BIODIESEL PRODUCTION VIA ALKALI-CATALYZED TRANSESTERIFICATION OF MUSTARD AND SOYBEAN OIL: EFFICIENCY ANALYSIS OF CuNPs AS CATALYSTS

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by

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DECLARATION

I, Megha Shah 23/MSCBIO/29 hereby certify that the work which is being presented in the thesis entitled "Biodiesel Production via Alkali-Catalyzed Transesterification of Mustard and Soybean Oil: Efficiency Analysis of CuNPs as Catalysts" in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2023 to 2025 under the supervision of Prof. Jai Gopal Sharma.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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SUPERVISOR'S CERTIFICATE

Certified that Megha Shah (23/MSCBIO/29) has carried out their search work presented in this thesis entitled "Biodiesel Production via Alkali-Catalyzed Transesterification of Mustard and Soybean Oil: Efficiency Analysis of CuNPs as Catalysts" for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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“BIODIESEL PRODUCTION VIA ALKALI-CATALYZED TRANSESTERIFICATION OF MUSTARD AND SOYBEAN OIL: EFFICIENCY ANALYSIS OF CuNPs AS CATALYSTS”

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ABSTRACT

Amid increasing global energy demand, biodiesel has emerged as a sustainable alternative to conventional fossil fuels. It is an eco-friendly, biodegradable, and non-toxic fuel. It can be produced through vegetable oil, non-edible oil, waste cooking oil, including other triglyceride sources. The choice of feedstock significantly influences both the process and biodiesel yield. Biodiesel can be produced through different methods, including pyrolysis, dilution, micro-emulsification, and transesterification. Out of all these methods, transesterification is the most commonly used technique, due to its efficiency and simplicity. This process can be catalyzed using homogeneous catalysts such as sodium hydroxide, potassium hydroxide, hydrochloric acid, and sulfuric acid or heterogeneous catalysts, including metal oxides, nanoparticles, etc. The selection of catalysts depends on the type of feedstock and its FFA content. Key parameters affecting the transesterification reaction include a molar ratio of TAG to alcohol, reaction time, reaction temperature, and catalyst concentration, with glycerol as a by-product. In this study, biodiesel was produced from mustard and soybean oil using an alkali-catalyzed transesterification reaction, and the efficiency of CuNPs as a catalyst was also evaluated. CuNPs were synthesized using both chemical and green synthesis, and further characterization was performed using FTIR.

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LIST OF SYMBOLS, ABBREVIATIONS

FAME - Fatty Acid Methyl Esters

TAG - Triacylglycerol

ASTM - American Society for Testing and Materials

FFA - Free Fatty Acid

UCO - Used cooking oil

NPs - Nanoparticles

CuNPs - Copper nanoparticles

EtOH - Ethanol

FTIR - Fourier Transform Infrared

RT - Room temperature

W - Weight (g)

Y% - Yield (%)

CHAPTER 1

INTRODUCTION

Energy is a key input in the economic development of any country(Rathnayaka et al., 2011). Currently, around 80% of energy demand relies on fossil fuels, with coal accounting for 70%, petroleum for 20%, and natural gas for 10%(Sadeghinezhad et al., 2013). According to a report, the world's proven coal reserves are estimated to last only 192 years, calculated through the R/P ratio (reserves to production). As the global energy demand continues to increase, attention is turning towards alternative energy sources. In addition to solar, wind, and tidal energy, bioenergy has emerged as a sustainable, renewable, and alternative source of energy. Bioenergy is the energy derived from biomass (organic matter), such as wood, agricultural crops, municipal waste, etc. It is a versatile source of energy, contributing 10% of the global energy demand. Major forms of bioenergy include biopower, biogas, and biofuels. Biofuels include renewable fuels like bioethanol, biodiesel, biogas, and advanced biofuels derived from organic and waste materials. These are the sustainable options for reducing oil dependency and increasing the energy security of a country(Demirbaş et al., 2004). Recognizing the urgent need to shift towards sustainable energy, India has taken significant steps towards a sustainable environment by implementing its National Policy on Biofuel (2018), which aims to target 20% ethanol blending (E20) and 5% biodiesel blending B5 up to 2030. FSSAI (Food Safety and Standards Authority of India) has also launched Repurpose Used Cooking Oil (RUCO) initiative to promote UCO for biodiesel Production(Mutabaruka et al., 2024).

Biodiesel is a cleaner substitute for conventional diesel. Biodiesel is a renewable, biodegradable, and non-toxic fuel produced through a transesterification reaction, using vegetable oil, animal fats, or waste cooking oil as a feedstock(Kwon & Yeom, 2015).Globally, the US is top producer of biodiesel using Soybean oil as a primary feedstock. In Europe, rapeseed and canola oil are widely used, while tree-borne jatropha oil is used in India for biodiesel production. Biodiesel has a higher flash point, a higher cetane number, low sulfur content, as compared to conventional diesel

(Vasudevan & Briggs, 2008b). Different catalysts are employed in the transesterification reaction for biodiesel production; commonly used are alkaline catalysts are sodium hydroxide (NaOH) and potassium hydroxide (KOH), as well as acidic catalysts hydrochloric acid (HCL), and sulfuric acid(H_2SO_4). These are classified as homogeneous catalysts, widely used due to their high reaction efficiency and faster kinetics. However, homogeneous catalysts have significant drawbacks, including their single-use nature, difficulty in separation from the product, low recovery rate, and saponification. To overcome this issue, heterogeneous catalysts are used, such as metal oxides, CaO, MgO, zeolites, etc. can be easily separated, reused, and often produce fewer by-products(Awogbemi et al., 2024). Recently, nanomaterials have emerged as a promising class of heterogeneous catalysts in biodiesel production due to their high surface area, tunable surface properties. Nanoparticles (e.g., CaO, TiO2, ZnO, CuNPs, etc.) offer significant advantages(Changmai et al., 2020).

CHAPTER 2

LITERATURE REVIEW

2.1. Biodiesel

Biodiesel is a renewable, biodegradable, non-toxic fuel produced from biological sources such as vegetable oil, animal oil/fat, and algae. It is a fuel consisting of monoalkyl esters of long-chain fatty acids or FAME produced from lipid sources through the transesterification process(Kalsoom et al., 2017). Biodiesel is a good alternative to conventional petroleum-based fuels like gasoline and diesel(S.Jaichandar & Annamalai, 2011). It is blended with diesel; B20 is the most common blend, 20% biodiesel mixed with 80% diesel. For a 20% blend, no change in the engine is required. Similarly, B5 blend is 5%biodiesel with 95% diesel, B100 (100% biodiesel), B15, etc(Jayashri, J Deepthi, 2013).

Biodiesel is an eco-friendly fuel and plays a significant role in climate change mitigation. Since the amount of carbon dioxide released during emission is equivalent to the amount of CO_2 that plants absorbed, it can be considered carbon-neutral. Oxygen content in biodiesel is around 11%, which lowers energy density and hence lowers particulate emissions(Sharp et al., 2005). Net sulfur content is zero, it does not release sulfur oxides during emissions, which are responsible for acid rain. By replacing diesel with biodiesel, it helps to reduce greenhouse gas (GHG) emissions, thus supporting sustainable development goals and cleaner air quality(Rony et al., 2023).

2.2. Sources For Biodiesel production

Biodiesel can be derived from a wide range of sources, including edible oils(Vegetable sources), non-edible oils, and animal fats(Bajpai & Tyagi, 2006). Edible oil contains 20-30% oil content, and it comes under first-generation feedstock. Common sources include vegetable oils such as palm, soybean, rapeseed, sunflower, mustard and coconut oil,(Xu et al., 2023). On the other hand, non-edible oil-bearing crops such as Jatropha (*Jatropha curcas*), Mahua (*Madhuca indica*), Karanja (*Pongamia pinnata*), Sal (*Shorea robusta*), Kokum (*Garcinia indica*), etc., are classified as secondgeneration feedstock(Abdul Hakim Shaah et al., 2021). In India, tree-borne oil Jatropha curcas has been promoted under the Jatropha mission. It is a drought-resistant perennial plant belonging to the Euphorbiaceae family with an oil content of around 37-40%(Dhyani et al., 2011). Additionally, microalgae like *Chlamydomonas reinhardtii* and *Chlorella Vulgaris* have high lipid content, around 21% and 14-22%, and are being explored as alternative feedstocks for the production of biodiesel(Gaurav et al., 2024). Waste cooking oil is another sustainable and economical feedstock option being utilized in various biodiesel initiatives.

2.3. Chemical Properties of biodiesel

1) Specific Gravity- Biodiesel's Specific gravity is around 0.86 to 0.90g/cc, making it slightly denser than petroleum diesel but lighter than water(Siraj et al., 2017).

2) Iodine Number- It indicates the degree of unsaturation in a fuel. A high iodine number reflects a greater presence of unsaturated fatty acids, containing one or more double bonds. These bonds are reactive and undergo oxidation, which leads to deposit formation and unstability. The maximum iodine number should be around 135; above this storage property of a fuel decreases. Biodiesel iodine number is around 115(Etim et al., 2022).

3) Cetane Number- It is a measure ignition quality of a fuel, indicating how readily it ignites upon injection into the combustion chamber. A high cetane number corresponds to a shorter ignition delay and better combustion, which is a desirable property in diesel engine fuel. Biodiesel has a cetane value of 48-55, while petroleum diesel has a cetane 46-47(Sadeghinezhad et al., 2013).

4) **Flash Point**- It is the minimum temperature at which a fuel vaporizes and ignite upon exposure to an open flame or spark. A lower flash point indicates higher flammability, while a higher flash point signifies lower flammability, offering safer storage and handling. For reference, petroleum diesel typically has a flash point around 52°C(Santanumurti et al., 2019).

5) Distillation temperature- It is a tolerable temperature range at which a fuel starts to evaporate and fully vaporize when heated. High distillation temperature means fuel is less volatile and has a slower evaporation, while lower distillation temperature means fuel is more volatile and has a faster evaporation rate. The distillation temperature range of biodiesel is around 300-350°C.

6) Flow Properties – Flow property of biodiesel includes Viscosity, Cloud point, and Pour point. Viscosity is a measure of fluid resistance to flow. Biodiesel exhibits higher viscosity than diesel. It refers to the negative property, as high viscosity means more unsaturation, resulting in poor atomization of the fuel spray, affecting engine performance. (Dunn, 2015).Cloud Point is the temperature range at which wax crystals begin to form in the fuel, giving cloudy appearance. Cloud point for biodiesel ranges from 0-15°C. Above cloud point, fuel flows normally. The Pour Point is, lowest temperature at which biodiesel remains capable of flowing. Biodiesel pour point is -3 to 12°C. Below this range, fuel stops flowing, which can lead to engine failure. (McCormick & Moriarty, 2023).

2.4. Methods for Biodiesel Production

Pyrolysis, or thermal cracking, is a process of thermally decomposing triglycerides (oil/fat) at high temperature ranging from 350-500°C, in oxygen free environment, producing biofuel, syngas, and biochar. Triglycerides at high temperature decompose into short-chain hydrocarbons (alkanes, alkenes, and aromatics), which are a key biofuel component and by-product like syngas (CO, CO_2, CH_4) and biochar (solid residue)(Chiarello et al., 2022).

Dilution method is used for small-scale production. This method involves directly diluting vegetable oil with a solvent like diesel, petroleum, or ethanol. The components are thoroughly blended to ensure homogeneity, and the mixture can be directly used as fuel in diesel engines. This method is simple, cost-effective, and does not require chemical catalysts. It significantly reduces the viscosity of vegetable oil, improving its flow property(Olusola et al., 2009).

Micro-emulsification or microemulsion is a thermodynamically stable mixture, formed by blending vegetable oil with alcohol such as methanol, ethanol, and a surfactant (butanol, hexanol, or detergents). It has lower viscosity than pure vegetable oil, and no catalyst is required, but it is not widely adopted, and may not meet all fuel standards(Attaphong et al., 2023).

Transesterification, also known as alcoholysis, is a chemical process in which triglycerides (oils/fats) react with alcohol typically methanol or ethanol in the presence of a catalyst to produce free fatty acid methyl esters (FAME) commonly known as biodiesel and glycerol as a by-product(Vasudevan & Briggs, 2008a). Industrial biodiesel is mainly produced through this reaction, using different catalysts. These catalysts can be alkali, acids, or enzymes(Buckner et al., 2016).

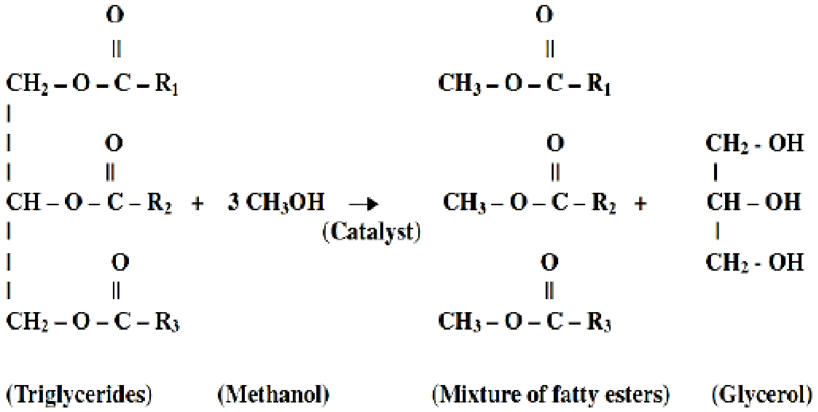


Fig.2.1. Transesterification reaction

2.5 Catalyst used in Transesterification

Homogenous Catalyst –

Base-catalyzed Transesterification reaction uses a base such as alkaline metal alkoxide and hydroxide (NaOH, KOH, CH_3ONa, sodium methoxide) as a catalyst. This process is less corrosive and offers a faster reaction rate compared to the acid-catalyzed method. However, it is highly sensitive to FFAs (saponification), which not only reduces biodiesel yield but also complicates separation and purification steps. FFA content for base-catalysed reaction should be below 1%(Chanakaewsomboon et al., 2020).

Fig.2.2. Base-catalyzed transesterification mechanism

$$\text{TAG + Alcohol} \xrightarrow{\text{KOH/ NaOH}} \text{Metal alkoxide}$$

$$\downarrow$$

(nucleophilic arrangement)

Tetrahydrate (Intermediate)

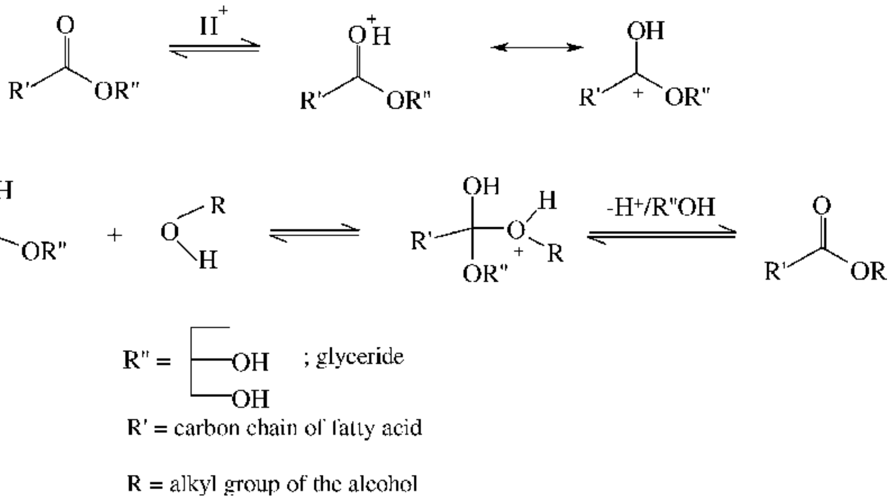
$\downarrow$ (rearrangement)

Diglyceride + 1 Monoalkyl ester

$\downarrow$

2 monoalkyl ester + glycerol

Acid-catalyzed transesterification reaction uses Bronsted acid, like BF_3, HCl, H_3PO_4, H_2SO_4, etc, as a catalyst. It is insensitive to FFAs (>2% use acid-transesterification). Reaction rate is 4000 times slower, and lower yield, and need high oil to alcohol molar ratio(Lotero et al., 2005). Fig.2.3. Acid-transesterification mechanism



The acid-base catalyzed transesterification process is typically employed for oils with high FFA. In the first step, an acid catalyst (such as sulfuric acid) in combination with methanol to esterify high FFA in oil, reducing FFA below 1-2%. Once FFA is reduced, a base catalyst is added to carry out standard transesterification.

$$\text{Triglyceride} + RCOOH \xrightarrow[H_2SO_4]{R_1OH} \text{Triglyceride} + RCOOR_1 + H_2O$$

$$\text{Triglyceride} + RCOOR_1 \xrightarrow[NaOH]{R_1OH} RCOOR_1 + \text{glycerol}$$

Fig2.4. Acid-Base Transesterification

2.6 Nanoparticles as a catalyst

Nanoparticles have emerged as advanced heterogeneous catalysts in biodiesel production, owing to their unique physicochemical properties, such as high surface area-to-volume ratio, tunable surface properties, and enhanced thermal and chemical stability, enhancing the transesterification reaction(Pan et al., 2020). Types of nanoparticles used in biodiesel production are metal oxide nanoparticles (MgO, CaO, ZnO, TiO2, ZrO2)(Awogbemi et al., 2024), magnetic NPs (Fe3O4, NiFe2O4), metal nanoparticles (Cu, Ni, Co), supported nanoparticles on porous materials (SiO2, Al2O3, Zeolites), etc(Dossin et al., 2006)(Yadav et al., 2022). Using nanoparticles as a catalyst, it reduces soap formation, making them suitable even for feedstock having high free fatty acid, and can be reused, with easy recovery, especially with magnetic nanoparticles. Using biogenic nanoparticles makes the approach even more eco-friendly and sustainable. Compared to other metal nanoparticles, CuNPs are relatively cost-effective and easier to synthesize. Copper can exist in multiple oxidation states, Cu^{0}, $Cu^{+,}$ and $Cu^{2+,}$ facilitating electron transfer during esterification steps, and further, it can also be reused after a reaction(Wang et al., 2022).

2.7. Synthesis of Nanoparticles

Nanoparticles are materials with dimensions typically ranging from 1-100 nanometres. They can be synthesized using two primary strategies: the top-down approach and the bottom-up approach. In the top-down approach, bulk materials are broken down into nanosized particles through physical and mechanical methods(Arole & Munde, 2014). Common techniques include lithography, laser ablation, Ball Milling, Etching, etc. In contrast, the bottom-up approach, involves self-assembly of atoms or molecules through chemical and biological processes. Techniques used in the bottom-up approach include chemical vapor deposition (CVD), Solvothermal and hydrothermal methods, sol-gel method, green synthesis, etc. Together top-down and bottom-up approach refers to the overall strategy to synthesize nanoparticles(Abid et al., 2021).

Nanoparticles are synthesized using physical, chemical, and biological methods(Kamal et al., n.d.). Physical methods typically involve mechanical, thermal, or electrical processes to produce NPs. Physical methods require high energy input and high operational costs, as advanced machinery is required. The chemical method involves chemical reactions to synthesize NP; however, in the chemical method, certain toxic chemicals, which are generally reducing agents (Sodium borohydride, Hydrazine, etc.), are used during the synthesis process. The chemical method is non-eco-friendly, along with waste disposal challenges(Abid et al., 2021). Both of these methods involve challenges, making them less sustainable and cost-effective. As a sustainable alternative to the conventional method of synthesis, biological synthesis is considered an ideal approach due to its cost-effectiveness, environmental friendliness, and non-toxic nature. The biological method is often referred to as the "Green Synthesis of Nanoparticles". Biogenic synthesis utilizes biological entities, bacteria, actinomycetes, fungi, yeast, and plants to produce nanoparticles. Plants are advantageous in nanoparticle synthesis as it contains natural phytochemicals including flavonoids, phenolic acids, tannins, terpenoids, alkaloids, and other compounds which act as both reducing and capping agents for nanoparticle synthesis. Plant-mediated green synthesis has been successfully employed in the synthesis of various nanoparticles, such as cobalt, copper, silver, gold, palladium, zinc oxide, magnetite, and many more, making it a promising approach for environmentally sustainable nanotechnology.(Pantidos, 2014).

2.8. Factors Affecting Transesterification

1) Oil composition and free fatty acid (FFA) – Edible oil contains free fatty acid, less than 1%, and is called high-grade oil. It does not contain toxic components. Non-edible oils typically contain high free fatty acid content, e.g., crude mahua oil has 20% FFA, and tobacco seed oil has 17% FFA, and are low-grade oils. Based on FFA content, different types of catalysts are loaded.

2) Alcohol to triglyceride molar ratio is generally (3:1), where three moles of alcohol react with one mole of TAG to produce three moles of fatty acid methyl esters (FAME) and glycerol(Jindal et al., 1960). In practice, (6:1), (9:1) ratios are used for base-catalyzed reactions. For acid-catalyzed reactions, this ratio can go higher. Beyond the optimal ratio, yield does not increase.

3) Reaction time- At the start of the reaction, the rate is relatively slow due to alcohol and oil mixing. In general reaction completes within 90 minutes, depending on the catalyst concentration, temperature, and mixing efficiency. Beyond the optimal range, the yield of biodiesel becomes constant, indicating completion of the forward reaction. Extended reaction time may lead to the start of the backward reaction.

4) Reaction temperature is a critical parameter that significantly affects reaction rate. Higher reaction temperature means faster reaction. The optimal temperature for a reaction is 60°C(Mathiyazhagan & Ganapathi, 2011).

CHAPTER 3

MATERIALS REQUIRED AND METHODOLOGY

3.1. Materials required:

For copper nanoparticle synthesis: Copper sulfate pentahydrate, Sodium hydroxide, ascorbic acid, moringa leaves, banana peel, distilled water.

Biodiesel production- Feedstock (Oil source), Mustard oil and soybean oil, methanol, CuNPs, distilled water, KOH

Instruments/Equipment- Magnetic Stirrer, Centrifuge, FTIR, Separating funnel, hot air oven etc.

3.2. Experimental Procedure

3.2.1. Sample extract preparation For the Green Synthesis of CuNPs

Sample collection: For biogenic synthesis of copper nanoparticles, moringa (*Moringa oleifera*) leaves were collected from DTU, and banana (*Musa acuminata*) peel were collected from food waste. Samples were carefully separated, washed to remove dirt and contaminants, and then dried in a hot air oven at 70 to 80°C. Dehydrated peels and leaves were finely ground using a mortar and pestle to obtain fine powder and preserved for subsequent use.

Fig.3.1. Banana peel

(*Musa acuminata*)

Fig.3.2. Moringa leaves

(Moringa Oleifera)

Fig.3.3. Banana peel powder

Fig.3.4. Moringa leaves powder

Preparation of the extract from the sample: Powder from each sample was added to 100 ml of distilled water and heated with continuous stirring at 60°C for approximately one hour. After thorough mixing, the solution was filtered using a Whatman No.1 filter paper to obtain an aqueous extract. The resulting extract was stored at 4°C until further use in the green synthesis of nanoparticles(Mali et al., 2020).

Fig.3.5. Banana extract preparation

Fig.3.6. Moringa extract preparation

Fig.3.7. Moringa extract (using filter paper)

3.2.2. Preliminary phytochemical screening: Phytochemical analysis was conducted to identify the presence of key bioactive compounds such as flavonoids, saponins, tannins, terpenoids, polyphenols, and glycosides in the extract of Moringa and banana. These compounds are known to facilitate the reduction of Cu^{+2} ions and aids stabilization of nanoparticles. Phytochemical test were conducted following the methods described in Shaikh and Patil (2020) paper as a reference(Shaikh & Patil, 2020).

Test for phenolic compounds: A ferric chloride assay was used to detect phenolic compounds. In this test, 2 mL of aqueous extract solution was mixed with 1-2 drops of 5% Ferric chloride solution. The appearance of dark bluish-black or /green color indicated the presence of phenolic compounds(Khoddami et al., 2013).

Test for Flavonoids: An alkaline reagent assay was performed by adding a few drops of 10% sodium hydroxide (NaOH) to 2 ml of aqueous extract. The solution turned intense yellow, which became colorless upon addition of dilute hydrochloric acid, (HCL)confirming flavonoids(Khoddami et al., 2013).

Test for Alkaloids: (Wagner's test) 2 ml of extract were treated with a few drops of Wagner's reagent. The formation of a brown or reddish precipitate indicated the presence of alkaloids(Shaikh & Patil, 2020).

Test for saponins: The froth test involved diluting 2 mL of aqueous extract with 4 mL of distilled water, followed by vigorous shaking to produce froth. A few drops of oil were added, aggressively agitated, and inspected for emulsion formation. The persistence of stable froth confirmed the presence of saponins(Shaikh & Patil, 2020).

Test for Tannins: The ferric chloride test was performed using 2 ml of aqueous extract mixed with 2 ml of distilled water and a few drops of 1 % ferric chloride. A brownish green or blue-black color indicates tannins(Shaikh & Patil, 2020).

Test for sterols: One millilitre of concentrated H2SO4 was added to each extract. The presence of red color (in the lower layer) indicates the presence of sterols(Shaikh & Patil, 2020).

Test for Coumarins: To 2 ml of aqueous extract, 3ml of 10%NaOH was added. The formation of yellow color indicates the presence of coumarins(Shaikh & Patil, 2020).

Test for Terpenoids: 3 mL of the sample's aqueous extracts were combined with 2 mL of chloroform and then with 2 mL of concentrated H2SO4. The occurrence of a reddish-brown colour at surface interaction revealed an abundance of terpenoids(Shaikh & Patil, 2020).

3.2.3. Biogenic Synthesis of Copper Nanoparticles (Cu NPs)

A stock solution of 0.1 M copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) was prepared by dissolving 2.49g of $CuSO_4.5H_2O$ in 100 mL of distilled water, resulting in formation of deep blue solution(Canassa et al., 2018). For the green synthesis of copper nanoparticles (CuNPs), extracts of moringa and banana peel were employed as reducing and stabilizing agents. The Copper sulfate solution was added dropwise to the plant extract under continuous stirring at 60°C, while maintaining appropriate volumetric ratios to ensure nanoparticle formation. For banana peel extract 2:1 ratio (extract: Cu solution), which means for 15 ml of extract, 7.5 ml of solution was added, whereas for moringa 1:2 (extract: Cu solution) ratio was used. After continuous stirring for about 30 to 40 minutes color change was observed from greenish brown to dark green, which serves as a preliminary indicator of nanoparticle formation. The resulting solution was allowed to rest overnight at room temperature. The mixture was centrifuged at 8000 rpm for 15 minutes at 10°C to pellet out synthesized nanoparticles. The Supernatant was discarded and washed with distilled water, followed by centrifugation at 8000 rpm for 15 minutes at 10°C. The washing step was repeated 2 to 3 times to remove impurities. The final pellet obtained was then dried at 80°C in a hot air oven, and dried CuNPs were then collected, stored, and used for further characterization(Mali et al., 2020).

Fig.3.8. CuNPs synthesis- dark blue color

Moringa and banana solution

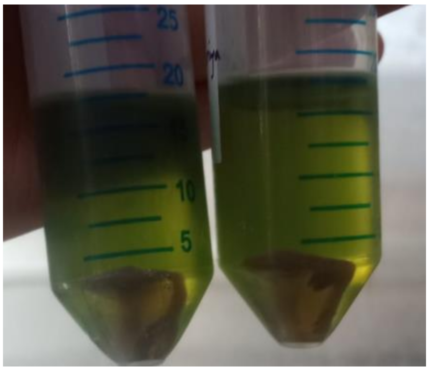


Fig.3.9. Biogenic CuNPs

3.2.4. Synthesis of CuNPs by chemical method

Copper nanoparticles were synthesized via a chemical reduction method, using $CuSO_4$. H_2O as a precursor salt, and starch as a capping agent. A 0.1M solution of $CuSO_4$. H_2O was added dropwise to 120 mL of 1.2 % starch solution, followed by continuous stirring for 30 minutes to ensure uniform dispersion. Subsequently, 50 mL of 0.2 M ascorbic acid-acting as the reducing agent, was added gradually to the mixture with continuous stirring. Thereafter, 30 mL of 1M NaOH solution was introduced dropwise to the mixture with constant stirring and heating at 80 °C for 2 hours. A visible color change from yellow to ochre indicated the successful formation of copper nanoparticles.

After the completion of the reaction, the solution was removed from the heat and left overnight. The supernatant was carefully decanted and washed with distilled water twice, centrifuged at 9000 rpm for 30 min. at RT. At last, the pellet was washed with ethanol. The final ochre-colored copper nanoparticles precipitate was air-dried at room temperature overnight. NPs were then stored in a clean, labelled glass vial for subsequent characterization(Khan et al., 2016).

Fig.3.10. CuSO4.5H2O solution Starch solution Ascorbic acid

Fig.3.11. Visible color change to ochre

Fig.3.12 CuNPs formation

3.2.5. Characterization of Copper Nanoparticles:

After synthesis, the characterization of nanoparticles is the next crucial step that provides essential information about their shape, size, surface area, chemical composition, and dispersity.

FTIR (Fourier transform infrared):

Molecular composition, functional groups, and chemical bonds can be characterized using Fourier Transform Infrared (FTIR)Spectroscopy. When infrared radiation is absorbed by a sample, it causes vibrational changes in the molecular bonds of the sample. FTIR measures these vibrations and converts the resulting data into an infrared spectrum, which provides information about the molecular composition and functional groups. The samples are scanned using infrared light with a wavelength range of 4000 to 400 cm-1.

3.2.6. Determination of Free Fatty Acids

The free fatty acid content of the oil was determined using the acid-base titration method. For this procedure, 2grams of the oil sample was dissolved in 25 mL of ethanol, and gently heated to ensure complete solubilization. Then, 1-2 drops of phenolphthalein indicator were added to the solution. A 0.1 N KOH solution was prepared(50ml) and added to the burette. Titration was carried out by slowly adding KOH solution to the mixture while stirring continuously. The endpoint was the appearance of pink color that should remain for at least 10 seconds(Khan et al., 2013).

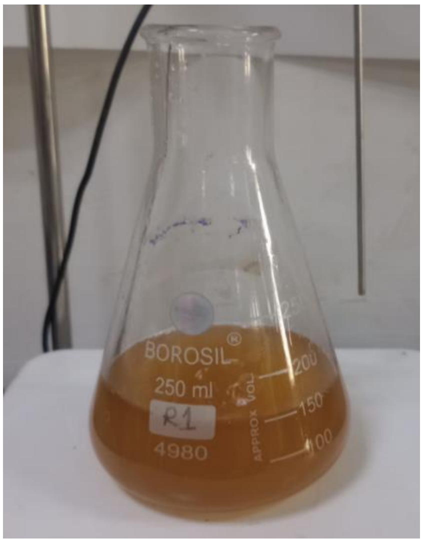
$$\text{FFA (as \% oleic acid)} = \frac{\text{Titre value X Normality of KOH X 56.1}}{\text{Weight of sample (g)}}$$

Fig.3.13. Faint pink color (titration)

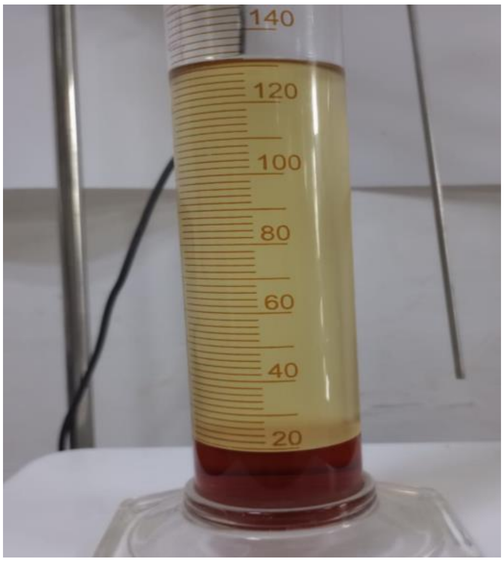
3.2.7. Biodiesel Production:

For biodiesel production, two different oil samples: soybean oil and mustard oil were utilized. Initially Oil sample were heated at 60°C for 15-20 minutes to eliminate moisture content. The transesterification reaction was performed using varied methanol to oil molar ratios l, 4:1, 6:1, 9:1, with 0.71% w/w KOH serving as a catalyst. For 6:1 methanol to oil molar ratio, 100 g of oil was preheated. Separately, 25.4 ml of methanol was mixed with 0.71% w/w KOH to form a methoxide solution, which was then added dropwise into the heated oil under constant stirring at 60°C for 1 hour. After the completion reaction, the product was allowed to settle at room temperature overnight to separate the biodiesel and glycerol layers.

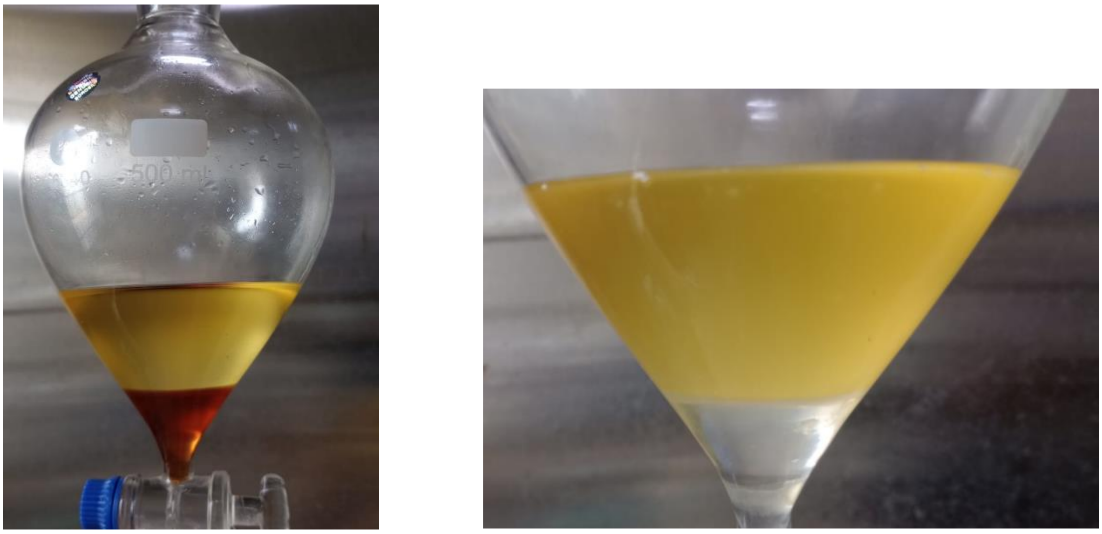
After settling, two distinct layers are formed: upper layer (biodiesel), and lower layer, (glycerol). These layers are carefully separated using a separating funnel, and biodiesel is washed 2-3 times with warm distilled water to remove residual methanol, catalyst, and soap impurities. After washing, the drying step is done in which biodiesel is heated and left uncovered to evaporate moisture. Biodiesel should appear bright and clear(Sajjad et al., 2022).



Transesterification Reaction



Biodiesel and Glycerol layer



Separation of both layers

Washing Step with warm distilled water

Fig.3.14. Steps in Biodiesel Production

From Copper Nanoparticles - The transesterification reaction was conducted using ratio of 9:1(methanol: oil). To initiate the process, 0.5% w/w CuNPs were dispersed in methanol and mixed vigorously to ensure homogeneous mixing. This methanol-CuNP solution was then added dropwise to the pre-heated oil at a temperature of 65-70°C. The reaction mixture was stirred continuously at 600- 800 rpm for a duration 2 hours. After the reaction, the mixture was allowed to settle overnight at room temperature. The biodiesel layer was carefully decanted, and CuNPs were settled in the glycerol phase. These nanoparticles can be recovered from the glycerol layer and used again but catalytic activity can decrease.

Fig.3.15. CuNPs along with a glycerol in bottom layer

CHAPTER 4

RESULT AND DISCUSSION

4.1. Preliminary Phytochemical Screening

Phytochemical screening of moringa and banana peel extract. Results of Phytochemical screening are identified in the Table.

S.No.	Bioactive/ Phytochemicals	Test/ Reagents	Banana peel (*Musa acuminata*)	Moringa leaves (*Moringa oleifera*)
1	Phenolic compounds	Ferric chloride test	+++	+++
2	Flavonoids	Alkaline reagent test	-	++
3	Alkaloids	Wagner's test	+	++
4	Saponins	Frothing test	+++	+++
5	Tannins	Ferric Chloride test	++	++
6	Sterols	-	++	+++
7	Coumarins	-	++	+
8	Terpenoids	Salkowski test	++	+++

Note: (+) least present, (++) Moderately present, (+++) Strongly present, (-) Absent

Both plant materials showed a strong presence of phenolic compounds and saponins. Moringa leaves contain high phenolic, sterols, and terpenoids content, Banana peel contains moderate tannins (++) and alkaloids (+), while moringa showed slightly higher alkaloid content. Coumarins were moderately present in banana peel (++) but were least present in moringa (+).

4.2. FTIR Analysis: For both biogenic synthesis and Chemical Synthesis of CuNPs

Fig.4.1. FTIR graph of CuNPs synthesized from Banana peel (*Musa acuminata*)

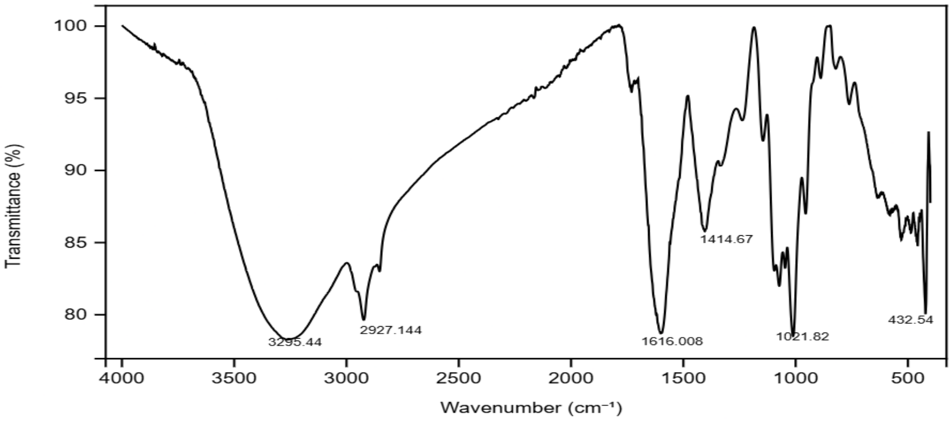


Fig.4.2. FTIR graph of CuNPs synthesized from Moringa leaves (*Moringa oleifera)*

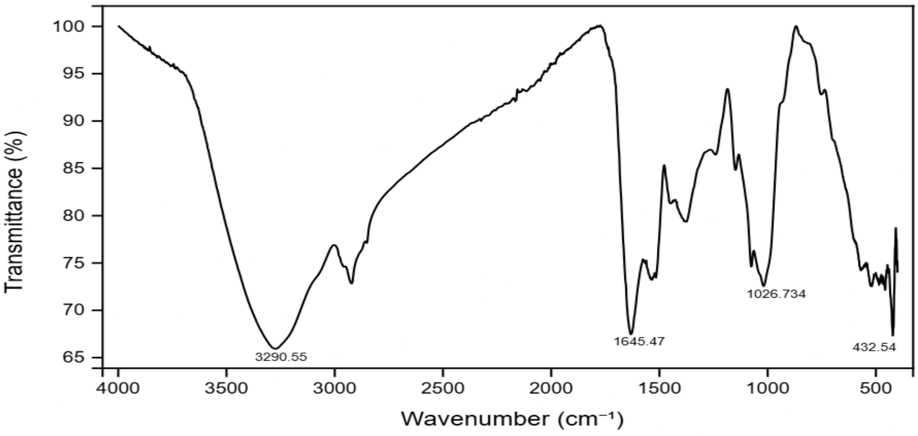
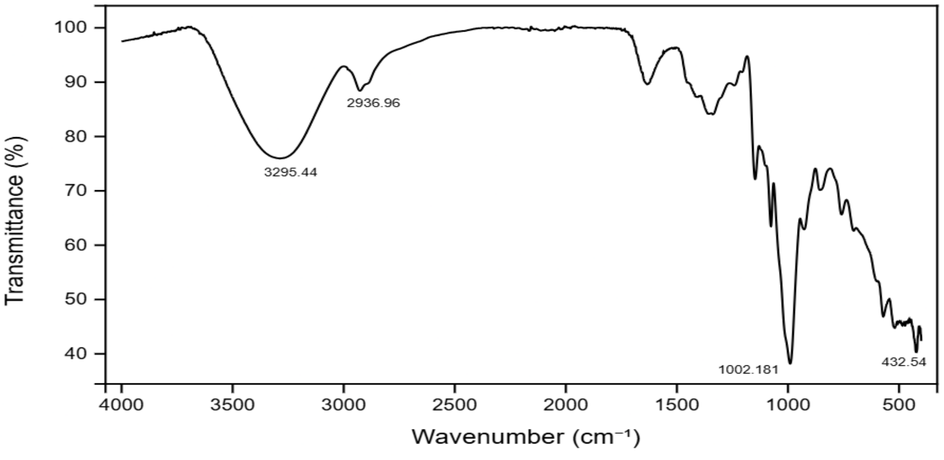


Fig.4.3. FTIR graph of CuNPs: from Chemical synthesis



Fourier Transform Infrared (FTIR) Spectroscopy was used to identify the functional groups responsible for the reduction and stabilization of CuNPs. The FTIR spectrum was recorded within the wavenumber range of 4000-400 cm^{-1}. A broad absorption band observed between 3400-3200 cm-1 is attributed to the O-H/N-H stretching vibrations, indicating the presence of hydroxyl groups, alcohols, and amines. In this study, a distinct peak was detected at 3295 cm-1, signifying the presence of hydrogen bonding interaction, possibly from polyphenolic compounds. Peaks at 2927 cm-1 and 2936 cm-1 correspond to the presence of C-H stretching vibrations of aliphatic -CH2 and -CH3 groups, typically from hydrocarbon chains. A notable absorption band in range around 1630-1650 cm-1 is associated with C=O stretching, corresponding to carbonyl groups. A band in the range of 1100–1020 cm^{-1} represents C–O or C–O–C stretching, which are commonly found in alcohols, ethers, or esters. Furthermore, band observed at 432 cm^{-1} falls below 700cm-1 corresponds to Cu–O stretching vibrations, confirming the formation of copper–oxygen bonds and thereby supporting the successful synthesis of copper nanoparticles(Mohamed, 2020).

4.3. Free Fatty Acid determination

Titre value of mustard oil was 0.1 mL, while that of soybean oil was 0.2mL. Free fatty acid was calculated using formula where M is the titre value, ml of KOH used, N is the normality of KOH, W is weight of sample used in grams.

$$\text{FFA (as \% oleic acid)} = \frac{\text{M X N X 56.1}}{\text{W (g)}}$$

FFA of mustard oil is 0.2805% while that of soybean oil is 0.561%. Both oil samples were suitable for base-catalyzed transesterification.

4.4. Biodiesel Yield-

Biodiesel (Y%) = mass of biodiesel obtained / mass of oil used

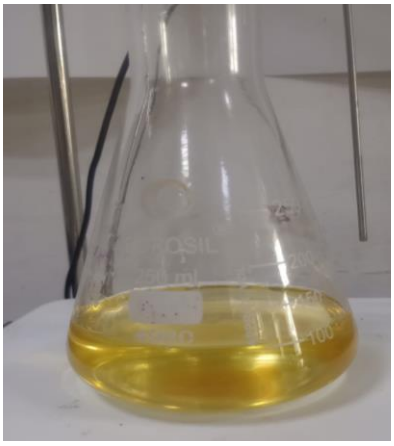
Biodiesel yield was calculated for both one-time used oil (mustard oil), and soybean oil at a constant concentration of the catalyst, 0.71% w/w KOH, and constant temperature at 60 °C. For 100 g of soybean oil (approximately 106 ml), biodiesel was obtained, around 102 ml, giving a yield of 89.76% for a ratio 6;1. Similarly, all other ratios were taken. For a mustard oil (6:1) ratio, 60 ml of mustard oil was taken, and 15 ml of methanol was used along with 0.392g KOH (0.71%), forming 58 ml biodiesel, giving 92.49% biodiesel yield.

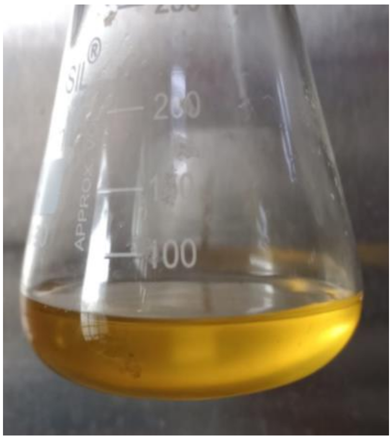
Ratio (methanol: oil)	Catalyst (KOH) (0.71% w/w)	mustard oil (0.28% FFA)	Soybean Oil (0.56% FFA)
4:1	0.71%	91.83%	88.88%
6:1	0.71%	92.49%	89.76%
9:1	0.71%	93%	90.64%

Copper nanoparticles for biodiesel yield-

20 ml of oil sample was taken in the ratio (9:1) with 0.5 g CuNPs (approximately 2.78%). Biodiesel formed was around 17 ml, and biodiesel yield% = 81.3%

The biodiesel sample appeared as a clear, light-yellow color (depending on the oil used) with no suspended particles. It has a mild, oily odor, but lacks a strong, pungent smell like petroleum diesel. During the ignition test, it took a few seconds to ignite, indicating a higher flash point. The combustion was clean, generating very little smoke and leaving no visible soot on a white ceramic surface held above the flame. These observations confirm that biodiesel burns more cleanly and safely than conventional fossil fuels.





Biodiesel formation from soybean oil.

Biodiesel formation from mustard oil

Fig4.4. Biodiesel Formation

CHAPTER 5

CONCLUSION AND FUTURE PROSPECT

The synthesis of copper nanoparticles was successfully carried out using both moringa leaf extract and banana peel extract. It was found out that moringa extract required a higher volume and concentration of copper sulfate solution than banana peel extract. It can be due to the variation in the phytochemical composition of both extracts. Banana peel extract contains a higher concentration of natural reducing sugars, and may provide better capping and stabilization of NPs even at a low metal solution ratio, while moringa extract is rich in bioactive compounds, than reducing sugars. Banana peel extract is more efficient in synthesizing CuNPs under the conditions performed in the experiment.

The study further compared the biodiesel production efficiency using mustard oil and soybean oil as feedstocks, under the same transesterification conditions. FFA content in mustard oil (0.28%) was lower compared to soybean oil (0.56%). Both samples were suitable for the alkali-catalyzed transesterification reaction. Lower FFA contents favour higher biodiesel yield; in mustard oil, biodiesel yield was around 93% in ratio (9:1), while in soybean oil, biodiesel yield was around 90% in ratio (9:1). Mustard oil is preferable for biodiesel production even at low methanol ratio. According to (ASTM D 6751), soybean oil shows 98% of biodiesel yield at a 6:1 ratio using 1% KOH as a catalyst. By increasing the catalyst ratio, or changing reaction time and temperature yield can vary. Further CuNPs were used as a catalyst in the transesterification with a yield of around 80%, suggesting as a potential heterogeneous catalyst. It shows less yield when compared to ZnO, CaO nanoparticles. CuNPs can be doped with other metals to improve catalytic efficiency. Further investigation into the reaction mechanism, kinetics, and thermodynamic behaviour of CuNPs can help in transesterification.

Algae oil has emerged as a promising and sustainable feedstock for biodiesel production. Algae can be used as a potential feedstock because of their high lipid content (some algae strains can contain up to 60% lipid), high oil yield, rapid growth, utilize wastewater and CO2, and can be grown in any conditions(Medipally et al., 2015). It does not compete with food crops, such as soybean oil, mustard oil, etc, making it a sustainable alternative. However, several challenges remain, including complexities involved in scale-up and commercialization, limited policy and incentives, difficulties in Strain selection and optimization, and high production costs associated with harvesting and oil extraction(Gaurav et al., 2024).

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LIST OF PUBLICATIONS

Presented a paper entitled “Toward Smart PGPR Systems for Millets: Bioinoculant Technology for Climate-Smart Agriculture” at the International Conference on Renewable, Environment and Agriculture(ICREA) held in Ajmer, India on 01 June, 2025.

The conference paper is accepted for further proceedings.

Paper ID: SA-CREA-AJMR-010625-7707

SA-CREA-AJMR-010625-7707

SARC
South Asian Research Center

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Renewable, Environment and Agriculture

CERTIFICATE

of

PRESENTATION

Presented to

Megha Shah

for presenting a paper entitled “Toward Smart PGPR Systems for Millets: Bioinoculant Technology for Climate-Smart Agriculture” at the International Conference on Renewable, Environment and Agriculture(ICREA) held in Ajmer, India on 01st June, 2025.

Conference Coordinator
South Asian Research Center

Managing Director
South Asian Research Center

Presented Poster Presentation on topic “Bio-inspired Nanomaterials for CO_2 Capture and Energy Storage”

Held at Rajdhani College, University of Delhi

International Conference

on

“Advances in Nanomaterials and Nanotechnology”

(ICANN – 2025)

Organized by

Department of Physiscs, Rajdhani College, University of Delhi, New Delhi

Sponsored by DRDO, Delhi

Sr. no.

ePP119

Certificate

This is to certify that Prof./Dr./Mr./Ms. **Megha Shah** from **Delhi Technological University** presented a paper titled **Bio-Inspired Nanomaterials for CO2 Capture and Energy Storage** in the International Conference held at Rajdhani College, University of Delhi, New Delhi, from 05 to 06 March 2025.

Prof. Rajesh Giri

Principal, Rajdhani College

Dr. Jasvir

Convenor, ICANN 2025

DELHI TECHNOLOGICAL UNIVERSITY
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PLAGIARISM VERIFICATION

Title of the Thesis: "Biodiesel production via alkali-catalyzed transesterification of mustard and soybean oil: Efficiency analysis of CuNPs as catalysts."

Total Pages: 44 Name of the Scholar: Megha Shah (23/MSCBIO/29).

Supervisor

Prof. Jai Gopal Sharma

Department of Biotechnology

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10% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Quoted Text
- Cited Text
- Small Matches (less than 10 words)

Match Groups

43 Not Cited or Quoted 10%
Matches with neither in-text citation nor quotation marks

0 Missing Quotations 0%
Matches that are still very similar to source material

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Matches that have quotation marks, but no in-text citation

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Matches with in-text citation present, but no quotation marks

Top Sources

6%	Internet sources
6%	Publications
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Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

0% detected as AI

The percentage indicates the combined amount of likely AI-generated text as well as likely AI-generated text that was also likely AI-paraphrased.

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Detection Groups

0 AI-generated only 0%
Likely AI-generated text from a large-language model.

0 AI-generated text that was AI-paraphrased 0%
Likely AI-generated text that was likely revised using an AI-paraphrase tool or word spinner.

Megha Shah

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ACADEMIC DETAILS

Year	**Degree/Exam**	**Institute**	**CGPA/Marks(%)**
2023-present	M.Sc Biotechnology	Delhi Technological University	9.04
2018 - 2021	B.Sc.(Hons) Biotechnology	Graphic Era University Dehradun	9.35
2018	I.S.C	St. Joseph's Convent School	8.3
2016	I.C.S.E	St. Joseph's Convent School	8.6

INTERNSHIP

- **Isolation and Identification of Bacterial Strain from Cheese Sample** *Feb 2021 (45 days)*
  Host Organization: Orbit Biotech
  Description: Completed Research Project from Orbit Biotech, Chandigarh on Isolation and Identification of Bacteria Strain from Cheese Sample. Learned **Wet Lab** Techniques, **Bacterial Culture and Isolation** (e.g-spreading, Streaking etc), **Biochemical Test**, **DNA Extraction** and **Gel electrophoresis**
  Instruments Used: **Autoclave**, **Centrifuge**, **Spectrophotometer**.
  Certificate Link: Orbit Biotech Certificate

CERTIFICATES

- **International Conference on Advances in Nanomaterials and Nanotechnology (ICANN) :**
  Presented poster presentation titled Bio-Inspired Nanomaterials for CO2 capture and Energy Storage held at Rajdhani College,University of Delhi ,New Delhi.

- **International Conference on Renewable, Envionment and Agriculture :**
  Presented a paper entitled " Toward Smart **PGPR** Systems for Millets:Bioinoculant Technology for Climate-Smart Agriculture held in Ajmer.

- **Molecular Biology and Biochemistry Techniques :**
  Completed offline workshop on Bio Informatics Techniques. Learned tools like **BLAST** for sequence alignment, **UniProt** for Gene/Protein annotation, and **MEGA** for Phylogenetic Analysis.
  Tools Used : BLAST, UniProt, MEGA.
  Certificate Link: Molecular Biology and Biochemistry Techniques Certificate

- **International Conference on Medicinal, Aromatic and Nutraceutical plants from the mountainous area :**
  Attended International Conference on Medicinal, Aromatic and Nutraceutical plants from Mountainous Areas organised by department of life science (Graphic Era, Dehradun) and ACMAP (USA).
  Certificate Link: Conference Certificate

- **Flagship Program (MoE) UNNAT BHARAT ABHIYAN(UBA) :**
  Participated in two days activity of painting and cleaning of Primary school at Bhullawala.Organized by Department of Life Sciences and Rural Development Group under UNNAT BHARAT ABHIYAN
  Certificate Link: Unnat Bharat Abhiyan Certificate

TECHNIQUES AND INSTRUMENTATION

- **Molecular Biology Techniques:** DNA Extraction, Gel Electrophoresis and PCR.
- **Microbiology Techniques:** Bacterial Culture (Streaking, Spreading) and Biochemical Tests.
- **Instrumentation:** Autoclave, Centrifuge and Spectrophotometer.
- **Bioinformatics Tools:** BLAST (Sequence Alignment), UniProt (Gene/Protein Annotation) and MEGA (Phylogenetic Analysis).