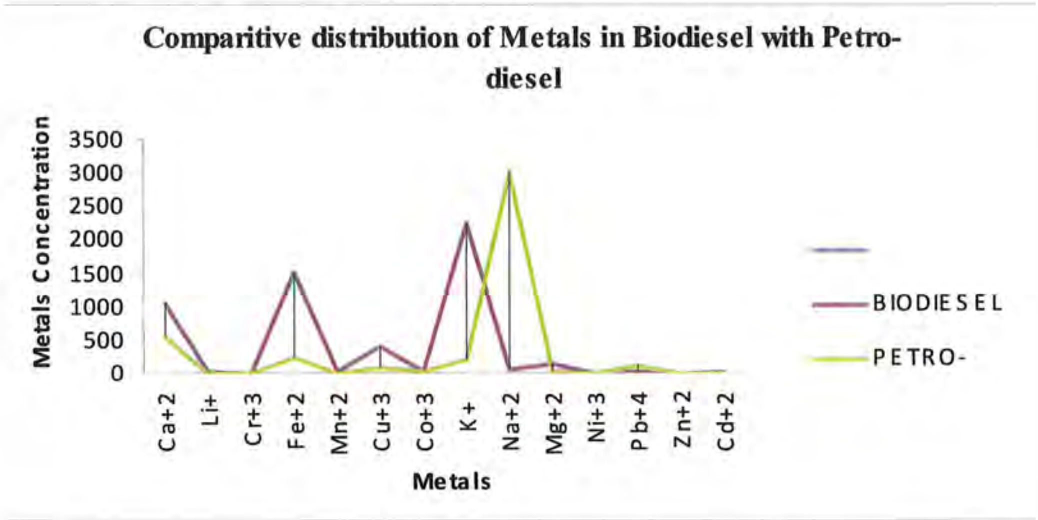


Fig.9. Comparative distribution of metals in Biodiesel with petro-diesel

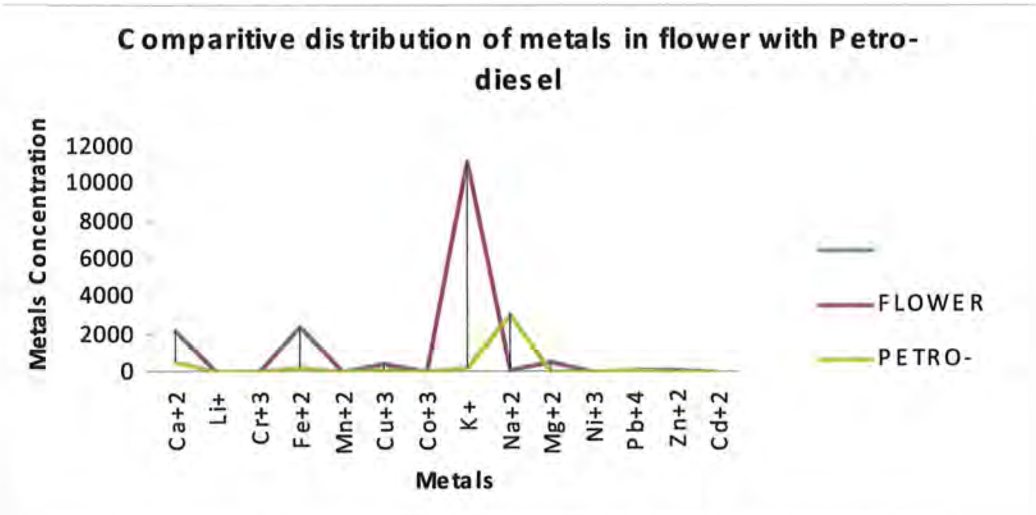


Fig.10. Comparative distribution of metals in flower with petro-diesel

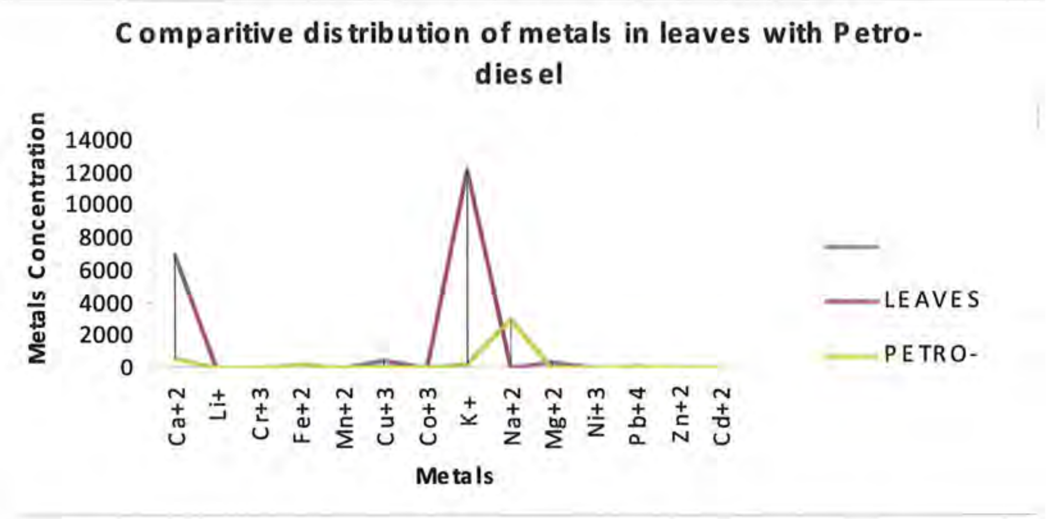


Fig.11. Comparative distribution of metals in leaves with petro-diesel

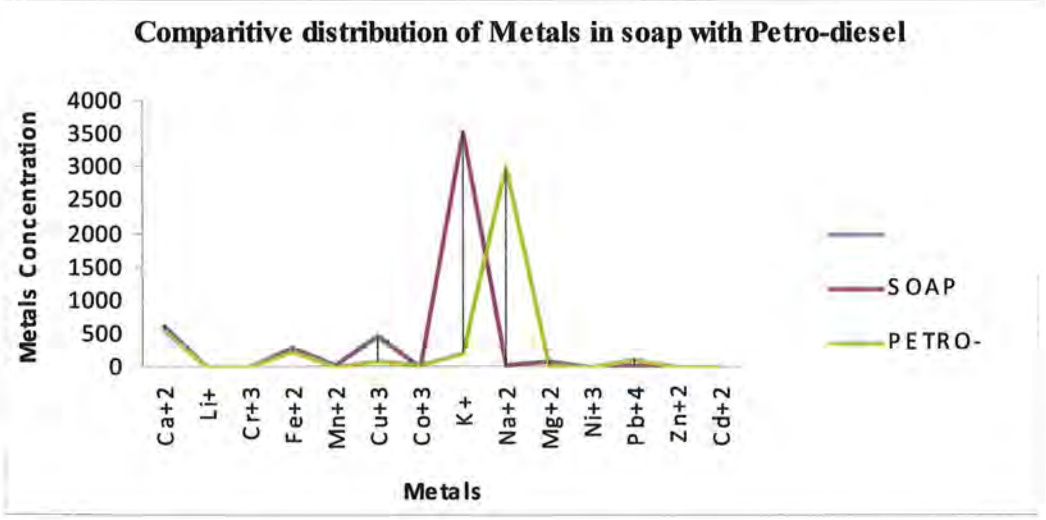


Fig.12. Comparative distribution of metals in soap with petro-diesel

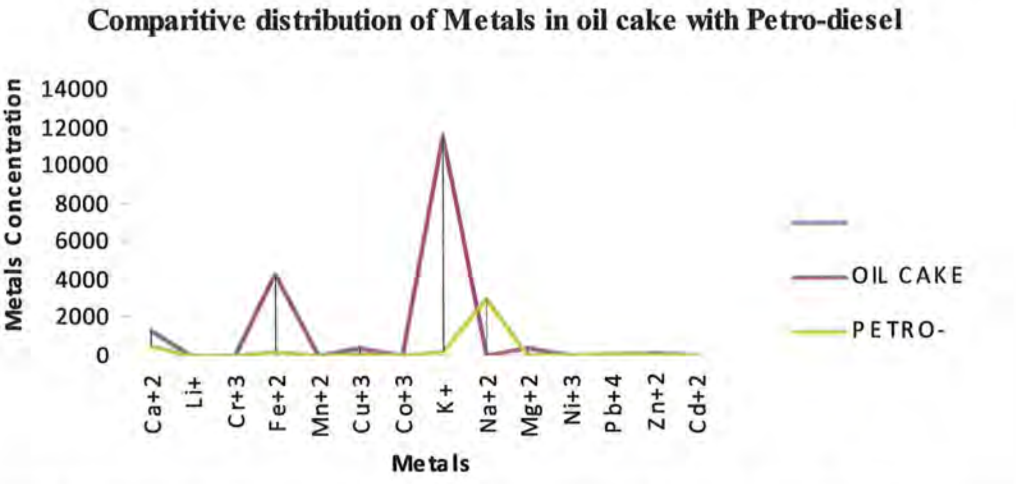


Fig.13. Comparative distribution of metals in oil cake with petro-diesel

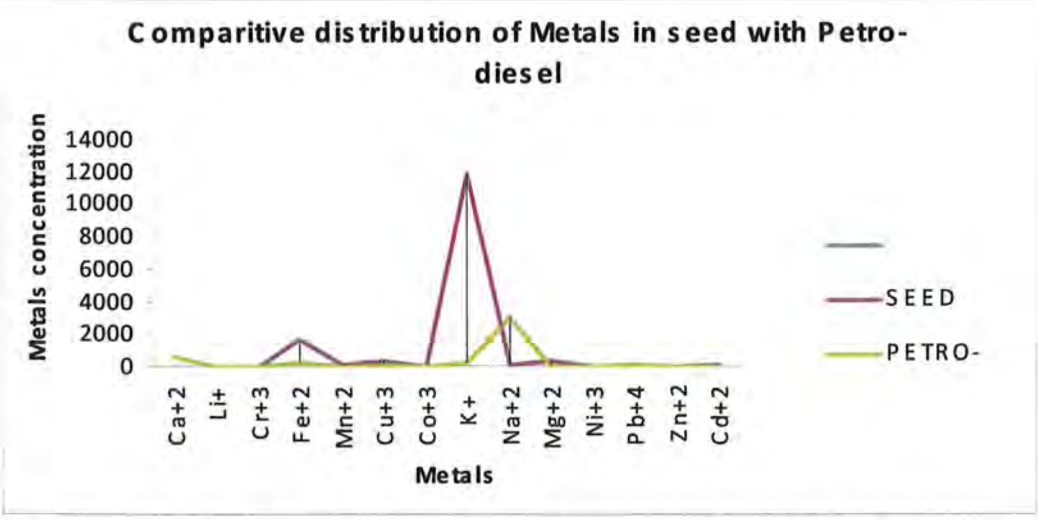


Fig.14. Comparative distribution of metals in seed with petro-diesel

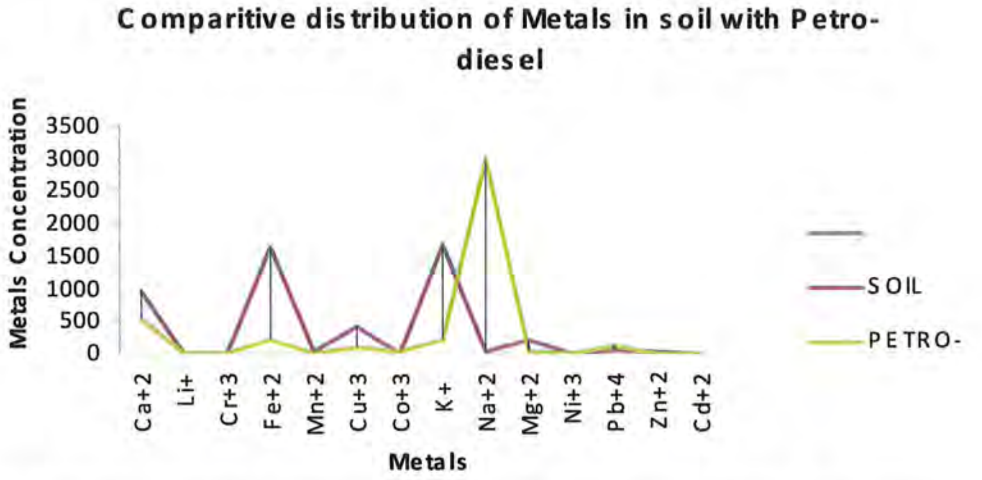


Fig.15. Comparative distribution of metals in soil with petro-diesel

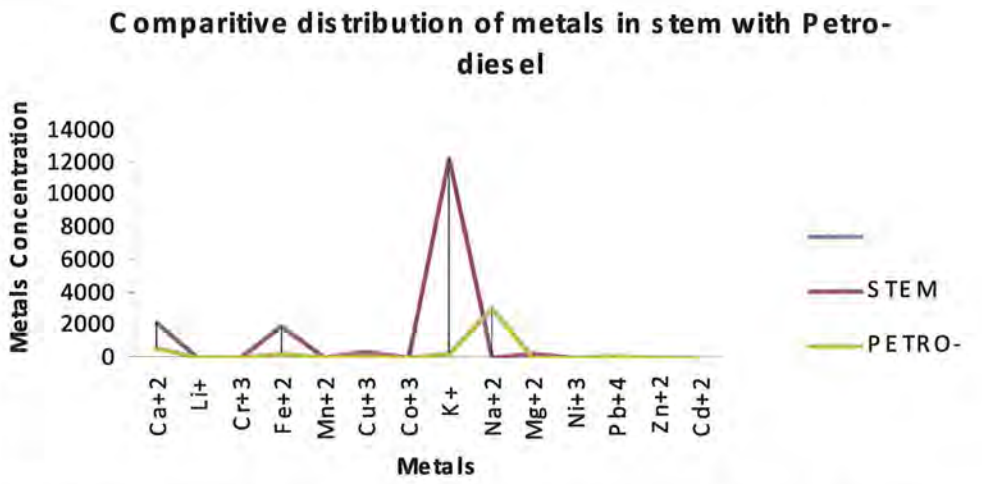


Fig.16. Comparative distribution of metals in stem with petro-diesel

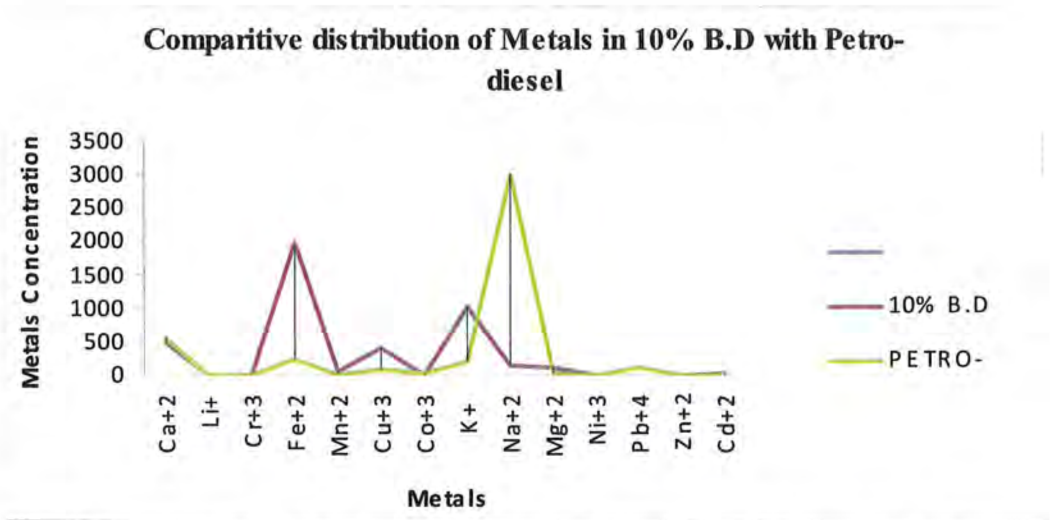


Fig.17. Comparative distribution of metals in 10% B.D with petro-diesel

3.4 ANALYTICAL METHODS USED IN BIODIESEL QUALITY ASSESMENT

Several analytical methods FT-IR, NMR and GC-MS were conducted for fuel quality and to verify the prescribed biodiesel standard.

3.4.1 FT-IR STUDY OF MUSTARD BIODIESEL

Biodiesel was characterised by FT-IR spectroscopy and its spectra shows the presence of important functional groups. The characteristic peak of ester is observed at 1741 cm^{-1}, of aliphatic CH at 2922 cm^{-1}, and of CO in finger print region at about 1168 cm^{-1}. The two peaks at 1461cm^{-1} and 1435cm^{-1} shows bending vibrations of CH_3 while 1244 cm^{-1} shows bending vibrations of CH_2.

3.4.2 NMR STUDY OF MUSTARD OIL

The Mustard oil biodiesel was characterized by NMR (^1H and ^{13} C).

3.4.2.1 ^{1}H NMR SPECTROSCOPY

The characteristic peak of methoxy proton is observed at 3.65ppm (singlet) and α-CH_2 protons at 2.26ppm (triplet). The two peaks are distinct peaks for confirmation of methyl esters present in biodiesel.

3.4.2.2 ^{13}C NMR SPECTROSCOPY

In ^{13}C NMR spectrum, the characteristic peaks of ester carbonyl (-COO-) and C-O are observed at 174.20 and 51.36ppm, respectively.

3.4.3 GC-MS STUDY OF MUSTARD OIL

GC-MS is used to study the chemical composition of the biodiesel product. Different peaks were observed in the spectrum. Each peak corresponds to a fatty acid methyl ester which was identified by library match software. The identified FAMEs with their retention time are shown in Table-2

Table-2 FAMEs Composition of Brassica campestris biodiesel B100.

Peak No.	Retention time(min)	FAMEs
1	2.81	Methyl 9-hexadecenoate (C16:1) $CH_3OOC(CH_2)_7CH=CH(CH_2)_5CH3$
2	2.87	Methyl hexadecanoate (C16:0) $CH_3OOC(CH_2)_{14}CH_3$
3	3.43	Methyl 9-octadecenoate (C18:1) $CH_3OOC(CH_2)_7CH=CH(CH_2)_7CH_3$
4	3.47	Methyl octadecanoate (C18:0) $CH_3OOC(CH_2)_{16}CH_3$
5	4.01	Methyl 11-eicosenoate (C20:1) $CH_3OOC(CH_2)_9CH=CH(CH_2)_7CH_3$
6	4.07	Methyl eicosanoate (C20:0) $CH_3OOC(CH_2)_{18}CH_3$
7	4.75	Methyl 13-docosenoate (C22:1) $CH_3OOC(CH_2)_{11}CH=CH(CH_2)_7CH_3$
8	4.84	Methyl docosenoate (C22:0) $CH_3OOC(CH_2)_{20}CH_3$
9	5.77	Methyl 15-tetracosenoate (C24:1) $CH_3OOC(CH_2)_{13}CH=CH(CH_2)_7CH_3$
10	5.91	Methyl tetracosanoate (C24:0) $CH_3OOC(CH_2)_{22}CH_3$

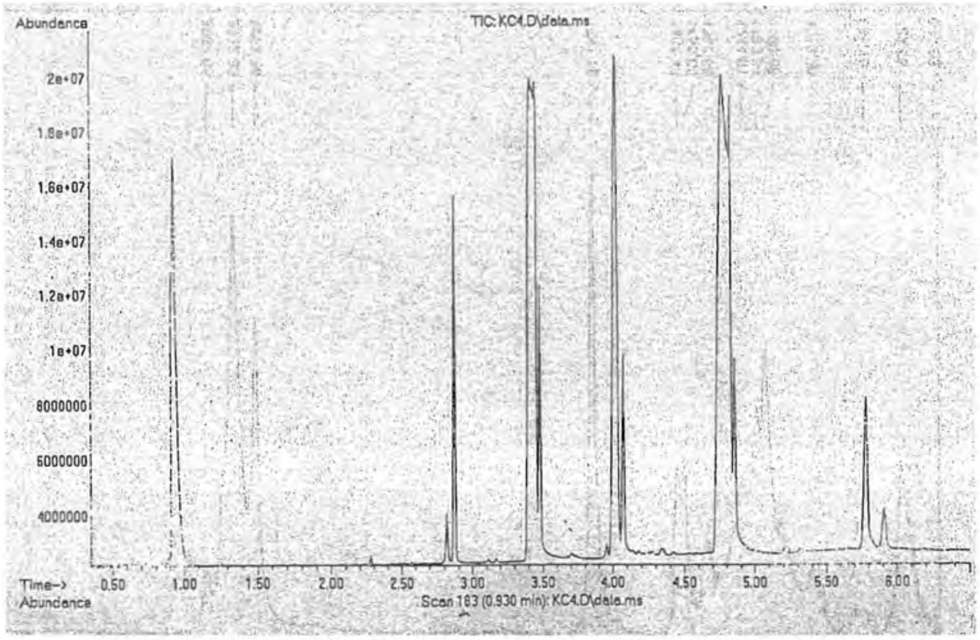


Fig No.18 GC-MS of Mustard Oil Biodiesel

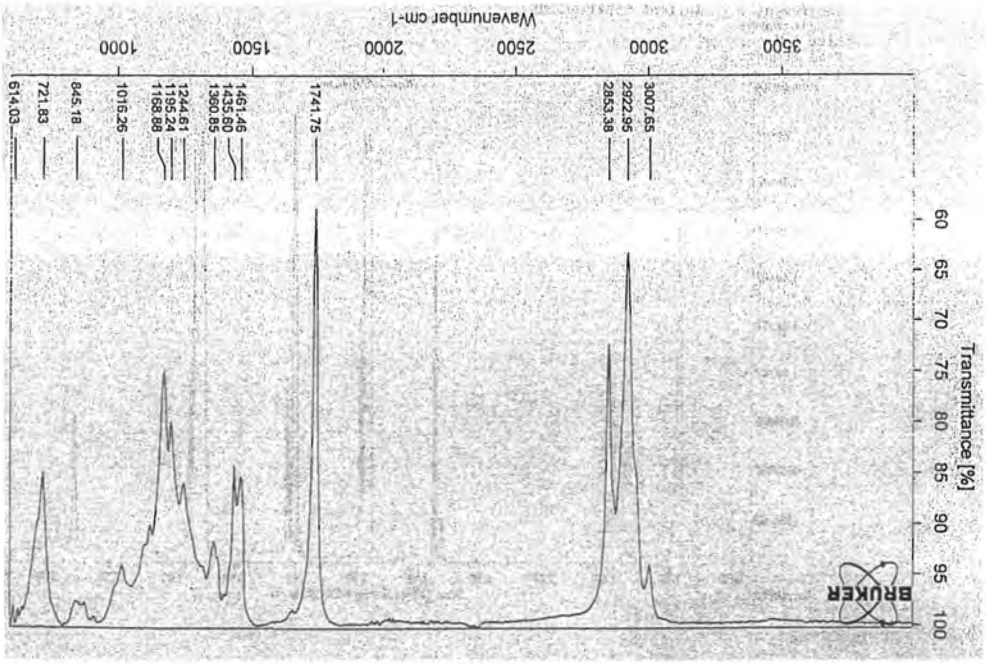


Fig No.19 FT-IR of Mustard Oil Biodiesel

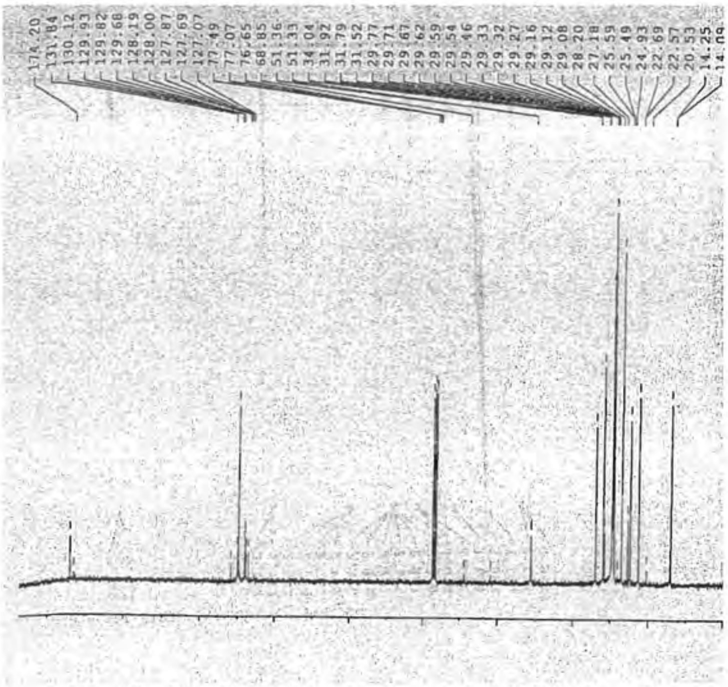


Fig No.20 HNMR of Mustard Oil Biodiesel

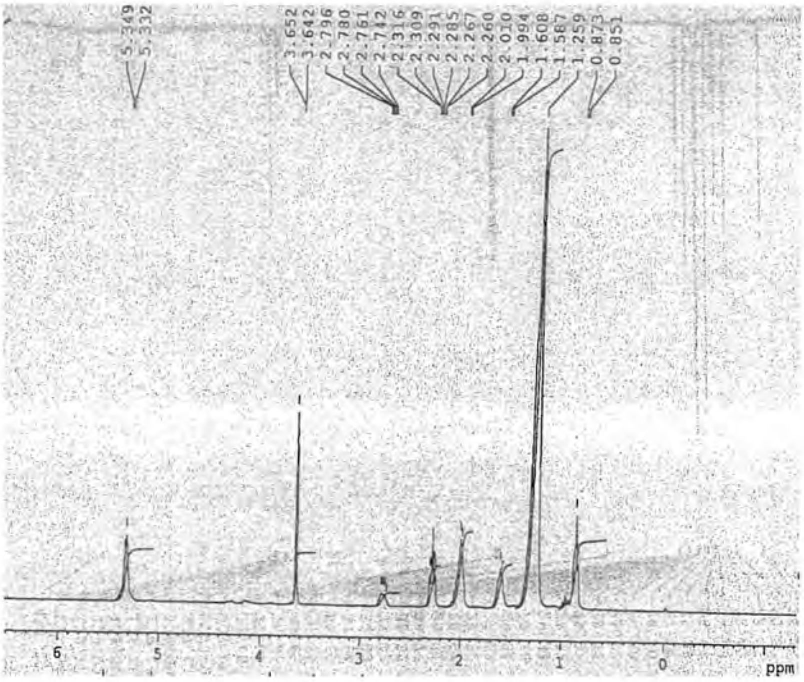


Fig No.21 CNMR of Mustard Oil Biodiesel

3.5 FUEL PROPERTIES

In this current project Mustard oil biodiesel and its different blends with high speed diesel are analysed for their physico-chemical properties. The results of biodiesel and its blends were found to be similar to ASTM (American Standard & Material Testing) as shown in Table: 3.

TABLE: 3. Fuel Properties of *Brassica compestriss* L. Biodiesel and its Blends

TEST	METHODS	B100%	B50%	B20%	B10%	HSD
Flash Point, °C	ASTM D-93	110	92	82	85	3.2642
Kinematic viscosity@ 40 °C (ny)	ASTM D-445	5.775	4.042	4.214	4.187	1.9-6
Color Comparison	Visual	2	2	2	2	2
Sulfur %wt	ASTM D-4294	0.0083	0.00651	0.0	0.00452	0.05
Pour Point, °C	ASTM D-97	-18	-10	-15	-12	-15,-35
Cloud Point, °C	ASTM D-2500	-6.3	-6	-9	-7	-5,-15
Cetane Number	ASTM D613	53	44	51	48	40-55
Density @ 15 °C kg/L	ASTM D-1298	0.871	0.836	0.8431	0.838	0.8343

HSD: High Speed Diesel

B100: 100% Biodiesel

B50: 50% HSD and 50% Biodiesel

B20: 80% HSD and 20% Biodiesel

B10: 90% HSD and 10% Biodiesel

DISCUSSION

&

CONCLUSION

4.1 BIODIESEL POTENTIAL TO MUSTARD OIL

In this current research work Mustard oil was analyzed for the production of biodiesel. It was found that the Mustard plant (*Brassica compestriss*) is a favourable and suiteable choice for biodiesel production. Prior to biodiesel production, determined the free fatty acid number of oil by aqueous acid base titration, the free fatty acid number of Mustard oil against sodium hydroxide was and against potassium hydroxide was. According to (Dorado *et al*., 2002), if the free fatty acid contents are more than 3% in the oil, transterification reaction would not occurs. The percentage of conversion from oil to biodiesel, Glycerin and Soap was different with different types and concentration of base catalyst (NaOH &KOH) as shown in table 2 and 3.

When 1.26g amount of NaOH catalyst is used, the percentage of biodiesel was (80%), Glycerin (14%), soap was (4.20%). The result of biodiesel with KOH reaction was (93%), Glycerin (3.6%) and soap (1.2%). By using 2.52g concentration of catalyst, the result of biodiesel with NaOH (82%) Glycerin (9%) and soap was (6.65%) along to it the result with KOH of biodiesel was (84%), Glycerin (6.8%) and soap (4%). When the concentration of base catalyst (NaOH & KOH) was 0.63g, the result was found in different amount. The result of biodiesel with NaOH catalyst was (67%), Glycerin (22%) and soap was (8%) and the result of biodiesel with KOH was (70%), Glycerin (12%) and soap was (7.2%). From the current research it was observed that the yield percentage was affected drastically by lowering the catalyst concentration under the same conditions, as NaOH and KOH gave maximum when the maximum amount of the catalyst is used and when we reduce the amount of catalyst it results decrease in yield. (Refaat *et al*., 2008).

The influence of catalyst type on the ester yields was also observed in our project and found that among the catalysts NaOH and KOH, the KOH was the best suited catalyst. These results agree with those obtained by other authors. Nye et al 1992, and Tomasevic and Silver-Marinkovic 2003.in a process of transterification of used frying oil also concluded that Potassium hydroxide was the best catalyst.

4.2 FUEL PROPERTIES OF MOB

Low or almost negligible sulphur content is a common trait of bio diesel derivedfrom any vegetable oil (Mohammad *et al* 2009). The sulfur contents in biodiesel are present in B100, B10 and B20 are 0.0087, 0.0045 and 0.0 whereas it is 0.05 in petro-diesel. Sulfur content is an important feature in term of reduction of SO_2 from the exhaust emission (Kumar, 2007). Hence, anoter brassicae family member taramira bio diesel is also showing no exception in terms of low sulphur content in comparison to mineral diesel fuel thus enabling it to be considered as a very environment friendly fuel. However, slightly higher sulphur content in comparison to canola bio diesel means that it may result in the release of more SOX compounds in the compression-ignition engine in comparison to its canola cousin (Chakrabarti & Ali, 2008).

Flash point of MOB (110) is quite high as compared to mineral diesel (60-80), High flash point makes it safer to store and transport (Kumar, 2007). The Kinematic viscosity was found to be 5.575, 4.187 and 4.21 in B100, B10 and B20 respectively and is comparable to diesel which is 4.1. The Kinematic viscosity of Mustard oil biodiesel is close to the upper limit for the American standard for testing materials (Anon., 2008). Both these values are very much high in comparison to petro-diesel.

The density of biodiesel found in B100, B10 and B20 are 0.871, 0.838 and 0.8431 respectively. These densities are close to density of high speed diesel 0.8343. Higher density for biodiesel results in the delivery of a slightly greater mass of fuel. While two other brassicae family members taramira & Canola bio diesel is also showing 0.881 and 0.88 respectively (Mohammad., *et al* 2009) that were close to the ASTM limit as well as the pure mineral diesel (Chakrabarti & Ali, 2008).The color comparison is also similar to international standard.

The cetane number provides a measure of the ignition characteristics of diesel fuel or bio diesel fuel in compression ignition engines (Anon., 2008). The CN of biodiesel is generally higher then conventional diesel (Bala, 2005). The higher the cetane index the shorter the delay interval and the greater the fuel combustibility. Fuels with low Cetane Numbers will result in difficult starting, noise and exhaust smoke. In general, diesel engines will operate better on fuels with Cetane Numbers above 50. The Cetane number of MOB found in B100, B10 and B20 are 53, 48 and 51 respectively and is comparable to diesel which is 40-55, so its ignition characteristics are likely to be same that of mineral diesel in the diesel engine.

The Cloud Point of any petroleum fuel is defined as the temperature at which a cloud of wax crystal first appears in the oil when it is cooled at a specific rate (Lang *et al* 2005). The Pour Point is the temperature at which the amount of wax out of solution is sufficient to gel the fuel, thus lowest temperature at which the fuel can flow. Biodiesel has higher Cloud Point and Pour Point compared to conventional diesel (Ali *et al.,* 1995). The Cloud Point of MOB was found to be -6.3, -7 and -9 in B100%, B10% and B20% and these values are lower than conventional petroleum diesel which ranges from -15 to -5. The Pour Point of biodiesel of B100%, B10% and B20 % are -18, -12 and -15 respectively whereas the pour point of diesel ranges from -35 to -15. The Cloud point determined by Sinha *et al.,* (2008) of rice bran oil biodiesel is 9 which is very much high than mineral diesel. The Pour point biodiesel studied by Sinha *et al.,* (2008) is -2 whereas Kumar (2007) found it to be -4.

4.3 CHEMISTRY OF MOB

Main analytical methods for the credible analysis of biodiesel non expensive to quantify the chemical composition, following analytical techniques were used in biodiesel i.e Infrared spectroscopy, Gas Chromatography and mass spectroscopy and ^{1}H NMR ^{13}C.

4.3.1 FT-IR STUDY OF MUSTARD BIODIESEL

Infrared spectroscopy is used in the evaluation of transterification reaction (Knot, 1999). In this study we characterised the biodiesel its spectra that shows the presence of important functional groups. The characteristic peak of ester is observed at 1741 cm^{-1}, of aliphatic CH at 2922 cm^{-1}, and of CO in finger print region at about 1168 cm^{-1}. The two peaks at 1461cm^{-1} and 1435cm^{-1} shows bending vibrations of CH_3 while 1244 cm^{-1} shows bending vibrations of CH_2.

4.3.2 NMR STUDY OF MUSTARD OIL

The Mustard oil biodiesel was characterized by NMR (^{1}H and ^{13} C).

4.3.2.1 ^{1}H NMR SPECTROSCOPY

The analysis of chemical constituents of biodiesel by by ^{1}H NMR was first reported by Gelbard *et al.,* 1995 to measure the protons of methylene group adjacent to ester moiety in tryglycerols and protons in the alcohol moiety of the methyl ester to assist the yield. Current research showed that the characteristic peak of methoxy proton is observed at 3.65ppm (singlet) and α-CH_2 protons at 2.26ppm (triplet). The two peaks are distinct peaks for confirmation of methyl esters present in biodiesel. Other peaks were at 0.85ppm of terminal methyl protons, a strong signal at 1.25 ppm related to methylene protons of carbon chain, a signal at 1.58ppm from β- methylene protons and 5.33 ppm assign to olefinic hydrogen.(Monteiro *et al.*, 2009 and Samios *et al.*, 2009)

4.3.2.2 ^{13}C NMR SPECTROSCOPY

In ^{13}C NMR spectrum, the characteristic peaks of ester carbonyl (-COO-) and C-O are observed at 174.20 and 51.36ppm, respectively.The peaks at 127.07-131.84 ppm showed unsaturation in methyl esters. Other peaks were at 14 ppm related to terminal carbon of methyl groups and 22.57-34.04 ppm signals related to methyl carbons of long carbon chain in FAMEs. (Soares *et al.*, 2008).

4.3.3 GC-MS STUDY OF MUSTARD OIL

GC-MS is used to study the chemical composition of the biodiesel product. Different peaks were observed in the spectrum. Each peak corresponds to a fatty acid methyl ester which was identified by library match software. The identified FAMEs with their retention time are shown in Table-2 .The identity of FAMEs was verified by mass spectrometric analysis and retention time data. The mass spectrum was obtained by an electron impact (EI) ion source. GC analysis confirmed the saturated and unsaturated FAMEs in Mustard oil biodiesel which were in accordance with the reported composition of the rocket seed oil (Bhandari *et al.*, 1996 and Yadava *et al.*, 1998). In Mustard oil biodiesel, five saturated and five unsaturated FAMEs were identified by GC-MS.

The electron impact spectra of saturated FAMEs were very similar, having characteristics peaks of fragment ions shown in Table-2. The molecular ion peak is usually weak. EachFAME was separated out at specific retention time by gas chromatography. So the retention time and mass fragmentation pattern help to identify and verify FAMEs. The base peak in all saturated FAMEs is observed at m/z 74 which is product of well known McLafferty rearrangement process, (McLafferty *et* *al.,* 1959). a peak $[M-31]^+$ due to α-cleavage (loss of methoxy group). These two peaks can be seen clearly in the spectra of all saturated FAMEs. Other characteristic ion include at m/z 87, the first member of carbomethoxy ion series due to β-cleavage [CH3OCO (CH2)n $]^+$, where n= 1, 2, 3, 4…….m/z 73,87,101,115,129,143………with difference of 14 a.m.u, a hydrocarbon series of ions(alkyl series) that are abundant in the low mass region. Thus β-cleavage and alkyl series support the identification of saturated FAMEs.

Monounsaturated FAMEs were observed in Mustard oil biodiesel B100. Monounsaturated FAMEs showed a similar fragmentation pattern and a characteristic base peak at m/z 55. There were two main processes in monounsaturated FAMEs which help to characterize them, α-cleavage on either side of C=O group and β-cleavage to the double bond. The fragment ion $[M-32]^+$ due to loss of methanol (methoxy group plus a hydrogen atom) and fragment ion $[M-74]^+$ due to loss of the McLafferty ion (Hallgren *et al.,* 1959) are also two distinct ions to identify monounsaturated methyl esters.

4.4 ELEMENTAL ANALYSIS

Our findings of literature tell that there is no systematic data of elemental analysis on plant by-product with reference to petro-diesel. In this respect, no data has been published on the determination of different metals in plant by-products with contrast to petro-diesel.

The need of the current research to perform an analysis of chemical composition of different parts of plants and also their by-products with reference to petro-diesel, the main advantage of metals is that, they are more stable at high temperature allowing non-volatile material, removing contamination and improving life cycle period.

As the final product is the ultimately burnt, the quality of the fuel is much important, not only structural analysis is important (Holcapek *et al.,* 2001).but the inorganic components are also be analyzed; the raw oil is generally low in inorganic contaminant and in sulfur, but the final product need to be analyzed to check that catalyst are low or any other kind of contamination is present.

Different kinds of elements that are present in catalyst residues are important to analyze as they lead to the ash content in the engine and can also cause corrosion, subsequently affecting the performance of engine and its life. Sulfur is much important as engine legislation and less sulfur requirements of modern engines. Different catalyst poison elements such as lead and vanadium also to be checked.

Phosphorus has been shown to damage the ability of after-treatment systems to reduce exhaust emissions as intended. The influence of phosphorus is cumulative; and as a result, very low levels of contamination over the significant amount of fuel consumed by an engine may lead to unexpected deterioration of the after treatment system.

Sodium and potassium hydroxides are utilized as catalysts and magnesium and calcium as absorbents in the production of biodiesel and should be removed through the biodiesel production process. These residual metals can form deposits in fuel injection system components and poison emission control after-treatment systems.

Many poisons are very dangerous and highly active even they are present in low quantity and with the increasing use of modern and advanced catalysts in biodiesel production, the need to check such elements is very important. Different other organo-metallic compounds are used in the fuels to enhance their fuel properties, such as Si (anti-foaming agent), Mn (a burn enhancer), and different other like Cr, Fe, Ni and they need to be checked to ensure that best amount is used.

The concentration of 14 metals K^{+}, Mg^{+2}, Ca^{+2}, Na^{+2}, Fe^{+2}, Co^{+3}, Mn^{+2}, Cu^{+3}, Cr^{+3}, Zn^{+2}, Ni^{+3}, $Li^{+}1$, Pb^{+4} and Cd^{+} was investigated in Mustard oil by-products. In the investigated metals Potassium (K) was the highest with (12211.6ppm) found in the sample of Mustard plant stem and the minimum value of Potassium (K) was found in 10% biodiesel with (1024.2ppm) while its amount in petro-diesel was 213.7ppm.

The highest concentration of Iron was calculated in oil cake (4338ppm) and the lowest was detected in leaves of the Mustad plant (245.8ppm). In petro-diesel its value is 224.1ppm. The amount of zinc determined in the flower of mustard plant was highest with (58ppm) and lowest was found in soap with (0.81ppm). Whereas the quantity of zinc detected in leaves, stem, crude oil, biodiesel and glycerin are 44ppm, 26ppm, 34ppm, 14ppm, and 18ppm respectively. The amount of zinc is 14ppm in Mustard biodiesel is higher in comparison with petro-diesel, which is 9.5ppm. Concentration of Chromium was high in biodiesel of Mustard plant (1.7ppm) and the lowest value was found in glycerine 0.3ppm whereas its amount investigated in petro-diesel was 2.6ppm.

The quantity of Magnesium in flower (483.2ppm) found highest and the lowest was in 50% biodiesel (10.2ppm). The highest value of Calcium (6922ppm) was detected in leaves and the minimum was (501.6ppm) in 20% biodiesel. The concentration of Sodium (356ppm) was the maximum in glycerin and minimum amount (0.6ppm) was investigated in rice stem of Mustard plant. Concentration of Cobalt in high rank was found in petro-diesel (21.1ppm) crude oil (43.6ppm) and in low rank (3ppm) observed in soil of Mustard plant.

The amount of Manganese found in leaves (58.4ppm) which is highest and the lowest was found in 50% biodiesel (6ppm). The maximum amount of Copper was (466ppm) found in soap and the minimum was in Mustard biodiesel (400ppm) whereas the amount of copper in petro-diesel is higher (98.4ppm) comparative to biodiesel.

The concentration of Nickel was highest in 50% biodiesel (36ppm) and lowest (0 ppm) present in soap, glycerine and soil of the Brassica compestriss. The amount of Lead (120ppm) was investigated maximum in 50% biodiesel whereas its lowest amount (26.2ppm) was found in Mustard stem. The high quantity of Lithium was found in glycerin (44ppm) whereas its low quantity is present in 10% biodiesel (0.7ppm). The presence of Cadmium was highest (137ppm) in seeds and its lowest presence (9.2ppm) was found in stem.

4.5 CONCLUSION

In the current research work Mustard plant i.e *Brassica campestriss* L. was analyzed for the production of Biodiesel. Results showed that this is a viable source for biodiesel production all over the world especially in Pakistan. The best results of Biodiesel production were achieved on constant 1:6 Molar ratio at 60°C, with different types and concentrations of Catalyst, Potassium hydroxide with 1.26 & 2.52 shown the best results. The Physico-chemical properties i.e Kinematic viscosity, specific gravity, flash point, cloud point and sulfer content of biodiesel present in accordance with the ASTM methods.

The distribution and concentration of Metals in different plant parts and their by-products in contrast to biodiesel were checked by the atomic absorption spectrophotometer. I4 metals were analyzed in all the samples. From the results it was observed that the concentration of metals present in plant parts and their by-products were comparatively high then that present in petro-diesel.

Different analytical techniques i.e GC-MS, NMR and FT-IR Spectra was used for the chemical analysis of MOB. Samples of plant biodiesel and petro-diesel shown different peaks .On the basis of these peaks determined the different types of fatty esters present in biodiesel.

From the results it is concluded that Mustard plant have the potential for biodiesel production and it is recommended to grow them on large scale for biodiesel production.

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