



PRODUCTION OF BIOPLASTIC FROM AZADIRACHTA INDICA, RICE STARCH AND INVESTIGATION OF IT'S BIODEGRADABILITY

B. Pavatharani	II, M. Sc., Microbiology, Department of Microbiology, Tiruppur Kumaran College for Women, Tirupur- 641687
Dr. S. Rashitha Parveen	Assistant professor, Department of Microbiology, Tiruppur Kumaran College for Women, Tirupur- 641687
K. Sneha	II M. Sc., Microbiology, Department of Microbiology, Tiruppur Kumaran College for Women, Tirupur- 641687

ABSTRACT Plastics are highly consumed materials made from fossil resources that cause environmental problems such as prolonged permanence in landfills, greenhouse gas emissions, etc. In recent years, environmental awareness has risen among people and governments, leading to the development of biodegradable plastic materials from renewable resources such as microbes and agricultural residues. In this study, we aimed to assess the possibilities for producing eco-friendly bioplastics from leaf extracts of *Azadirachta indica* along with some degradable blending materials. In addition to its performance and abundant sources, starch from rice extract is the solution for an alternative. Starch, as a biodegradable polymer, becomes a reasonable material for the production of bioplastics because of its low cost. Bioplastic was prepared with *Azadirachta indica*, and its sustainability and retention time were analyzed. Added to that, qualitative biodegradability tests, soil burial tests and various physicochemical parameters have been studied.

KEYWORDS : *Azadirachta indica*, Rice starch, Eco-friendly, Bioplastic, Biodegradable

1. INTRODUCTION

Plastics have been nominated the most generally used materials at the global position. The disposal of plastics is one of the least honored and most largely problematic areas and creates plastic's ecological impact. Replacing petroleum- grounded plastic with bioplastic is a accessible idea as it's eco-friendly to the terrain and can be attained at a low cost. Bioplastics are plastics that are made from a renewable resource and are suitable to break down naturally. Biodegradable plastics allow enhanced end- of- life scripts for disposal and recycling. The bioplastics are biodegradable due to the presence of bacterial organisms in the soil. The development of bioplastics has been given considerable attention as an eco-friendly and biodegradable volition to hydrocarbon- grounded plastics. During the natural degradation process, the are converted into natural composites similar as water, CO₂, and compost. The circumstance of declination is characterized by an increase in percent weight loss or a drop in the residual weight of bioplastic.

The agrarian product of *Azadirachta indica* leaves for the production of bioplastics could help in reducing the environmental damages that are caused by petroleum or hydrocarbon grounded conventional plastics. Hence, the increased quality and value of Neem bioplastics can be attained by perfecting their parcels with the most abundant and biodegradable materials.

2. MATERIALS AND METHODS

2.1. Collection Of Plant Material

The leaves of *Azadirachta indica* were collected from Rakkiyapalayam town, Tirupur, Tamil Nadu, and India. The analytical grade chemicals were used for this study, and the chemicals were procured from Hi-Media, Chemical Pvt. Ltd., India.

2.2. Development Of Bioplastic Preparation

2.2.1. Neem Based Bioplastic

To the 20 ml of Neem extract, 0.75g of glucose, 1.125g of gelatin, 0.565g of agarose and 1.8 ml of glycerol were added and kept on the magnetic stirrer at 60°C for 2 hours. [11]

2.2.2. Rice Starch Bioplastic

Rice was boiled for 30 minutes in water. The rice was strained, and the starchy supernatant was separated. The starchy supernatant was then heated on a hot plate at 220°C with frequent stirring until its volume was reduced by 50%. To 10 grams of this rice starch powder, 3 ml of 5% acetic acid and 0.8 ml of glycerol were added. It acted as a plasticizer. The bioplastics, which were produced without plasticizer, were used as a control [10]

2.2.3. Casting For Bioplastic

The neem based and rice starch -based blend preparation was then cast on a clean, dry butter paper and left to dry for 48 hours. It was then peeled.

2.3. Isolation And Identification

2.3.1. Collection Of Soil Sample

Soil samples were collected from dry lands, without stone or hard materials, and the soil was used for further study.

2.3.2. Isolation Of Bacteria From Soil

A stock solution was prepared by adding 1 g of the soil sample to 9 ml of sterile distilled water. From the stock solution, a serial dilution was made up to 10⁻⁷ using sterile distilled water. All the dilutions were spread on nutrient agar plates and incubated at 37°C for 24 hours. The isolated colonies were selected for further processing. [7]

2.3.3. Identification Of Isolates

The isolate was identified on the basis of Gram's reaction and biochemical characteristics, and the results were identified with the help of Bergey's manual of systematic bacteriology.

2.4. Characterization Of Bioplastic

2.4.1. Qualitative Biodegradability Test

1 ml of bacterial culture was poured into a sterile Petri plate, and plates were added with 20 ml of nutrient agar media. The Petri dish was also stirred to make the mixture homogeneous, and kept for solidification. After that, samples of bioplastic (needed size), synthetic plastic(negative control), and sludge paper (positive control) were placed onto solid media surface. 100 µl of culture was spread inversely onto the bioplastic sample and both controls. Bioplastic samples and controls were incubated at 30°C for 10 days. And the results were observed. [3]

2.4.2. Fourier Transform- Infrared Analysis (ftir)

The Fourier transform infrared (FTIR) spectrophotometer is a technique used for detecting functional groups in substances. The synthesized bioplastic crude was sent to the CRL lab at Vellalar College for Women, Thindal, Erode, Tamil Nadu- 638012, for FTIR analysis. [1]

3. RESULT AND DISCUSSION

3.1. Collection Of Plant Material

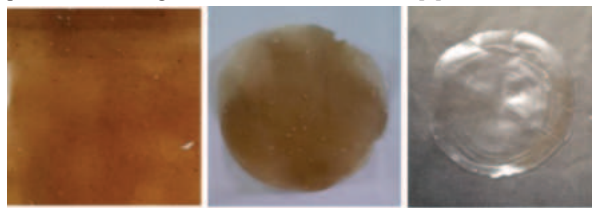
Azadirachta indica leaves were collected from the Tirupur region.

3.2. Development Of Bioplastic Preparation

3.2.3. Casting For Bioplastic

A light brownish colored extract was derived from the leaf extract of *Azadirachta indica*. The blended extract was poured into a piece of butter paper and kept for 48 hours. After 48 hours, a slime layer was developed on the butter paper, and when it was peeled, it looked like a

thin plastic layer. The results were observed as a thin bioplastic layer. This finding result was showed light brownish in color due to the presence of the huge amount of starch molecules. [2]



A. Neem extract bioplastic B. Rice starch bioplastic C. Starch bioplastic

3.3. Isolation And Identification

A total of 4 strains (*Serratia* sp., *Bacillus* sp., *Pseudomonas* sp., and *Pseudomonas* sp.) were isolated from soil in various fields. The isolated strains were identified by a biochemical test following gram staining and were identified given in table 1.

Table.1. Identification Of The Soil Isolate

S.NO	TEST PARAMETERS	RESULT			
		ISOLATE 1	ISOLATE 2	ISOLATE 3	ISOLATE 4
1	Gram staining	Negative	Positive	Negative	Negative
2	Nitrate	+	+	+	+
3	Carbohydrate fermentation				
	Glucose	Acid/Gas	Acid/Gas	Acid	Acid/Gas
4	Sucrose	Acid	Acid/Gas	Acid	Acid/Gas
5	Maltose	Acid/Gas	Acid/Gas	Acid	Acid/Gas
6	Mannitol	Acid	Acid	Acid	Acid
7	Lactose	Acid/Gas	Acid	Acid	Acid/Gas
8	Identification	<i>Serratia</i> sp.,	<i>Bacillus</i> sp.,	<i>Pseudomonas</i> sp., 1	<i>Pseudomonas</i> sp., 2

3.4. Bioplastic Characterization

3.4.1. qualitative Biodegradability Test

The results of the biodegradability test with the modified ASTM G21-70 method on nutrient agar media shows the bioplastic degradation. On day 1st, there was no bacterial growth *Serratia* sp., and *Bacillus* sp., began to grow on the 2nd day; they grew only on the nutrient agar surface. On day 5th day *Serratia* sp., and *Bacillus* sp., began covering the surface of both neem-based bioplastic and nutrient agar. Bacterial growth began to cover the bioplastic surface on the 7th day, bioplastic will be ruptured by the growth of microorganisms especially in Neem based bioplastic. After days, they degrade partially. [8]

3.4.2. Fourier Transform- Infrared Analysis (ftir)

FT-IR analysis was performed to analyze the presence of functional groups in neem-based bioplastic. Based on figure.1, FTIR spectrophotometer with a scan range of 400-4000cm⁻¹ for *Azadirachta indica*. The FT-IR spectra and their functional groups are shown in figures and tables. A peak at 3751.56 cm⁻¹ was indicated -O-H stretch due to the presence of alcohol and phenol groups. The highest peak of prepared bioplastics contains alcohol and phenolic compounds, which increase soil biodegradability as well as the mechanical and thermal properties of the bioplastic films. The FTIR spectra confirmed the presence of various bio molecules compounds. The results were obtained by[4]

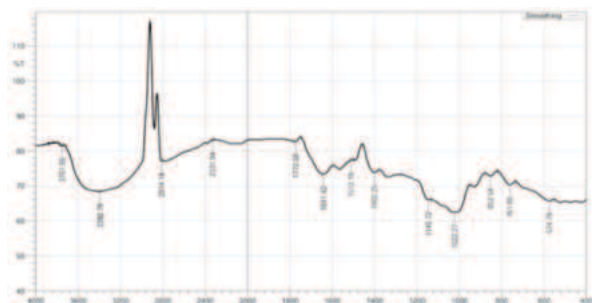


Figure.1. FTIR Analysis of Neem based bioplastic

S. NO	FREQUENCY BOND	BOND	FUNCTIONAL GROUP
1	3751.56	-O-H stretch, free hydroxyl	Alcohols, Phenol
2	3392.79	N-H stretch	Primary, secondary amines
3	2814.14	H-C=O: C-H stretch	Aldehydes
4	2331.94	O=C=O stretch	Carbon dioxide
5	1772.58	C=O stretch	Carbonyls
6	1641.42	-C=C- stretch	Alkenes
7	1512.19	N-O asymmetric stretch	Nitro compounds
8	1402.25	C-C stretch (in ring)	Aromatics
9	1145.72	C-N stretch	Aliphatic amine
10	1022.27	C-N stretch	Aliphatic amine
11	852.54	C-H "oop"	Aromatics
12	761.85	C-CC stretch	Alkyl halides
13	574.79	C-CC stretch	Alkyl halides

4. CONCLUSION

A ground-breaking invention that uses natural resources that are both renewable and biodegradable to address environmental problems and produce bioplastics at a lower cost. The demand for environmentally friendly alternatives to materials derived from natural sources has propelled this forward-thinking development. The aforementioned states that research on bioplastics will be carried out and that bioplastic based on rice starch and neem have a wide range of medical applications.

REFERENCES

- Ghozali, S. Z., Ismail, M. N., & Ahmad, N. H. (2018). Characterisation of Silver Nanoparticles using a Standardised *Catharanthus roseus* Aqueous Extract. *Malaysian Journal of Medicine and Health Sciences*, 14(102).
- Gnanavel, V., Roopan, S. M., & Rajeshkumar, S. (2019). Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. *Aquaculture*, 507, 1-6.
- Kasmuri, N., & Zait, M. S. A. (2018). Enhancement of bio-plastic using eggshells and chitosan on potato starch based. *Int. J. Eng. Technol.*, 7, 110-115.
- Maulida, Kartika, T., Harahap, M. B., & Ginting, M. H. S. (2018, February). Utilization of mango seed starch in manufacture of bioplastic reinforced with microparticle clay using glycerol as plasticizer. In *IOP Conference Series: Materials Science and Engineering* (Vol. 309, p. 012068). IOP Publishing.
- Missio, A. L., Gatto, D. A., & Tondi, G. (2019). Exploring tannin extracts: Introduction to new bio-based materials. *Revista Ciência da Madeira (Brazilian Journal of Wood Science)*, 10(1).
- Nissa, R. C., Fikriyyah, A. K., Abdullah, A. H. D., & Pudjiraharti, S. (2019, May). Preliminary study of biodegradability of starch-based bioplastics using ASTM G21-70, dip-hanging, and Soil Burial Test methods. In *IOP Conference Series: Earth and Environmental Science* (Vol. 277, No. 1, p. 012007). IOP Publishing.
- Oladunmoye, O. O., Aworh, O. C., Ade-Omowaye, B., & Elemo, G. (2017). Substitution of wheat with cassava starch: effect on dough behaviour and quality characteristics of macaroni noodles. *Nutrition & Food Science*, 47(1), 108-121.
- Oluwasina, O. O., Olaleye, F. K., Olusegun, S. J., Oluwasina, O. O., & Mohallem, N. D. (2019). Influence of oxidized starch on physicochemical, thermal properties, and atomic force micrographs of cassava starch bioplastic film. *International journal of biological macromolecules*, 135, 282-293.
- Shafiqat, A., Tahir, A., Khan, W. U., Mahmood, A. D. E. E. L., & Abbasi, G. H. (2021). Production and characterization of rice starch and corn starch based biodegradable bioplastic using various plasticizers and natural reinforcing fillers. *Cellulose Chemistry and Technology*, 55, 867-881.
- Shellikeri, A., Kaulgud, V., Yaradoddi, J., Ganachari, S., Banapurmath, N., & Shettar, A. (2018, June). Development of neem-based bioplastic for food packaging application. In *IOP Conference Series: Materials Science and Engineering* (Vol. 376, No. 1, p. 012052). IOP Publishing.

Table. 5. FTIR Functional Group