

PRODUCTION AND OPTIMIZATION OF HUMIC ACID FROM PRE-FERMENTED LIGNITE AND ITS APPLICATION ON ARACHIS HYPOGAEA, ABELMOSCHUS ESCULENTUS, AND CAPSICUM FRUTESCENS SEED GERMINATION

PROJECT WORK

Submitted in partial fulfillment of the requirement for the award of Bachelor of
Science degree in Microbiology

By,

JENIFER J (41300016)

ZULFIQAR AHAMED S (41300054)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING
SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY -1 UNIVERSITY BY UGC

Accredited with grade A++ by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI,

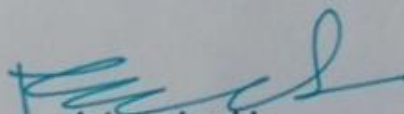
CHENNAI – 600 119

APRIL – 2024

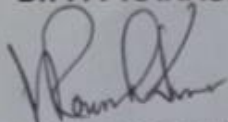
DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **JENIFER J (41300016)**, **ZULFIQAR AHAMED S (41300054)** who carried out the project entitled "PRODUCTION AND OPTIMIZATION OF HUMIC ACID FROM PRE-FERMENTED LIGNITE AND ITS APPLICATION ON ARACHIS HYPOGAEA, ABELMOSCHUS ESCULENTS AND CAPSICUM FRUTRSCENS SEED GERMINATION" under my supervision from January 2024 to April 2024.


Internal guide

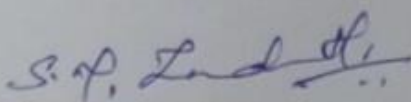
Dr. P. PRAKASH

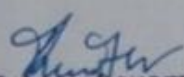


Head of the Department

DR. V. RAMESH KUMAR

Submitted for the viva voice examination held on 26.04.2024


Internal examiner

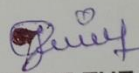

External examiner

DECLARATION

We, **JENIFER J (41300016)**, **ZULFIQAR AHAMED S (41300054)** hereby declare that the project reported entitled “**Production and optimization of humic acid from pre-fermented lignite and its application on Arachis hypogaea, Abelmoschus esculentus and Capsicum frutescens seed germination**” done by us under the guidance of **Dr. P PRAKASH** internal at sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirement for the award of bachelor of Science in **Microbiology**.

DATE: 26.04.2024

PLACE: CHENNAI



SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **Board of Management of Sathyabama Institute of Science and Technology** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey our thanks to **Dr. Rekha Chakravathi, Dean**, School of Science and Humanities and **DR. V. Ramesh Kumar M.Phil., PhD**, Head of the Department, Dept. of Biotechnology for providing us necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my project Guide **Dr. P. Prakash** (Assistant Professor) for his valuable guidance, suggestions and constant encouragement paved way for the successful completing on my project work.

I wish to express my thanks to all Teaching and Non- teaching staff members of the Department of biotechnology who were helpful in many ways for the completion of the project.

INDEX

CHAPTER NO.	TITLE	PAGE NO.
	ABSTRACT	i
	LIST OF ABBREVIATIONS	ii
	LIST OF FIGURES	iii
	LIST OF TABLES	iv
1.	INTRODUCTION	1
1.1	Humic acid	1
1.1.1	Source of humic acid	2
1.1.2	Benefits of humic acid	2
1.2	Organic fertilizer	3
1.2.1	Benefits of organic fertilizer	3
1.3	Paddy straw	4
2.	LITERATURE SURVEY	5
3.	AIM & SCOPE	8
3.1	Aim	8
3.2	Scope	8
4.	MATERIALS & METHOD	9
4.1	Materials	9
4.2	paddy straw extraction	10
4.3	Humic acid production	10
4.4	Formulation of rice straw soaked with Humic acid	10
4.5	Sample collection	11
4.6	Sample preparation	11
4.7	Placket Burman model	12

4.8	Humic acid estimation	14
4.9	Characterization studies	15
4.9.1	FTIR test	15
4.9.2	SEM test	15
5	Seed germinationn	16
5	RESULTS AND DISCUSSIONS	17
6.1	Paddy straw extraction	17
6.2	Humic acid production	18
6.3	Formulation of rice straw soaked with humic acid	18
6.4	Characterization studies	19
6.4.1	FTIR test paddy straw extract and paddy straw extract + humic acid	19
6.4.2	SEM test paddy straw extract and paddy straw extract + humic acid	23
6.5	Seed germination	27
6.5.1	Seed germination using paddy straw extract	27
	Seed germination of Arachis hypogaea seeds	28
	Seed germination of Abelmoschus esculentus seeds	31
	Seed germination of Capsicum frutescens seeds	34
6	SUMMARY AND CONCLUSION	35
7.1	Summary	36
7.2	Conclusion	36
	REFERENCE	37

ABSTRACT

The composting waste, lignite, and soil all contain the organic molecule humic acid. Humic acid plays the role of improving agricultural output and soil fertility. Both chemical and biological methods can be used to create humic acid. The purpose of this study is to produce humic acid using a semi-fermented technique and to examine its effects on seed germination. Important components were observed in the lignite and other nutrients that were subjected to the Placket Burman Model. As inoculum, the lignite was partially broken down by the extract of paddy straw. The resulting humic acid underwent seed germination. The maximum seed germination was observed 0.3% of paddy straw extract.

LIST OF ABBREVIATION

mg - milligram

kg - kilogram

% - percentage

Ug - microgram

ml - millilitre

cm - centimetre

HA - Humic acid

HS - Humic substance

NaOH - Sodium hydroxide

MgSO₄ - Magnesium sulphate

MnSO₄ - Manganese (II) sulphat

CaCl₂ - Calcium chloride

KH₂PO₄ - Monopotassium phosphate

KOH - Potassium hydroxide

R_f - retention factor

H - high concentration

L - low concentration

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO
1.1	Humic acid	1
1.2	Paddy straw	4
5.1	Paddy straw soaked in water one day	17
5.2	Paddy straw extract	17
5.3	Humic acid	18
5.4	Formulated of rice straw soaked with humic acid	18
5.5	Arachis hypogaea seeds germination	27
5.6	Day 1 seed germination Arachis hypogaea using paddy straw extract	28
5.7	Day 3 seed germination Arachis hypogaea using paddy straw extract	29
5.8	Day 5 seed germination Arachis hypogaea using paddy straw extract	30
5.9	Day 1 seed germination Abelmoschus esculents using paddy Straw extract	31
5.10	Day 3 seed germination Abelmoschus esculentus using paddy Straw extract	32
5.11	Day 5 seed germination Abelmoschus esculentus using paddy Straw extract	33

5.12	Day 1 seed germination <i>Capsicum frutescens</i> using paddy straw extract	34
5.13	Day 3 seed germination <i>Capsicum frutescens</i> using paddy straw extract	35
5.14	Day 5 seed germination <i>Capsicum frutescens</i> using paddy straw extract	36

LIST OF TABLES

FIGURE NO.	TITLE	PAGE NO
4.6	Placket Burman model	12
4.6.1	Yield of reacted paddy straw extract	13
4.6.2	Yield of unreacted paddy straw extract	13
4.7	Placket Burman design	19
5.6	Day 1 seed germination <i>Arachis hypogaea</i> using Paddy straw extract	28
5.7	Day 3 seed germination <i>Arachis hypogaea</i> using Paddy straw extract	29
5.8	Day 5 seed germination <i>Arachis hypogaea</i> using Paddy straw extract	30
5.9	Day 1 seed germination <i>Abelmoschus esculentus</i> using paddy straw extract	31
5.10	Day 3 seed germination <i>Abelmoschus esculentus</i> using paddy straw extract	32
5.11	Day 5 seed germination <i>Abelmoschus esculentus</i> using paddy straw extract	33
5.12	Day 1 seed germination <i>Capsicum frutescens</i> using paddy straw extract	34
5.13	Day 3 seed germination <i>Capsicum frutescens</i> using paddy straw extract	35
5.14	Day 5 seed germination <i>Capsicum frutescens</i> using paddy straw extract	36

CHAPTER 1

INTRODUCTION

1.1 HUMIC ACID

Humic acid, a vital component of soil organic matter, plays a crucial role in soil fertility, plant growth, and environmental sustainability. It is a complex mixture of organic molecules formed through the decomposition of organic matter in soil over time. Humic acid is characterized by its dark color, high molecular weight, and ability to chelate or bind with nutrients, making them more available to plants. Enhancing the texture and structure of soil is one of humic acid's primary uses. It improves soil aggregation, water retention, and aeration, which helps plants absorb nutrients and produce stronger roots. Humic acid also functions as a pH buffer in the soil, lessening the negative effects of acidic and alkaline conditions on plant growth. Furthermore, nitrogen, phosphorus, potassium, and micronutrients are among the vital plant nutrients that humic acid stores. Humic acid ensures that these nutrients remain available to plants throughout time by building complexes with them that shield them from leaching and immobilization. Because of its ability to store nutrients, humic acid is very useful in agricultural systems where controlling nutrient levels is essential to increasing crop yields and reducing environmental effects. All things considered, humic acid is a sustainable and natural way to boost plant growth, maintain food security in a changing environment, and improve soil health. For farmers, agronomists, and environmentalists looking to maximize agricultural productivity while reducing environmental effects,



Fig 1.1 Humic acid

1.1.1 SOURCE OF HUMIC ACID

A naturally occurring organic compound, humic acid is created when organic matter in soil breaks down over an extended period of time. Peat, lignite coal, and leonhardite all of which are high in organic matter—are popular places to find it. The main sources of humic acid are as follows: 51% to 57% C, 4% to 6% N, 0.2% to 1% P, and trace amounts of other micronutrients are all present in humic acid.

1.1.2 BENEFITS OF HUMIC ACID

- Expensive the expansion and growth of beneficial microorganisms in the soil
- stimulates and boosts the synthesis of plant enzymes
- improves nutrient uptake and stimulates root growth, particularly vertically
- increases the plants' mineral and vitamin content
- increases the seedlings' viability and germination
- promotes the growth of plants
- Boost the production of chlorophyll.

Because humic acid molecules are small, they can pass through the plant's plasma membrane, which effectively absorbs toxic heavy metals, and help release nutrients from the soil for use by the plant when needed. Many chemical and biological processes are influenced by the pH of the soil. It is a crucial factor in plant nutrition since high acidity reduces the absorption of minerals including calcium, Sulphur, phosphorus, potassium, and nitrogen, and high alkaline conditions reduce the absorption of iron, manganese, boron, copper, and zinc.

1.2. ORGANIC FERTILIZER

A wide range of plant-derived materials, including animal manure, litters, dried or fresh plant material, and agricultural byproducts, are used as organic fertilizers (Wohlfarth and Schroeder, 1979; Das and Jana, 2003; Kumar et al., 2004). Different source materials have different nutritional contents in organic fertilizers; those that degrade quickly serve as superior providers of nutrients. When compared to chemical fertilizers, the nitrogen and phosphorus concentrations of organic fertilizers is often lower, sometimes significantly lower. Another element that lowers or dilutes the concentrations of phosphorus and nitrogen in organic fertilizers is moisture content. Therefore, long-distance transportation of high-moisture organic fertilizer may not be cost-effective. However, if using locally accessible sources is in line with the production strategy, then doing so makes great sense. Compared to agricultural byproducts, animal manures have a more variable nutritional value. Manure nutrient content is dependent on a number of factors, including the animal's diet, the kind and use of bedding material, the age of the manure, and how it was kept. These characteristics might vary seasonally within farms as well as regionally or across a wider geographic area. Agricultural by-products, on the other hand, have a less changeable nutrient content, though it can still be influenced by the industrial method used to manufacture it. Nonetheless, it's always a good idea to analyze the organic fertilizer's nutrient content.

1.2.1. BENEFITS OF ORGANIC FERTILIZER

- Organic molecules increase the soil's ability to hold water and lighten its structural makeup.
- Fertilizers for organic farming use soil organic carbon, which feeds good bacteria that break down and cycle nutrients.
- The steady release of natural plant feed allows nutrients to reach the crops as they grow, resulting in a long-lasting effect.
- In contrast to synthetic fertilizers, which frequently overfeed crops and run off, natural fertilizers are consumed by plants at slower rates and seldom run off.
- Unlike synthetic fertilizers, which mostly rely on fossil fuels and contribute to environmental damage, organic fertilizers usually breathe new life into natural materials that would otherwise end up in the trash.

1.3. PADDY STRAW

In theory, rice straw can be processed and utilized in agriculture to create materials for industrial applications like silica and bio-fiber, as well as to enhance soil through carbonization and composting. Any biomass that remains in the field after grains and When rice is harvested, a byproduct known as paddy straw is created. During harvest, it is taken out along with the rice grains and, depending on whether it was harvested by hand or by machine, is either stacked up or strewn out in the field. Another important source of carbon is cropping residues, which make up roughly 40% of the biomass overall on a dry weight basis. Because they can be utilized as mulch for different crops and as feed for cattle, these were valued as precious commodities that should never be thrown away. Straw to paddy ratios vary from 0.7 to 1.4, contingent upon the variety and growth the annual production of 731 million tones, rice straw is one of the lignocellulosic waste products that is most prevalent worldwide. This quantity of rice straw has the capacity to yield the most amount of bioethanol from a single biomass feedstock—205 billion liters per year (Bohlmann, 2006). It is possible to produce biofuel feedstock economically on less ideal land without putting pressure on prime grain crop regions by choosing high- biomass yielding species that also have good fertilizer and water usage efficiency (Jakob et al., 2009). In India, the three main types of land used to grow rice are rainfed upland (16%), lowland (42%) and irrigated land (42%). In rainfed uplands, the average rice production is 0.6 MT/ha (1 MT = 1000 kg), whereas in medium land that is irrigated, it is 1.3 and 1.7 MT/ha, respectively.



Fig 1.2 Paddy straw

CHAPTER 2

LITERATURE SURVEY

Humic substances (HS) are the remains of decomposing plant and animal parts, including lignin, tannins, cellulose, and cutins (Tan et al., 2000; Billingham, 2012; Hayes and Swift, 2020). Considerable levels of HS remain in the soil even after adding harvested leftovers (Wiesler et al., 2016). The majority of arable land has seen a decline in harvested leftovers due to increasing livestock and biogas production, which has reduced soil HS levels. Over the past few decades, researchers have tried to use external applications to make up for the diminished HS (Rose et al., 2014; Gerke, 2018). The most popular commercial sources of HS are soils, coal, lignite, and organic materials (Gollenbeek and Van Der Weide, 2020; Yang et al., 2021)

Humic acids are important because they improve the structure, texture, water holding capacity (WHC), and microbial population of soil, which increases soil physical and biochemical activities (Nardi et al., 2017, 2021; Fuentes et al., 2018; Shah et al., 2018); they also increase the availability of soil nutrients, especially micronutrients, by chelating and co-transporting micronutrients to plants (Yang et al., 2021). By raising plant growth hormones like auxin and cytokinin, which support photosynthesis, nutrient metabolism, stress resistance, and Canellas et al., 2020, Nardi et al., 2021, and van Tol de Castro et al., 2021, humic acids increase crop growth. Additionally, some research has shown that HA has little impact on soil health or crop growth (Albiach et al., 2001; Bybordi and Ebrahimian, 2013).

The functions of HA are associated with their source-dependent structures, as per many research (Rupiasih, 2005; Garcíá et al., 2016; Garca et al., 2019; Nardi et al., 2021; van Tol de Castro et al., 2021). The carboxylic (COOH) and phenolic (OH) groups are the most common functional groups in the HA structure, while there are other functional groups as well (Nardi et al., 2021). For HA actions like improving soil physical and chemical properties and promoting plant development, COOH and OH functional groups are crucial (De Melo et al., 2016; Nardi et al., 2021). The hydrophilic and hydrophobic portions, respectively, are represented by the polar and non-polar ends that are formed when these functional groups separate (Mirza et al., 2011); The processes that confer desired HA characteristics involve both ends. According to Billingham (2012), the hydrophobic end is linked to repelling properties, while the hydrophilic end is linked to chelating capacities. The polar end of the anionic component forms complexes with cationic metals in the soil by electrostatic bonding when the OH and COOH groups dissolve, keeping these metals in the soil. The water-loving, hydrophilic component forms micelles that increase soil WHC. Contrarily, the non-polar end repels water molecules, reducing water penetration and boosting the stability of the clay aggregate (Billingham,

2012). Rice yield increased as a result of improved N absorption and soluble sugars caused by the aromatic and aliphatic functional groups of HA. In a prior study, García et al. (2016) discovered that rice seedling roots grew more quickly when humic compounds with aliphatic and aromatic functional groups were present.

The source of the HS determines how HA affects crops and soils (Rose et al., 2014; Gollenbeek & Van Der Weide, 2020). Numerous factors, such as the nutritional makeup, manufacturing process, functional group composition, and intended usage, influence the type of HA that is employed. The five primary HA sources that were investigated for their impact on crop agronomic parameters were compost from manure, compost from green waste, soil, brown coal, and peat (reviewed by Rose et al., 2014).

According to Martinez Balmori et al. (2014), the bioactivity potential of HA that is separated from different organic sources differs. Research has demonstrated that HA synthesised from waste materials is more effective than HA produced commercially (Arancon et al., 2006). Additionally, Jindo et al. (2020) found that HA derived from composted materials was successful in increasing the agronomic and physiological activity of plants. When Khan et al. (2018) used HA derived from coal and plants, they did not find any significant differences in wheat output between treatments. The chemical and nutritional compositions of different sources of HA can influence how efficiently they function in the soil. Different functional group concentrations were found in HA generated from Hill soil, Su Wane River, peat, leonardite, and Elliot soil by Garca et al. (2019).

In a pot experiment, Hamad and Tantawy (2018) used three different sources of HA: compost, podrite, and clayey soil. They discovered a relationship between the growth of the sorghum root and shoots and the quantity of aromatic, aliphatic, and carboxyl functional groups present in the different HA sources. Moreover, a positive correlation was found between high concentrations of the HA carboxylic functional group and N intake (Piccolo et al., 1992; Nardi et al., 2000). However, HA sources with rich aromatic functional groups demonstrated a negative link with this ability when several HA sources (such as lignite, soil, compost, coal, and peat) were examined for their capacity to kill fungus (Wei et al., 2018).

HS are categorized as fulvic acids (FA), humins, and humic acids (HA) according to how soluble they are in aqueous, acidic, and alkaline solutions (De Melo et al., 2016). Because the HA and FA fractions can boost soil fertility and health quickly and the humin percentage in HS doesn't degrade, researchers have focused on them. The HA and FA fractions of HS have beneficial functions in soils and plants due to their chemical reactivity and resistance to microbial activities (Billingham, 2012). Because of its long-term stability against degradation and amphiphilic characteristics, HA can form complex cations (Wood, 1996). About 60% of the HA portion is organic carbon (C), which is necessary for soil microbial growth (Sible and others, 2021). HA also contains Sulphur (S), hydrogen (H), oxygen (O), and nitrogen (N), aside from C.

This implies that selecting HA sources must to be done with a clear objective in mind. Barley plants with significantly increased N and P concentrations were the outcome of a pot experiment conducted by Laskosky et al. (2020) using three different HA-containing sources with different chemical properties: humiliate, peat, and biochar. More research is required because there aren't enough studies that evaluate and compare the effects of different HA sources on crop agronomic metrics in both lab and field settings.

According to Ferrara et al. (2008), humic acid is the primary constituent of humic substances and is regarded as a crucial component of soil. Humic acid's dark color enhances its ability to absorb sunlight and its thermal characteristics, which is beneficial in the early stages of the growing season.

Humic substances may modify the chemical compounds found in plant cells, including those involved in the Krebs cycle, nitrate and phosphorus metabolism, glycolysis, and photosynthesis (Roomi et al., 2018). They also influence the expression of genes.

Humic acid has been shown to enhance *Brassica campestris* L. growth, oil content, and yield in low-fertile alkaline-calcareous soils. Research indicates that applying humic acid to peanut plants has enhanced their yield each year, with the biggest yield occurring in 2018. The number, weight, and pot size of the peanut plants have all grown. It also enhanced the amount of protein and fat in the mixture. The availability of micronutrients has risen due to humic materials, particularly for iron and zinc. By storing more nitrogen in the soil, the use of humic acid in crops increases soil exchangeable NH_4^+ and accessible NO_3^- and improves the usage of urea nitrogen (Yusuff et al 2009).

The production of ornamental crops has grown due to humic acid's increased capacity for photosynthetic activity and nutrient absorption. These days, humic compounds can be artificially produced, primarily through the alkaline extraction of leonardite. Humate is used as an animal nutritional supplement when the soil humus percentage falls below 2%, which prevents crops from receiving the necessary quantity of humic acid. They are present in both fulvic and humic acid. Humic acid functions as a dilator to improve the permeability of the cell wall while fulvic acid acts as a chelator to transport the mineral. Increased permeability makes it easier for minerals to get from blood to bone and cells. (BAG. de Melo et al., 2016).

CHAPTER 3

AIM & OBJECTIVES

3.1. AIM

- Production and optimization of humic acid from pre-fermented lignite and its application on *Arachis hypogaea*, *Abelmoschus esculentus*, and *Capsicum frutescens* seeds germination.

3.2. OBJECTIVES

- To isolate microorganisms from paddy straw extract.
- To optimize humic acid by placket Burman method and response research methodology
- To characterize the humic acid by FTIR and SEM-EDOX.

CHAPTER 4

MATERIALS & METHODS

4.1. MATERIALS:

- Paddy straw
- Lignite
- Acetone
- Dextrose
- Yeast extract
- Potassium hydroxide
- Magnesium sulfate
- Manganese (II) sulfate
- Calcium chloride
- Burette
- Distilled water
- Filter paper
- Conical flask
- Funnel
- Petri plates
- Cotton
- Spray bottle
- Scissor
- Water
- Cuvette

SAMPLE PREPARATION:

4.2. PADDY STRAW EXTRACTION

- Collection of paddy straw
- A 2-liter conical flask was filled with 150 grams of paddy straw and another 2-liter conical flask.
- Was kept for soaking for 24 hours.
- After soaking it for 1 day the paddy straw extract was collected, a total of 1200ml paddy straw extract was collected.
- After being boiled in a heating mantle, the paddy straw extract was ready.

4.3. HUMIC ACID PRODUCTION

- Collected the sample lignite 10 grams lignite was mixed with 150ml distilled water.
- KOH was added to the sample dropwise while it was being sonicated.
- With the use of a magnetic stirrer, the samples were heated while being continuously stirred.
- Allowed to settle for day and collected the supernatant.
- Allow it settle for 24 hours.
- Let it dry in the hot air oven after passing it through some filter paper.

4.4. FORMULATION OF RICE STRAW SOAKED WITH HUMIC ACID

- Humic acid and paddy straw extract were combined.
- A 100 ml conical flask containing 1 gram of powdered humic acid and paddy straw extract was combined.

4.5. COLLECTION OF SAMPLES

Lignite

Paddy straw

4.5.1 LIGNITE

- Lignite, a type of brown coal, is indeed collected from Neyveli, a region in the Cuddalore district in Tamil nadu.
- Neyveli is renowned for its lignite reserves, which are among the largest in the country.
- Lignite is formed from the accumulation and partial decomposition of organic matter in swampy environments over millions of years.
- Neyveli lignite mines are operated by neyveli lignite corporation (NLC).

4.5.2 PADDY STRAW

- Paddy straw is collected from the local agriculture land.

4.6. PLACKET BURMAN DESIGN

The medium was optimized using the placket burman design to increase the solubilization rate. The media exhibited varying concentration of the following:

- Lignite (1.5 gm to 2.5gm)
- Dextrose (1mg to 2mg)
- Yeast extract (0.2gm to 0.3gm)
- KH_2PO_4 (0.02gm to 0.05gm)
- MgSO_4 (0.01gm to 0.03gm)
- MnSO_4 (0.1gm to 0.2gm)
- CaCl_2 (0.01gm to 0.02gm)

The solubilization assay was preferred according to the previous instruction after 0.5 ml of paddy straw extract suspension was added to the media.

4.6.1 PLACKET BURMAN MODEL

Plackett Burman model was used to develop the medium in order to increase lignite's solubilization. Seven factors were employed in this investigation. Among these, there was a considerable favorable effect for glucose, yeast extract, and lignite. Magnesium sulfate, calcium chloride, magnesium hydrogen phosphate, and potassium orthophosphate were all detrimental.

The Plackett-Burman approach is a statistical technique for determining the important variables impacting a process or product. When producing humic acid, the Plackett-Burman method can be applied to identify the critical components that affect the product's yield or quality.

The synthesis of humic acid depends on several factors, such as temperature, pH, substrate concentration, incubation time, etc. By using the Plackett-Burman method, researchers may efficiently filter these characteristics to identify the ones that have a major impact on the production of humic acid.

RUN	A	B	C	D	E	F	G
1	H	H	H	L	H	L	L
2	L	H	H	H	L	H	L
3	L	L	H	H	H	L	H
4	H	L	L	H	H	H	L
5	L	H	L	L	H	H	H
6	H	L	H	L	L	H	H
7	H	H	L	H	L	L	H
8	L	L	L	L	L	L	L

Table :4.6 Plackett Burman Model

A) YIELD OF REACTED IN PADDY STRAW EXTRACT:

S. NO	REACTED SAMPLE %	LIGNITE (g)
1	2.2	1
2	1.86	0.75
3	1.2	0.75
4	88.1	1
5	72.1	0.75
6	86.8	1
7	84	1
8	0.4	0.75

B) YIELD OF UNREACTED PADDY STRAW EXTRACT:

S. NO	UNREACTED SAMPLE %	LIGNITE (g)
1	64.8	1
2	69.7	0.75
3	30.9	0.75
4	37.6	1
5	69.6	0.75
6	5.9	1
7	38.1	1
8	23	0.75

4.7. HUMIC ACID ESTIMATION

The measurement of humic acid content is subject to variation based on various parameters, including the sample matrix, extraction procedure, and analytical method utilized. To guarantee reliable and repeatable outcomes, it is crucial to adhere to established processes and quality control techniques. The estimation procedure can also be optimized for certain sample types and research aims by looking up pertinent literature or getting advice from subject-matter specialists.

- It is necessary to prepare the sample (such as soil, compost, or a commercial humic acid product) for analysis. Drying, grinding, and homogenizing the sample may be necessary to guarantee representative outcomes.
- Chemical solutions are usually used to extract humic compounds from the sample. Alkaline solutions like sodium hydroxide (NaOH) or sodium pyrophosphate are frequently used as extractants.
- Following extraction, the humic materials are frequently separated into several fractions according to how soluble they are in various pH ranges. Humin fractions, fulvic acid (FA), and humic acid (HA) separation are a few examples of this.
- Next, the extract's humic acid content is ascertained. These methods frequently entail precipitating and quantifying the humic acid fraction or measuring the absorption of particular light wavelengths by the humic acid molecules.
- It's crucial to precisely measure the humic acid level using calibration curves or standard reference materials to guarantee accuracy.
- Lastly, using the extraction and quantification data, the concentration of humic acid in the original sample is computed, accounting for any dilutions or adjustments done throughout the investigation.

4.8. CHARACTERIZATION STUDIES:

4.8.1 FTIR Test (Fourier Transform Infrared Spectroscopy):

An effective analytical method for determining the chemical makeup of a variety of materials, including lignite—a kind of coal with a high moisture content and comparatively low carbon content—is Fourier Transform Infrared Spectroscopy (FTIR). FTIR can be used to characterize lignite in the following ways:

- **Identification of Functional Groups:** FTIR can be used to identify the functional groups present in lignite.
- **Quantification of Functional Groups:** The amount of various functional groups in lignite can be quantified using FTIR spectra.
- **Characterization of Structural Features:** FTIR can shed light on the degree of carbonization, aromaticity, and Aliphaticity, among other structural characteristics of lignite.
- **Evaluation of Thermal Properties:** FTIR can also be used to investigate the thermal characteristics of lignite, including its reactivity and thermal stability.
- **Monitoring Chemical Changes:** FTIR can be used to track changes in lignite's chemical composition brought on by different treatments or environmental factors.

4.8.2 SEM Test (Scanning Electron Microscopy):

Another effective analytical method for characterising lignite is SEM (Scanning Electron Microscopy), which provides high-resolution information on the material's morphological, textural, and surface characteristics. The following is the specific way that SEM can be used for lignite characterization:

- The surface morphology of lignite samples may be seen at high magnification thanks to SEM.
- The combination of SEM and energy-dispersive X-ray spectroscopy (EDS) allows for the mapping and identification of the mineral phases found in lignite samples.
- The size distribution and morphological features of lignite particles can be examined using SEM.
- SEM makes it possible to analyze lignite samples in great detail, looking at structural heterogeneities, pore networks, and microcracks.
- SEM can shed light on lignite's surface chemistry by observing surface characteristics and interactions with other materials

5. SEED GERMINATION

- To study the effects of paddy straw, humic acid and paddy straw + humic acid on ground nut seed to check the growth of the seeds and how rapidly the seed sprouts.
- The paddy straw extract was mixed with water and a total of 10 concentrations were prepared.
- From 0.1 to 9.1 to 1.0 to 9.0. 0. For seed germination, a total of ten concentrations were tested using the 3-sample paddy straw extract, humic acid, and paddy straw extract plus humic acid. 1 ml of the extract was combined with 9 ml of water in this manner.
- The ground nut, ladiesfinger and chilly seeds were surface sterilized with 30% ethanol.
- They soaked the crushed nut seeds for one hour. In each concentration range of 0.1 to 9.9, 10 seeds were soaked; the same for all concentrations.
- 30 petri plates in all were employed, ten of which were used for the germination of paddy straw extract seeds. 10 petri plates were utilized for the germination of humic acid seeds, and another 10 petri plates were used for the germination of paddy straw extract plus humic acid seeds.
- A control sample was placed on a different petri plate.
- Water was used to soak the control seeds for one hour.
- Each petri plate was layered with filter paper on top of it.
- The ground nut seeds were placed on petri dishes and watched for a week following an hour-long soak in the concentration.
- Using a spray bottle, water was sprayed daily for 7 days, and the growth was observed.

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 PADDY STRAW EXTRACTION

In a conical flask, the paddy straw was submerged in water for a full day. One day after 150 grams of paddy straw were soaked in two liters of water, the paddy straw extract was taken out the next day as shown in figure 5.1 and 5.2



Fig 5.1 paddy straw soaked in water for one day



Fig 5.2 paddy straw extract

5.2 HUMIC ACID PRODUCTION

The sample was agitated in a magnetic stirrer, let to settle for a day, passed through filter paper, and allowed to dry in a hot air oven after the lignite and 150 ml of distilled water were combined. The sample was then sonicated with KOH.



Fig 5.3 Humic acid

5.3 FORMULATION OF RICE STRAW SOAKED WITH HUMIC ACID

The extract from paddy straw was combined with humic acid. 100ml of paddy straw extract and 1ml of humic acid were combined.



Fig 5.4 Rice straw soaked with humic acid

PLACKETT BURMAN DESIGN:

RUN	A	B	C	D	E	F	G	RESPONSE %
1	H	H	H	L	H	L	L	64.8
2	L	H	H	H	L	H	H	69.3
3	L	L	H	H	H	L	H	30.9
4	H	L	L	H	H	H	L	37.6
5	L	H	L	L	H	H	H	69.6
6	H	L	H	L	L	H	H	5.9
7	H	H	L	H	L	L	H	38.1
8	L	L	L	L	L	L	L	23

Table:4.6.1

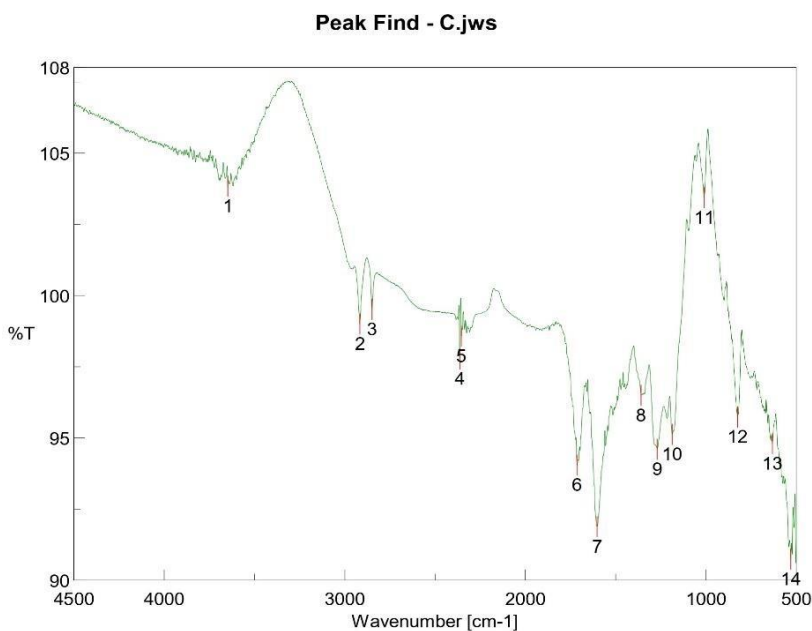
The 5th run in the Plackett-Burman model yielded the highest lignite solubilization rate among the eight runs conducted. This outcome indicates the significance of the variables tested and their respective levels in influencing the solubilization process. By identifying the specific combination of variables that led to maximum solubilization, this finding offers valuable insights for optimizing lignite solubilization processes. Further investigation into the individual contributions of each variable and their interactions could enhance our understanding and potentially lead to more efficient and cost-effective methods for lignite processing.

The significance components are dextrose, lignite, yeast extract, MgSo₄, MnSo₄ and CaCl₂.

Prakash et.al., 2014 reported that the extraction of humic acid was optimized using response surface methodology (RSM) - central composite design (CCD) by altering the concentration of the key constituents such as lignite, Potassium hydroxide and Sodium hydroxide. Among 20 trails, the maximum humic acid was extracted of lignite 5g, KOH 4g and NaOH 1g.

4. CHARACTERIZATION STUDIES

4.1 FTIR test paddy straw extract and paddy straw extract + humic acid



3647.7 cm^{-1} : Broad peak, likely indicative of O-H stretching vibrations in alcohols and phenols.

2916.81 cm^{-1} : Sharp peak, characteristic of C-H stretching vibrations in alkanes.

2849.31 cm^{-1} : Sharp peak, also indicative of C-H stretching vibrations in alkanes.

2362.37 cm^{-1} : Peak, possibly related to $\text{C}\equiv\text{C}$ stretching vibrations in alkynes.

2354.66 cm^{-1} : Peak, potentially associated with $\text{C}\equiv\text{C}$ stretching vibrations in alkynes.

1714.41 cm^{-1} : Peak, suggesting the presence of C=O stretching vibrations in carbonyl groups.

1602.56 cm^{-1} : Peak, indicative of C=C stretching vibrations in aromatic compounds.

1359.57 cm^{-1} : Peak, characteristic of bending vibrations of -CH₃ groups.

1269.9 cm^{-1} : Peak, indicating C-N stretching vibrations in amines or amides.

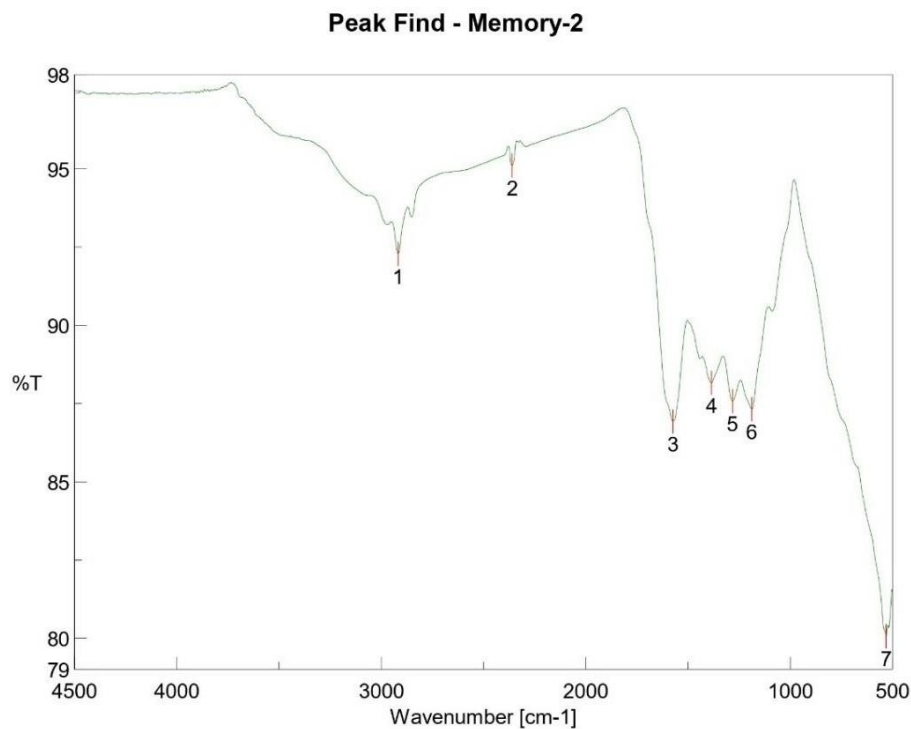
1186.97 cm^{-1} : Peak, possibly related to C-N stretching vibrations in amines or amides.

1010.52 cm^{-1} : Peak, suggestive of C-H bending vibrations in aromatic compounds.

825.384 cm^{-1} : Peak, indicating C-H bending vibrations in alkyl groups.

632.537 cm^{-1} : Peak, characteristic of C-H bending vibrations in alkyl groups.

530.328 cm^{-1} : Peak, indicative of C-Cl stretching vibrations in alkyl chloride



2917.77 cm⁻¹: Sharp peak, characteristic of C-H stretching vibrations in alkanes.

2359.48 cm⁻¹: Peak, potentially associated with C≡C stretching vibrations in alkynes.

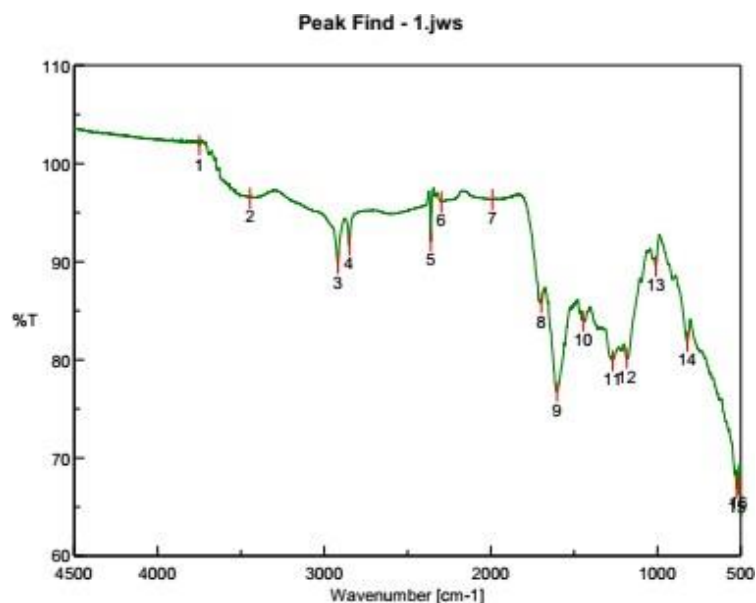
1573.63 cm⁻¹: Peak, indicating C=C stretching vibrations in aromatic compounds.

1384.64 cm⁻¹: Peak, characteristic of bending vibrations of -CH₃ groups.

1282.43 cm⁻¹: Peak, possibly related to C-N stretching vibrations in amines or amides.

1187.94 cm⁻¹: Peak, indicative of C-N stretching vibrations in amines or amides.

531.293 cm⁻¹: Peak, characteristic of C-Cl stretching vibrations in alkyl chlorides.



748.94 cm^{-1} (Broad peak): This broad peak suggests the presence of O-H stretching vibrations, which are commonly found in alcohols and phenols. This indicates the presence of hydroxyl groups in the humic acid structure, which are essential for its solubility and reactivity in aqueous environments.

3444.24 cm^{-1} (Peak): This peak may be related to N-H stretching vibrations in primary amines or O-H stretching vibrations in carboxylic acids. It suggests the presence of amine or carboxylic acid functional groups in the humic acid molecule, which are important for its interactions with other molecules and its role in biological and environmental processes.

2917.77 cm^{-1} and 2849.31 cm^{-1} (Sharp peaks): These sharp peaks are characteristic of C-H stretching vibrations in alkanes, indicating the presence of alkyl groups in the humic acid structure. Alkyl groups contribute to the hydrophobicity and stability of humic acid molecules.

2360.44 cm^{-1} and 2295.84 cm^{-1} (Peaks): These peaks are potentially associated with $\text{C}\equiv\text{C}$ stretching vibrations in alkynes. While less common in organic matter, the presence of alkynes in humic acid may indicate the incorporation of unsaturated carbon bonds.

1990.18 cm^{-1} (Peak): This peak suggests the presence of $\text{C}\equiv\text{C}$ stretching vibrations in alkynes or $\text{C}=\text{C}$ stretching vibrations in aromatic compounds. It indicates the presence of unsaturated carbon-carbon bonds, which contribute to the aromaticity and reactivity of humic acid.

1697.05 cm^{-1} (Peak): This peak is indicative of $\text{C}=\text{O}$ stretching vibrations in carbonyl groups, suggesting the presence of carbonyl functional groups in the humic acid molecule. Carbonyl groups are important for the reactivity and complexation properties of humic acid.

1602.56 cm^{-1} (Peak): This peak indicates the presence of $\text{C}=\text{C}$ stretching vibrations in aromatic compounds, further confirming the aromatic nature of humic acid molecules.

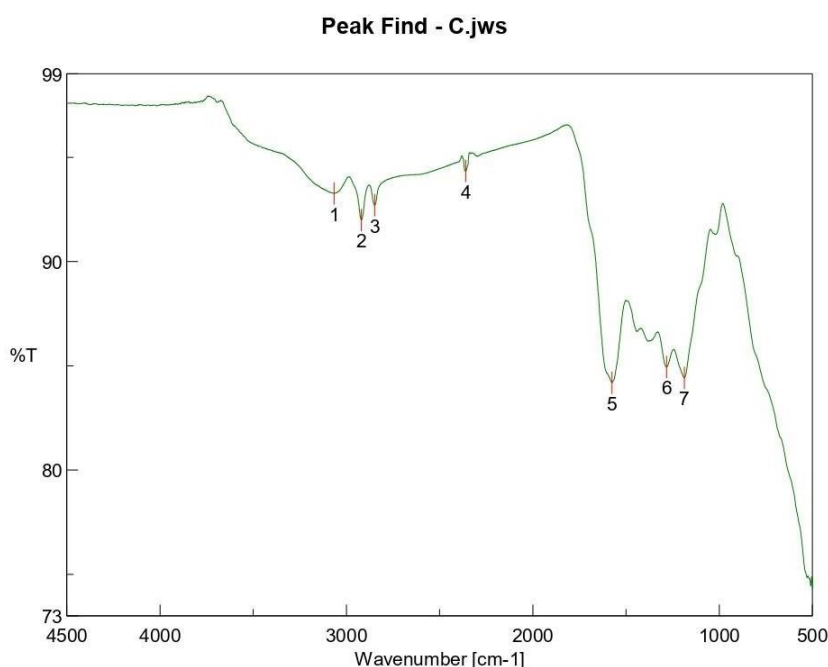
1444.42 cm^{-1} (Peak): This peak is characteristic of bending vibrations of $-\text{CH}_3$ groups, suggesting the presence of methyl groups in the humic acid structure.

1268.93 cm^{-1} and 1184.08 cm^{-1} (Peaks): These peaks suggest the presence of C-N stretching vibrations in amines or amides, indicating the presence of nitrogen-containing functional groups in the humic acid molecule.

1010.52 cm^{-1} (Peak): This peak indicates C-H bending vibrations in aromatic compounds, further confirming the presence of aromatic rings in the humic acid structure.

821.527 cm^{-1} (Peak): This peak suggests C-H bending vibrations in alkyl groups, providing additional evidence of the presence of alkyl chains in the humic acid molecule.

523.579 cm^{-1} and 513.936 cm^{-1} (Peaks): These peaks are characteristic of C-Cl stretching vibrations in alkyl chlorides, suggesting the presence of chlorine-containing functional groups in the humic acid structure.



3066.26 cm^{-1} : Peak, indicative of C-H stretching vibrations in alkenes or alkynes.

2919.7 cm^{-1} : Sharp peak, characteristic of C-H stretching vibrations in alkanes.

2848.35 cm^{-1} : Sharp peak, also indicative of C-H stretching vibrations in alkanes.

2359.48 cm^{-1} : Peak, potentially associated with $\text{C}\equiv\text{C}$ stretching vibrations in alkynes.

1574.59 cm^{-1} : Peak, indicating $\text{C}=\text{C}$ stretching vibrations in aromatic compounds.

1282.43 cm^{-1} : Peak, characteristic of C-N stretching vibrations in amines or amides.

1186.97 cm^{-1} : Peak, possibly related to C-N stretching vibrations in amines or amides.

4.2 SEM test of paddy straw extract + humic acid

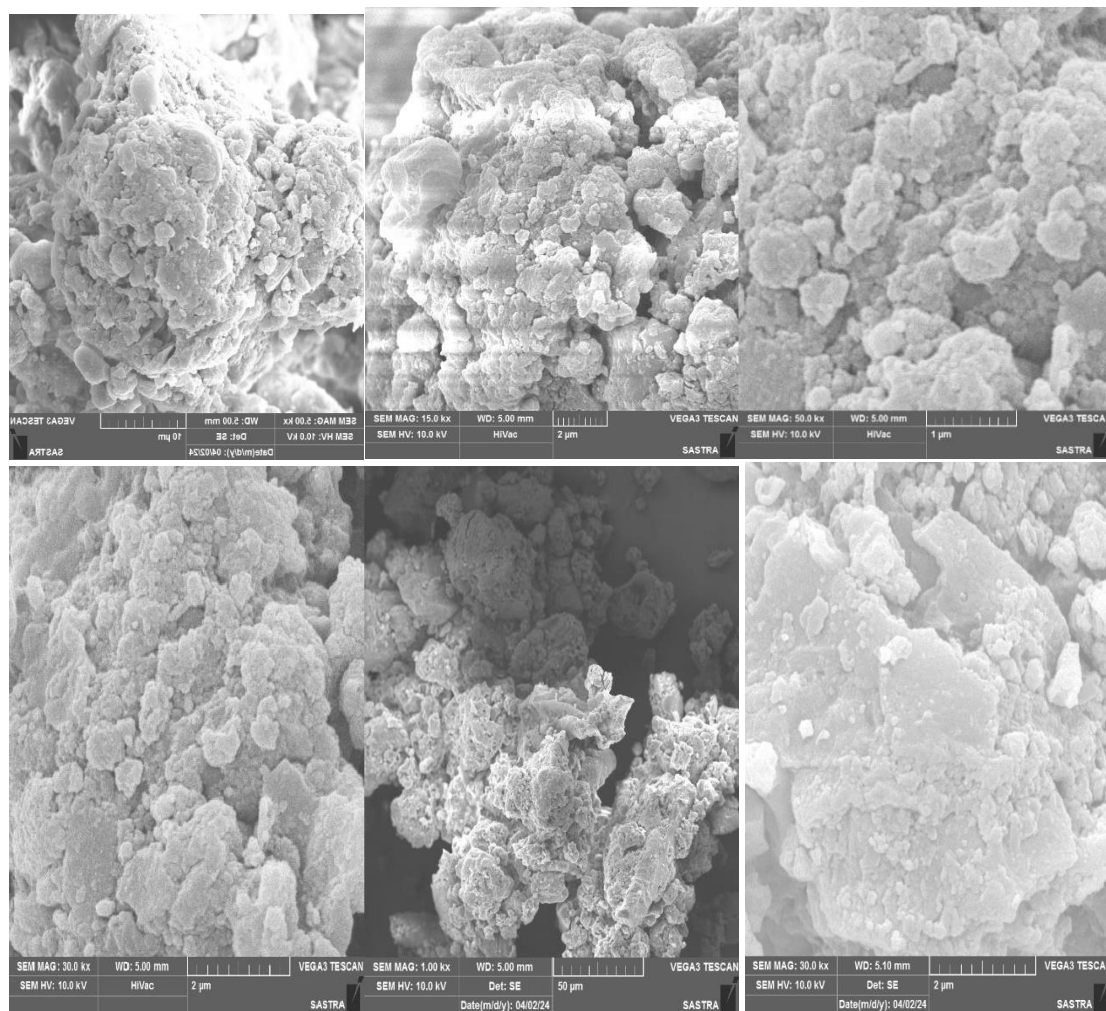


Fig:4.2.1 Day:1 SEM image of paddy straw extract from lignite A)10μm, B)2μm, C)1μm, D)2μm, E)50μm, F)2μm

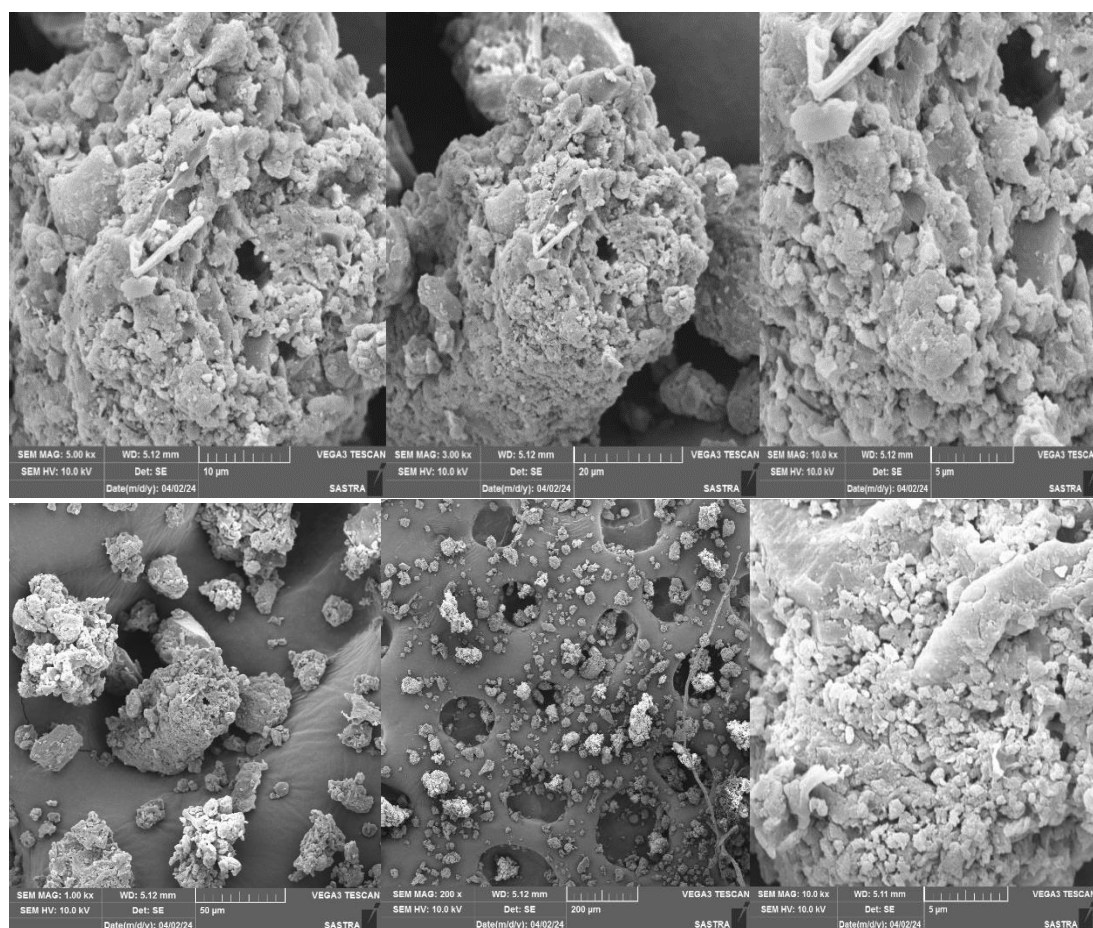


Fig:4.2.2 Day:7 SEM image of paddy straw extract of lignite A)10 μ m, B)20 μ m, C)5 μ m, D)50 μ m, E)200 μ m, F)5 μ m

In this study, the lignite sample was subjected to treatment with a paddy straw extract containing microbes, leading to the degradation of 65% of the lignite. Following this treatment, both initial images of the untreated lignite and final images depicting the altered lignite particles after degradation were observed using scanning electron microscopy (SEM). The SEM images revealed significant changes in the morphology and structure of the lignite particles, indicating the transformative effects of microbial activity on the lignite sample.

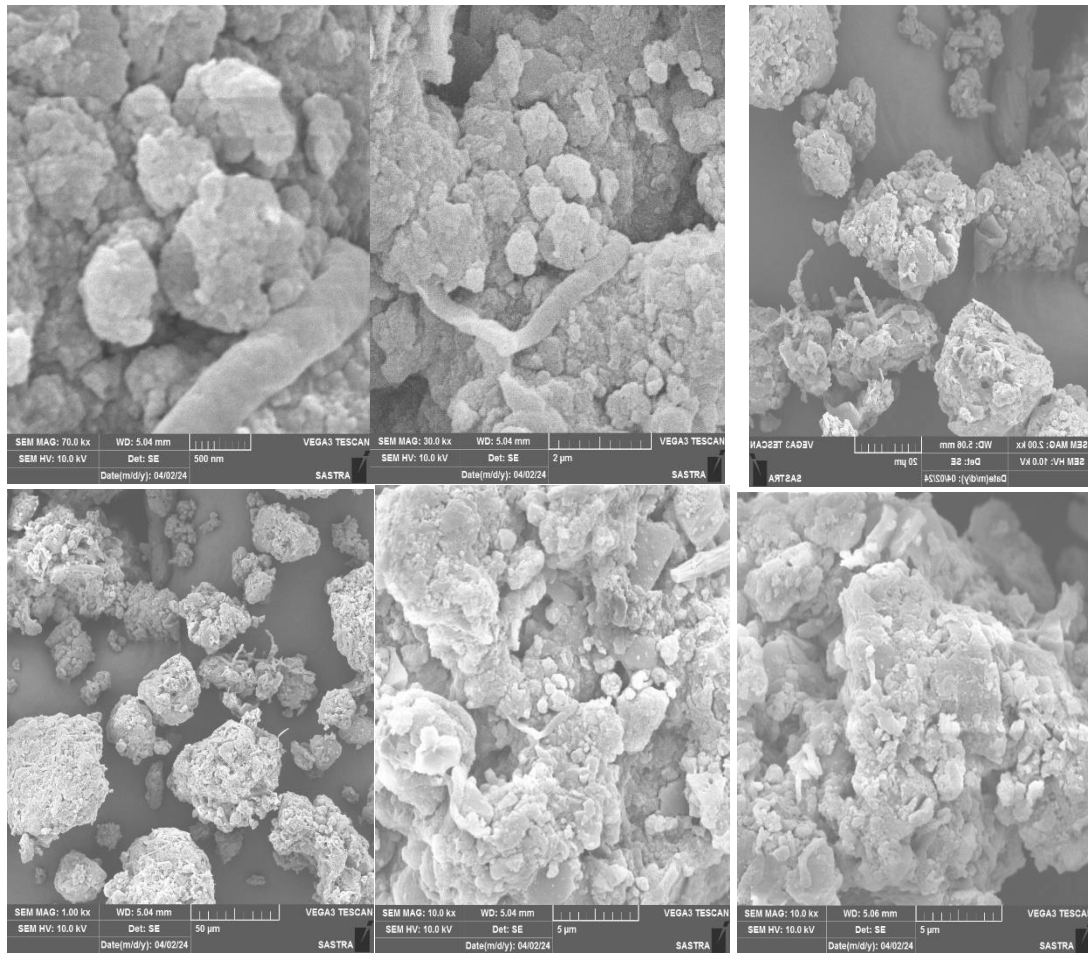


Fig:4.2.3 Day:1 SEM image of paddy straw extract of lignite A)500µm, B)2µm, C)20µm, D)50µm, E)5µm, F)5µm

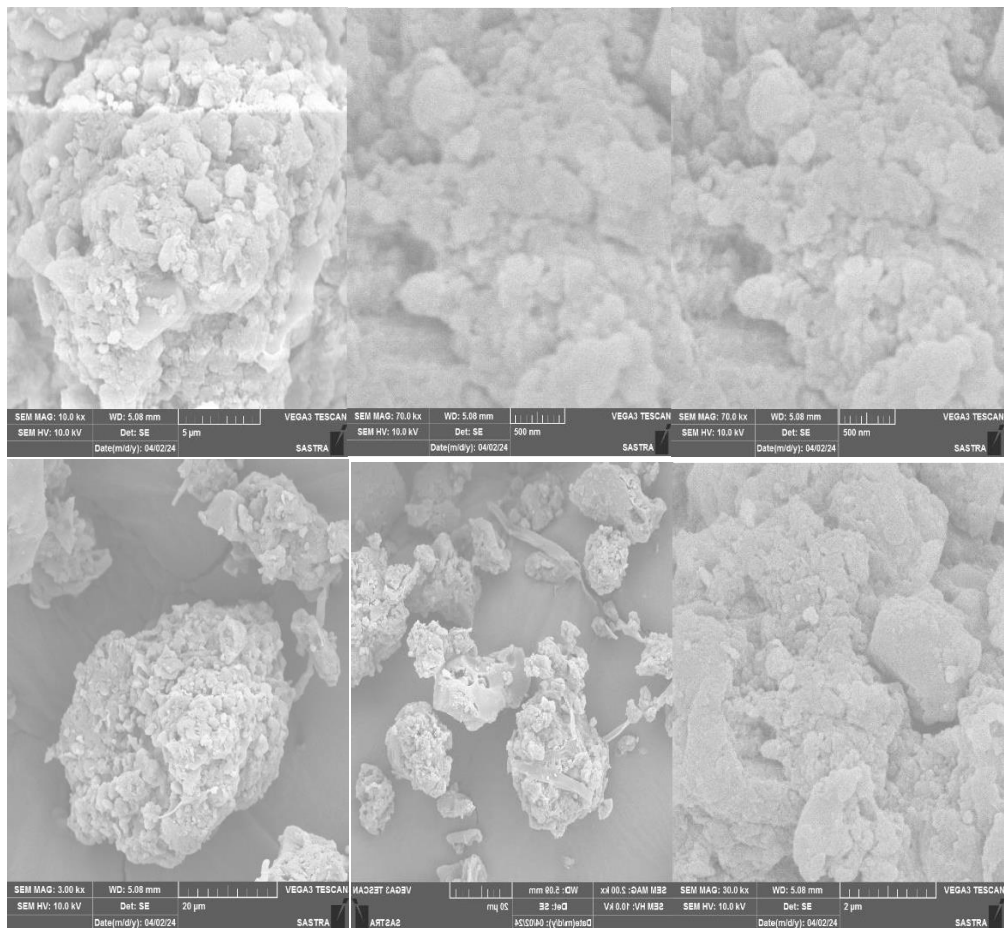


Fig:4.2.4 Day:7 SEM image of paddy straw extract of lignite A)5µm, B)500nm, C)500nm, D)20µm, E)20µm, F)2µm

5. SEED GERMINATION

1. SEED GERMINATION USING PADDY STRAW EXTRACT

The process of seed germination involved utilizing all three samples, beginning with the extracted sample from paddy straw. A total of 100 ground nut seeds were utilized, with 10 seeds allocated to each concentration, ranging from 0.1 to 9.9 to 1.0 to 9.0. The seeds were immersed in each of the ten concentrations for an hour. Using a water sprayer, water was sprayed on the ground nut seeds after they had been watched for seven days, during which time the seed growth was observed.

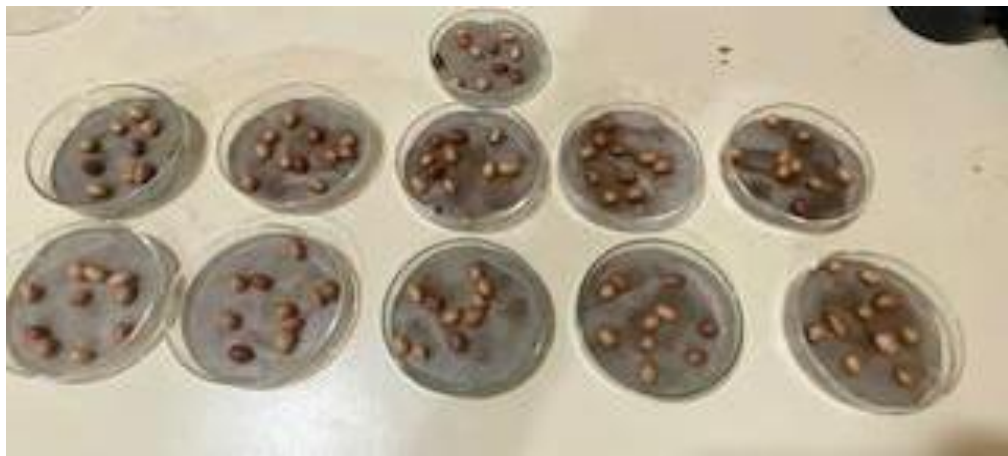
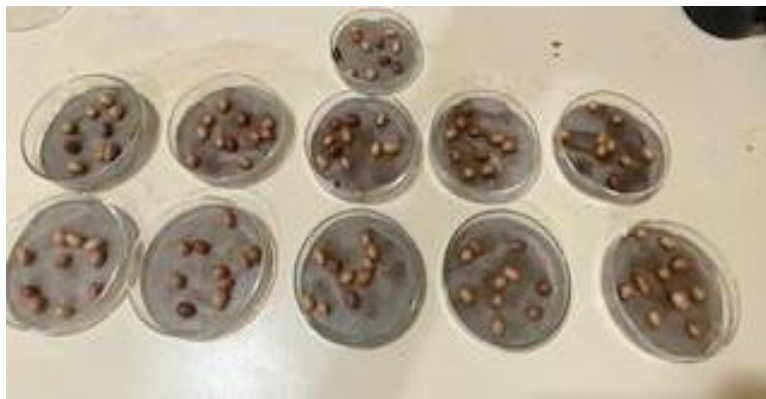


FIG 5.5 ARACHIS HYPOGAEA SEED GERMINATION

Prakash et.al., 2014 revealed that humic acid with the concentration of 0.25% showed maximum seed germination (100%) and the optimum shoot and root length was recorded as 6.175cm and 11.46cm respectively after 60 minutes soaking.

Table 5.1 Day 1 seed germination *Arachis hypogaea* using paddy straw extract

Paddy straw extract (ml)	Water (ml)	No. of seed Grown %	No. of seeds contaminated	No. of seeds not Grown %
0.1	9.9	70	-	30
0.2	9.8	10	-	90
0.3	9.7	80	-	20
0.4	9.6	70	-	30
0.5	9.5	70	-	30
0.6	9.4	50	-	50
0.7	9.3	60	-	40
0.8	9.2	60	-	40
0.9	9.1	40	-	60
1.0	9	80	-	20
Control		-	-	100



The investigation of groundnut seed germination with quantities of paddy straw extract ranging from 0.1% to 1% produced some fascinating findings. Interestingly, germination rates of 80% were seen at concentrations of 0.3% and 1% paddy straw extract, which was significantly greater than both lower concentrations and the control group in which no seeds germinated. According to this, components in paddy straw extract at concentrations of 0.3% and 1% may help to encourage the germination of groundnut seeds. It is imperative to acknowledge that the control group did not exhibit seed germination, suggesting a lack of proper germination conditions. Subsequent investigations may concentrate on clarifying the precise components in paddy straw extract that augment groundnut seed germination and refining application techniques to fully utilize its agricultural advantages.

Table 5.2 Day 3 seed germination *Arachis hypogaea* using paddy straw extract

Paddy straw extract (ml)	Water (ml)	No. of seed Grown %	No. of seeds contaminated	No. of seeds not grown %
0.1	9.9	100	-	-
0.2	9.8	100	-	-
0.3	9.7	100	-	-
0.4	9.6	100	-	-
0.5	9.5	100	-	-
0.6	9.4	90	-	10
0.7	9.3	100	-	-
0.8	9.3	90	-	10
0.9	9.2	80	-	20
1.0	9.1	100	-	-



The experiment examining groundnut seed germination using various concentrations of paddy straw extract revealed intriguing results. Notably, a concentration of 0.9% exhibited a high germination rate of 80%, indicating the potential of this concentration to promote groundnut seed germination effectively. However, concentrations of 0.3% and 1% showed no seed germination, contrasting sharply with the positive outcomes observed at other concentrations. This discrepancy underscores the importance of understanding the precise concentration thresholds for optimal seed germination when using paddy straw extract.

Table 5.3 Day 5 seed germination of *Arachis hypogaea* using paddy straw extract

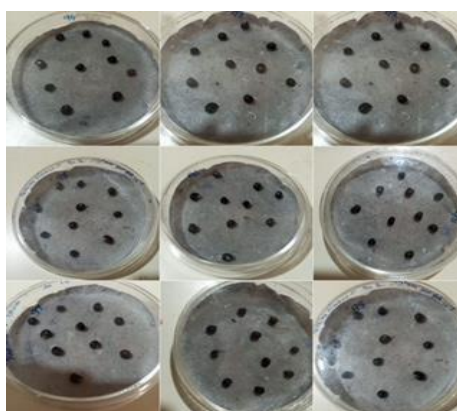
Paddy straw extract (ml)	Water (ml)	No. of seeds Grown %	No. of seeds contaminated	No. of seeds not grown %
0.1	9.9	100	-	-
0.2	9.8	100	-	-
0.3	9.7	100	-	-
0.4	9.6	100	-	-
0.5	9.5	100	-	-
0.6	9.4	100	-	-
0.7	9.3	100	-	-
0.8	9.2	90	-	10
0.9	9.1	100	-	-
1.0	9	100	-	-
control		40	-	60



The groundnut seed germination experiment utilizing paddy straw extract concentrations ranging from 0.1% to 1% yielded compelling findings. Notably, concentrations of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, and 0.9% resulted in 100% seed germination, highlighting the potency of these concentrations in promoting groundnut seed germination effectively. Additionally, a concentration of 0.8% exhibited a high germination rate of 90%, further emphasizing the efficacy of paddy straw extract as a growth stimulant. In contrast, the control group displayed a lower germination rate of 40%, underscoring the importance of supplemental treatments like paddy straw extract for enhancing seedling establishment. These results suggest that paddy straw extract, particularly within specific concentration ranges, can significantly improve groundnut seed germination rates compared to standard germination conditions.

Table 5.5 Day 1 seed germination of *Abelmoschus esculentus* using paddy straw extract

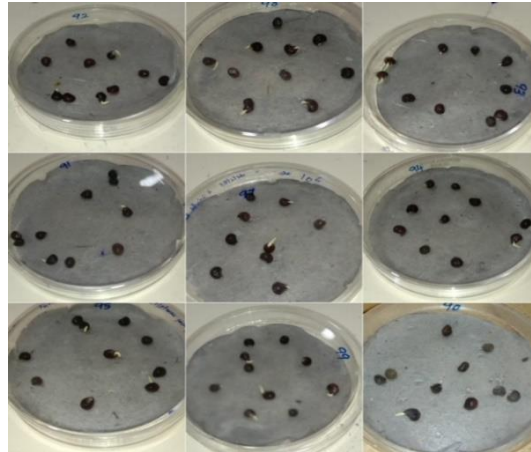
Paddy straw extract (ml)	Water (ml)	No. of seeds Grown %	No. of seeds contaminated	No. of seeds not grown %
0.1	9.9	-	-	100
0.2	9.8	10	-	90
0.3	9.7	-	-	100
0.4	9.6	-	-	100
0.5	9.5	-	-	100
0.6	9.4	-	-	100
0.7	9.3	-	-	100
0.8	9.2	-	-	100
0.9	9.1	-	-	100
1.0	9	-	-	100
control		-	-	100



Notable results were from the experiment examining ladies' finger seed germination using quantities of paddy straw extract ranging from 0.1% to 1%. Remarkably, a 0.2% concentration unexpectedly produced no seed germination, in contrast to the control group where no seeds sprouted at all. This is noteworthy because a 1% concentration of paddy straw extract produced a moderate 10% germination rate. According to this, the control condition and the 0.2% paddy straw extract do not contain the components required to promote seed germination in the given experimental setup. Improved standard germination conditions are required, as evidenced by the lack of seed germination in the control group. Enhancing seed germination processes may be achieved by delving further into the elements preventing germination at 0.2% paddy straw extract concentration.

Table 5.6 Day 3 seed germination of *Abelmoschus esculentus* using paddy straw extract

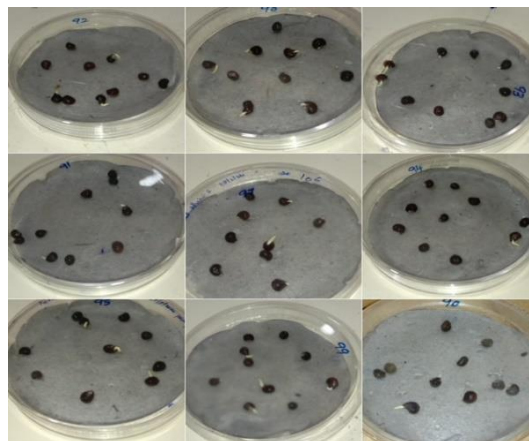
Paddy straw extract (ml)	Water (ml)	No. of seeds Grown %	No. of seeds contaminated	No. of seeds not grown %
0.1	9.9	50	-	50
0.2	9.8	50	-	50
0.3	9.7	60	-	40
0.4	9.6	20	-	80
0.5	9.5	30	-	70
0.6	9.4	20	-	80
0.7	9.3	40	-	60
0.8	9.2	30	-	70
0.9	9.1	30	-	70
1.0	9	10	-	90
control		10	-	-



The ladies' finger seed germination experiment with different paddy straw extract concentrations produced some interesting findings. Significantly outperforming both lower and higher concentrations as well as the control group, the ideal concentration of 0.3% demonstrated a 60% germination rate. This shows that the extract from paddy straw may be useful as a ladies' finger seed germination booster, with a 0.3% concentration demonstrating encouraging results. However, the control group's 10% germination rate is noteworthy and suggests that conventional germination conditions need improvement.

Table 5.7 Day 5 seed germination of *Abelmoschus esculentus* using paddy straw extract

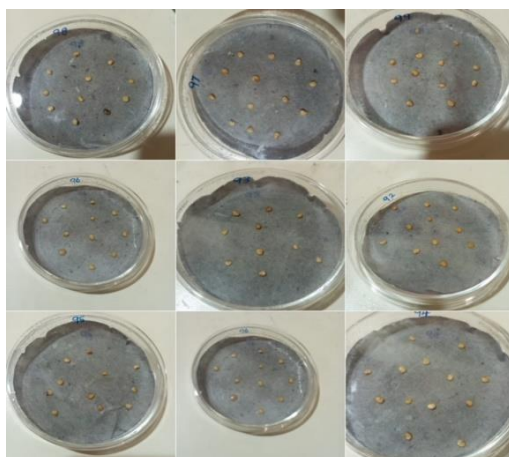
Paddy straw extract (ml)	Water (ml)	No. of seeds Grown %	No. of seeds contaminated	No. of seeds not grown %
0.1	9.9	70	-	30
0.2	9.8	70	-	30
0.3	9.7	80	-	20
0.4	9.6	50	-	50
0.5	9.5	50	-	50
0.6	9.4	40	-	60
0.7	9.3	60	-	40
0.8	9.2	50	-	50
0.9	9.1	40	-	60
1.0	9	30	-	70
control		30	-	70



The ladies' finger seed germination experiment with different paddy straw extract concentrations produced interesting findings. Notably, 0.3% concentration outperformed both lower and higher concentrations as well as the control group, exhibiting the greatest germination rate at 80%. This implies a range of concentrations that work best to encourage seed germination. It's interesting to note that the control group's germination rate was equal to that of the highest dose tested (1%), suggesting that using excessive quantities may have unfavorable effects. Overall, our results point to the possibility of using paddy straw extract as a ladies' finger seed germination stimulant; nevertheless, more investigation is needed to clarify underlying mechanisms and improve administration techniques for positive effects on agriculture.

Table 5.8 Day 1 seed germination of *Capsicum frutescens* using paddy straw extract

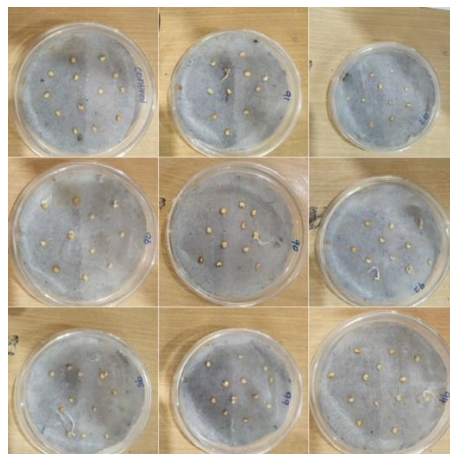
Paddy straw extract (ml)	Water (ml)	No. of seeds Grown %	No. of seeds contaminated	No. of seeds not grown %
0.1	9.9	-	-	100
0.2	9.8	-	-	100
0.3	9.7	-	-	100
0.4	9.6	-	-	100
0.5	9.5	-	-	100
0.6	9.4	-	-	100
0.7	9.3	-	-	100
0.8	9.2	-	-	100
0.9	9.1	-	-	100
1.0	9	-	-	100
control		-	-	100



The results of the experiment investigating chili seed germination with varying concentrations of paddy straw extract indicate a lack of seed germination across all tested concentrations, ranging from 0.1% to 1%, as well as in the control group. This unexpected outcome suggests that paddy straw extract, within the tested concentration range, may not possess the necessary compounds or nutrients to support chili seed germination. The absence of seed germination in both the experimental and control groups underscores the need for further research to identify alternative substances or methods that can effectively promote chili seed germination. Understanding the specific requirements for successful chili seed germination is crucial for improving agricultural practices and enhancing crop productivity in chili cultivation.

Table 5.9 Day 3 seed germination of *Capsicum frutescens* using paddy straw extract

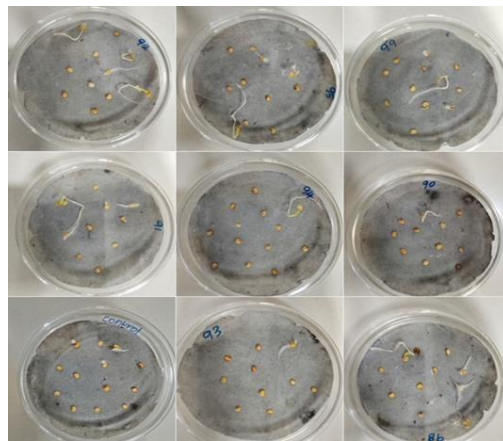
Paddy straw extract (ml)	Water (ml)	No. of seeds Grown %	No. of seeds contaminated	No. of seeds not grown %
0.1	9.9	20	-	80
0.2	9.8	50	-	50
0.3	9.7	30	-	70
0.4	9.6	10	-	90
0.5	9.5	40	-	60
0.6	9.4	30	-	70
0.7	9.3	20	-	80
0.8	9.2	10	-	90
0.9	9.1	10	-	90
1.0	9	20	-	80
control		-	-	-



The experiment on chili seed germination utilizing paddy straw extract concentrations ranging from 0.1% to 1% yielded intriguing findings. Notably, concentrations of 0.4%, 0.8%, and 0.9% resulted in a modest but notable 10% seed germination rate, indicating some potential for paddy straw extract to promote chili seed germination within this range. Additionally, a concentration of 0.5% exhibited a higher germination rate of 40%, suggesting that this concentration may be more conducive to chili seed germination compared to others tested. However, it's important to note that the control group showed no seed germination, emphasizing the necessity of supplemental treatments like paddy straw extract for enhancing seedling establishment.

Table 5.10 Day 5 seed germination of *Capsicum frutescens* using paddy straw extract

Paddy straw extract (ml)	Water(ml)	No.of.seeds grown %	No.of seeds contaminated	No.of seeds not grown %
0.1	9.9	40	-	60
0.2	9.8	70	-	30
0.3	9.7	50	-	50
0.4	9.6	30	-	70
0.5	9.5	60	-	40
0.6	9.4	50	-	50
0.7	9.3	40	-	60
0.8	9.2	20	-	80
0.9	9.1	20	-	80
1.0	9	40	-	60
control		30	-	70



The experiment examining chilli seed germination using paddy straw extract concentrations ranging from 0.1% to 1% revealed interesting results. Particularly, a concentration of 0.2% paddy straw extract demonstrated a relatively high seed germination rate of 70%, indicating its effectiveness in promoting chilli seed germination. However, concentrations of 0.8% and 0.9% resulted in lower germination rates of 20%, suggesting a potential decline in efficacy at higher concentrations. In comparison, the control group exhibited a seed germination rate of 30%. These findings suggest that paddy straw extract, particularly at 0.2% concentration, may have the potential to enhance chilli seed germination.

CHAPTER 6

SUMMARY AND CONCLUSION

SUMMARY

In our laboratory, we generated paddy straw extract by immersing the straw in water for a day. Similarly, we synthesized humic acid using a series of steps involving KOH addition, sonication, centrifugation, and drying. The combination of paddy straw extract and humic acid was created by blending them together. We conducted seed germination experiments using groundnut seeds, treating them with three different samples: paddy straw extract alone, humic acid alone, and a combination of paddy straw extract and humic acid, each with varying concentrations ranging from 0.1 to 9.9 to 1.0 to 9.0. A total of 300 groundnut seeds were employed, with 100 seeds for each sample, while 10 seeds were kept as controls. We observed the sprout length over a week.

FTIR and SEM were carried out on all two samples, and the results were compared with the control of the samples.

The study suggests that microorganisms found in paddy straw exhibit promising abilities to solubilize lignite. Additionally, the paddy straw extracts demonstrated significant nutritional qualities and facilitated seed germination. These findings indicate that the biological humic acid derived from the solubilized lignite could serve as effective fertilizer for foliar spraying. Moreover, the solubilized lignite sample can be readily converted into chemical humic acid, potentially reducing the required concentration of KOH in the production process.

The study found that microorganisms present in paddy straw show potential in breaking down lignite into humic acid. Lignite was treated with these microorganisms from paddy straw, resulting in the conversion of lignite into humic acid. The researchers then optimized the medium using the Plackett-Burman model to enhance the solubilization of humic acid. The humic acid produced was tested for its impact on seed germination and further analyzed using SEM and FTIR for confirmation.

REFERENCE:

- Ampong, Kwame, Malinda S. Thilakaranthna, and Linda Yuya Gorim. "Understanding the role of humic acids on crop performance and soil health." *Frontiers in Agronomy* 4 (2022): 848621.
- Albiach, R., Canet, R., Pomares, F., and Ingelmo, F. (2001). Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. *Bioresour. Technol.* 76, 125-129. doi: 10.1016/S0960-8524(00)00090-0999
- Arancon NQ, Edwards CA, Lee S, Byrne R. Effects of humic acids from vermicomposts on plant growth. *European journal of soil biology.* 2006 Nov 1;42:S65-9.
- Billingham, K. L. (2012). "Humic products-potential or presumption for agriculture? Can humic products improve my soil?" in 27th Annual Conference (Orange, NSW: Grassland Society of NSW Inc.).
- Canellas, L. P., et al. "Humic acids crossinteractions with root and organic acids." *Annals of Applied biology* 153.2 (2008): 157-166.
- Cheshire, M. V., Cranwell, P. A., Falshaw, C. P., Floyd, A. J., & Haworth, R. D. (1967). Humic acid—II: Structure of humic acids. *Tetrahedron*, 23(4), 1669-1682.
- Calvo, P., Nelson, L. and Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. *Plant and Soil.* 383(1): 3-41.
- de Melo, Bruna Alice Gomes, Fernanda Lopes Motta, and Maria Helena Andrade Santana. "Humic acids: Structural properties and multiple functionalities for novel technological developments." *Materials Science and Engineering: C* 62 (2016): 967-974
- Ekin, Z. (2019). Integrated use of humic acid and plant growth promoting rhizobacteria to ensure higher potato productivity in sustainable agriculture. *Sustainability.* 11(12):1-13.
- Fuentes, M., Baigorri, R., González-Gaitano, G., and García-Mina, J. M. (2018). New methodology to assess the quantity and quality of humic substances in organic materials and commercial products for agriculture. *J. Soils Sediments* 18, 1389-1399. doi: 10.1007/s11368-016-1514-2
- Ferrara, G. and Brunetti, G. (2008). Influence of foliar applications of humic acids on yield and fruit quality of table grape cv. Italia. *Journal International Science Vigne Vin.* 42(2): 79-87.
- Garciá, A. C., De Souza, L. G. A., Pereira, M. G., Castro, R. N., Garciá Mina, J. M., Zonta, E., et al. (2016). Structure-property-function relationship in humic substances to explain the biological activity in plants. *Sci. Rep.* 6, 20798. doi: 10.1038/srep20798

- Gollenbeek, L., and Van Der Weide, R. (2020). Prospects for Humic Acid Products from Digestate in the Netherlands. Report WPR-867, 5-40. doi: 10.18174/541280
- Haworth, R. D. "The chemical nature of humic acid." *Soil Science* 111.1 (1971): 71-79.
- Haumaier, Ludwig, and Wolfgang Zech. "Black carbon—possible source of highly aromatic components of soil humic acids." *Organic Geochemistry* 23.3 (1995): 191-196.
- Hayes, M. H. B., and Swift, R. S. (2020). Vindication of humic substances as a key component of organic matter in soil and water. *Adv. Agron.* 163, 1-37. doi: 10.1016/bs.agron.2020.05.001
- Islam, K. M. S., Schuhmacher, A., & Gropp, J. M. (2005). Humic acid substances in animal agriculture. *Pakistan Journal of nutrition*, 4(3), 126-134.
- Jindo, K., Olivares, F. L., Malcher, D. J., da, P., Sánchez-Monedero, M. A., Kempenaar, C., et al. (2020). From lab to field: role of humic substances under open-field and greenhouse conditions as biostimulant and biocontrol agent. *Front. Plant Sci.* 11, 426. doi: 10.3389/fpls.2020.00426
- Joshi, Rakesh, Jaswinder Singh, and Adarsh Pal Vig. "Vermicompost as an effective organic fertilizer and biocontrol agent: effect on growth, yield and quality of plants." *Reviews in Environmental Science and Bio/Technology* 14 (2015): 137-159.
- Joshi, R., Singh, J., and Vig, A. P. (2014). Vermicompost as an effective organic fertilizer and biocontrol agent: effect on growth, yield and quality of plants. *Rev. Environ. Sci. Biotechnol.* 14, 137-159. doi: 10.1007/s11157-014-9347-9341
- Khan, R. U., Khan, M. Z., Khan, A., Saba, S., Hussain, F., and Jan, I. U. (2018). Effect of humic acid on growth and crop nutrient status of wheat on two different soils. *J. Plant Nutr.* 41, 453-460. doi: 10.1080/01904167.2017.1385807
- Khaled, H. and Fawy, H.A. (2011). Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil & Water Research.* 6(1): 21-29
- Lee, Jongtae. "Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production." *Scientia Horticulturae* 124.3 (2010): 299-305.
- MacCarthy, P., et al. "An introduction to soil humic substances." *Humic substances in soil and crop sciences: Selected readings* (1990): 1-12.
- Martinez-balmori, D., Spaccini, R., Novotny, E. H., and Canellas, L. P. (2014). Molecular characteristics of humic acids isolated from vermicomposts and their relationship to bioactivity. *J. Agri. Food Chem.* 62, 11412-11419. doi: 10.1021/jf504629c

- Nardi, S., Schiavon, M., & Francioso, O. (2021). Chemical structure and biological activity of humic substances define their role as plant growth promoters. *Molecules*, 26(8), 2256.
- Pal, Samir, and M. B. Sengupta. "Nature and properties of humic acid prepared from different sources and its effect on nutrient availability." *Plant and Soil* 88 (1985): 71-91.
- Rose, M. T., Patti, A. F., Little, K. R., Brown, A. L., Jackson, W. R., and Cavagnaro, T. R. (2014). A meta-analysis and review of plant-growth response to humic substances: Practical implications for agriculture. *Adv. Agron.* 124, 37-89. doi: 10.1016/B978-0-12-800138-7.00002-4
- Senesi, N., Valeria D'Orazio, and G. Ricca. "Humic acids in the first generation of EUROSOLS." *Geoderma* 116.3-4 (2003): 325-344.
- Sarlaki, E., Paghaleh, A. S., Kianmehr, M. H., & Vakilian, K. A. (2021b). Valorization of lignite wastes into humic acids: Process optimization, energy efficiency and structural features analysis. *Renewable Energy*, 163, 105-122. <https://doi.org/10.1016/j.renene.2020.08.096>
- Stevenson, F. J., and J. H. A. Butler. "Chemistry of humic acids and related pigments." *Organic Geochemistry: Methods and Results*. Berlin, Heidelberg: Springer Berlin Heidelberg, 1969. 534-557.
- Vašková, J., Veliká, B., Pilátová, M., Kron, I., & Vaško, L. (2011). Effects of humic acids in vitro. *In Vitro Cellular & Developmental Biology-Animal*, 47, 376-382.
- Waqas, M., Ahmad, B., Arif, M., Munsif, F., Khan, A., Amin, M., Sang MoKang, S.M., Yoon-HaKim, Y. and Lee, I. (2014). Evaluation of humic acid application methods for yield and yield components of mungbean. *American Journal of Plant Sciences*. 5: 2269-2276.
- Wu, S., Li, R., Peng, S., Liu, Q., and Zhu, X. (2017). Effect of humic acid on transformation of soil heavy metals. *IOP Conf. Ser. Mater. Sci. Eng.* 207, 012089. doi: 10.1088/1757-899X/207/1/012089
- Yang, Fan, and Markus Antonietti. "Artificial humic acids: sustainable materials against climate change." *Advanced Science* 7.5 (2020): 1902992.