

DEVELOPMENT OF NOVEL FERTILIZER FOR CROP GROWTH USING HUMIC ACID ENRICHED RICE STRAW-SOAKED WATER

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Biotechnology

By

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

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Anusha R (40770005) who carried out the project entitled "**Development of Novel Fertilizer for crop Growth Using Humic Acid Enriched Rice Straw-Soaked Water**" under my supervision from September 2022 to May 2023.

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I (40770024) and **Anusha R** (40770005) hereby declare that the project report entitled “**Development of Novel Fertilizer for Crop Growth Using Humic Acid Enriched Rice Straw Water**” done by us under the guidance of **Dr. P PRAKASH** internal at Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science in biotechnology.

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INDEX

CHAPTER NO.	TITLE	PAGE
	Abstract	iv
	List of abbreviation	v
	List of figures	vi
	List of tables	viii
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	3
2.	LITERATURE SURVEY	5
3.	AIM & SCOPE	9
3.1	Aim	9
3.2	Scope	9
4.	MATERIALS & METHOD	10
4.1	Materials	10
4.2	Paddy straw extraction	11
4.3	Humic acid production	11
4.4	Formulation of rice straw soaked with humic acid	11
4.5	Characterization studies	11
4.5.1	Macro and micronutrient test	11
4.6	Seed germination	12

4.7	Pot studies	13
4.8	Biochemical analysis	13
4.8.1	Thin layer chromatograph	13
4.8.2	Estimation of protein by Lowry's method	14
5.	RESULT AND DISCUSSION	15
5.1	Paddy straw extraction	15
5.2	Humic acid production	16
5.3	Formulation of rice straw soaked with humic acid	16
5.4	Characterization studies	17
5.4.1	Macro and micronutrient test of paddy straw extract	17
5.4.2	Macro and micronutrient test of paddy straw extract + humic acid	18
5.5	Seed germination	19
5.5.1	Seed germination using paddy straw extract	19
5.5.2	Seed germination using paddy straw extract + humic acid	24
5.5.3	Seed germination using humic acid	20
5.6	Pot studies	35
5.6.1	Day 1 pot studies	35
5.6.2	Week 1 pot studies	37
5.6.3	Week 2 pot studies	39
5.7	Biochemical analysis	43
5.7.1	Thin layer chromatography	43
5.7.2	Estimation of protein by Lowry's method	47
6.	SUMMARY AND CONCLUSION	48
6.1	Summary	48

6.2	Conclusion	48
	REFERENCE	49

ABSTRACT

Humic acid is a substance which are present in humus. Humus is naturally occurring organic matter present in soil formed by degradation of plant matter, which leads to the formation of brown coal. In India humic acid is chemically extracted from lignite commonly known as brown coal. The aim of the study is formulation of paddy straw extract with humic acid and its effect on seed germination followed by pot studies. The paddy straw which is used in mushroom production in which a total of 60% of water is used as a substrate for soaking purpose and later discarded to the environment. The discarded paddy straw water contains carbon, nitrogen, calcium, potassium, magnesium, phosphorus, zinc and other essential nutrients which are required for plant growth instead of discarding it to the environment the paddy straw water is combined with humic acid to check is it beneficial for the development of the seed and plant and can it be used as fertilizer. Seed germination is done to see check how fast the seed sprout. Further the formulated paddy straw extracts subjected to Pot studies is done. Macro and micronutrients were done in order to evaluate the nutrient content. Also, Biochemical analysis is done in order to estimate the amount of protein present in paddy straw extract.

LIST OF ABBREVIATION

mg - milligram

kg – kilogram

% - percentage

µg – microgram

ml – millilitre

cm - centimetres

HA - Humic acid

HS - Humic substances

FA – Fulvic acid

NaOH - sodium hydroxide

BSA - Bovine Serum Albumin

TLC - Thin Layer Chromatography

R_f - retention factor

nm – nanometre

meq/l - milliequivalents per litre

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 for one day	15
5.2	Paddy straw extract	15
5.3	Humic acid	16
5.4	Formulation of rice straw soaked with humic acid	16
5.5	Ground nut seeds soaked in paddy straw extract concentration for 1 hour	19
5.6	Day 1 seed germination using paddy straw extract	20
5.7	Day 3 seed germination using paddy straw extract	21
5.8	Day 5 seed germination using paddy straw extract	22
5.9	Day 7 seed germination using paddy straw extract	23
5.10	Day 1 seed germination using paddy straw extract + humic acid	25
5.11	Day 3 seed germination using paddy straw extract + humic acid	26
5.12	Day 5 seed germination using paddy straw extract + humic acid	27
5.13	Day 7 seed germination using paddy straw extract + humic acid	28
5.14	Day 1 seed germination using humic acid	30

5.15	Day 3 seed germination using humic acid	31
5.16	Day 5 seed germination using humic acid	32
5.17	Day 7 seed germination using humic acid	33
5.18	Day 1 paddy straw extract plant growth	35
5.19	Day 1 humic acid plant growth	35
5.20	Day 1 paddy straw extract + humic acid plant growth	36
5.21	Day 1 control plant growth	36
5.22	Week 1 paddy straw extract plant growth	37
5.23	Week 1 humic acid plant growth	37
5.24	Week 1 paddy straw extract + humic acid plant growth	38
5.25	Week 1 control plant growth	38
5.26	Week 2 paddy straw extract plant growth	39
5.27	Week 2 humic acid plant growth	39
5.28	Week 2 paddy straw extract + humic acid plant growth	40
5.29	Week 2 control plant growth	40
5.30	Thin layer chromatography of paddy straw extract plant	43
5.31	Thin layer chromatography of humic acid plant	44
5.32	Thin layer chromatography of paddy straw extract + humic acid plant	45
5.33	Thin layer chromatography of control plant	46
5.34	Protein estimation standard graph	47

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
5.1	Result of macro and micronutrient of paddy straw extract	17
5.2	Result of macro and micronutrient of paddy straw extract + humic acid	18
5.3	Day 1 seed germination using paddy straw extract	20
5.4	Day 3 seed germination using paddy straw extract	21
5.5	Day 5 seed germination using paddy straw extract	22
5.6	Day 7 seed germination using paddy straw extract	23
5.7	Length of the sprout of paddy straw extract seed germination	24
5.8	Day 1 seed germination using paddy straw extract + humic acid	25
5.9	Day 3 seed germination using paddy straw extract + humic acid	26
5.10	Day 5 seed germination using paddy straw extract + humic acid	27
5.11	Day 7 seed germination using paddy straw extract + humic acid	28
5.12	Length of the sprout of paddy straw extract + humic acid	29
5.13	Day 1 Seed germination using humic acid	30
5.14	Day 3 seed germination using humic acid	31

5.15	Day 5 seed germination using humic acid	32
5.16	Day 7 seed germination using humic acid	33
5.17	Length of sprout of humic acid seed germination	34
5.18	Length of the paddy straw extract plant	41
5.19	Length of the humic acid plant	41
5.20	Length of the paddy straw extract + humic acid plant	42
5.21	Thin layer chromatography of paddy straw extract plant	43
5.22	Thin layer chromatography of humic acid plant	44
5.23	Thin layer chromatography of paddy straw extract + humic acid plant	45
5.24	Thin layer chromatography of control plant	46
5.25	Protein concentration of all the four sample	47

CHAPTER 1: INTRODUCTION

1.1 HUMIC ACID

The most ubiquitous naturally occurring organic molecule on Earth, humic acid is also regarded as the "most important component of a healthy fertile soil" and is one of the most biochemically active substances found in soil. (Calvo, 2014). The word humic acid refers to the brown-black, polymeric, alkali-soluble acids found in soils, plants, seagrasses, fungi, sediments, and terrestrial and marine waters (Susic *et al.*, 2016). Humic acid is the most active component of soil and compost organic matter and is the predominant portion of humic substances (HS) (Ferrara *et al.*, 2008). Humic acid is also a naturally occurring chemical in soil and a by-product of organic matter decomposition that is effectively employed in crop cultivation (Ekin *et al.*, 2019). Humic acid has a high molecular weight, is dark brown in colour, and is soluble in alkali solutions. Humic acids are a broad family of chemical compounds with similar properties that are results of organic matter transformations by soil microbes (Roy *et al.*, 2017). Humic acids are an excellent substance to employ as a supplement to synthetic or organic fertilisers (Khaled *et al.*, 2011).



Fig 1.1 humic acid

1. SOURCE OF HUMIC ACID

It is crucial to return plant waste from harvested crops to the soil since humic acids are often generated in senescent plant matter (Susic *et al.*, 2016). In agriculture, low rank coal may be effectively exploited as a rich supply of humic acids (Huculak-Maczka *et al.*, 2018). Huge quantities of lignite coal, a rich source of humic acids, were employed in agriculture by several developing nations (Susic *et al.*, 2016). Humic acid includes 51% to 57% C, 4% to 6% N, and 0.2% to 1% P, as well as trace levels of other micronutrients (Waqas *et al.*, 2014).

2. BENEFITS OF HUMIC ACID

Humic acid has several positive benefits, including:

1. Adding organic matter to soils with a lack of organic matter.
2. Improving nutrient absorption.
3. Increasing chlorophyll production.
4. Improve root health.
5. Better germination of seeds.
6. Greater ability to retain fertiliser.
7. Stimulate virtuous microbial activity.
8. Plants that are healthier and produce more.

Humic acids help to free up nutrients in the soil so that they may be used by the plant as needed (Khaled *et al.*, 2011). Because humic acid molecules are tiny, they may "achieve the plant plasma membrane, where they effectively influence nutrient assimilation" (Quilty, 2011). Humic acid also efficiently collects harmful heavy metals (Sinha *et al.*, 2011). HA can improve chemical, biological, and physical soil qualities while increasing nutrient availability (Meganind *et al.*, 2015). The direct and indirect effects of HA on cell membranes result in improved protein synthesis, increased microbial populations, plant hormone-like activity, promoted photosynthesis, modified enzyme activities, solubility of micro- and macro-elements, and reduced active levels of toxic minerals, among other positive effects on plant growth and development (Hamideh *et al.*, 2013).

2. ORGANIC FERTILIZERS

Organic fertilisers are mineral sources that are naturally available and include a moderate amount of necessary plant nutrients. Compost, blood meal and humic acid, natural enzyme-digested proteins, fish muck, and other organic fertilisers can be natural (manure and slurry) or processed (Ciesielczuk *et al.*, 2015). Organic fertilizers function as slow-release fertilisers, providing nutrients in smaller amounts over a longer period of time. The three primary macronutrients required for plant development are nitrogen (N), phosphorus (P), and potassium (K). Synthetic or quick-release fertilizers include a high concentration of soluble nitrogen, which dissolves readily in water. The bulk of organic fertilizers, on the other hand, contain a balanced quantity of raw nitrogen and hence function as slow-release fertilizers. Organic fertilizers, by definition, reduce the danger of eutrophication, ground water pollution, and overfertilization (Hitha *et al.*, 2021).

1. BENEFITS OF ORGANIC FERTILIZER

- Improve soil parameters (microbiological, physicochemical, and biochemical) and hence impact soil quality.
- Assist in restoring organic matter loss in the short and long term, hence maintaining soil fertility.
- They improve the current soil nutrients, allowing for healthy development with low nutrient densities.
- Minimise environmental impact while increasing crop yields and achieving sustainable agricultural production levels

1.3 PADDY STRAW

The vegetative portion of the rice plant that is left over after the grains are harvested is known as paddy straw. For more than half of the world's population, rice (*Oryza sativa* L.) serves as their main source of nutrition. It is thought that rice was the first crop ever to be cultivated. 90-95% of the Asia produces the majority of the rice that is produced worldwide (Arunakumara *et al.*, 2013; Rathna Priya *et al.*, 2019). The three most important food crops in the world are rice, wheat, and maize, which together account for 51% of all calories consumed (Madhukar, 2022).

Rice is one of the world's most widely consumed grains. China leads the world in rice consumption, with India coming in second (Shahbandeh, 2022). The global rice production in 2021-2022 is estimated to be 509.87 million tons, 2.63 million tons higher than the previous year, 2020-2021 ("Production volume of milled rice worldwide 2021/22, Statista", 2022). The total rice production in India in 2020-21 is expected to be 121.46 million tonnes. It is 9.01 million tonnes higher than the five-year average of 112.44 million tonnes (Ministry of Agriculture & Farmers Welfare, 2021). Approximately 13.95% of the nation's total rice production is produced in West Bengal, with Punjab (12 million tonnes) and Uttar Pradesh (13.75 million tonnes) vying for second and third place, respectively. The other major rice-producing states in India include Tamil Nadu, Andhra Pradesh, Bihar, Chhattisgarh, Odisha, Assam, and Kerala (TractorGuru, 2021).



Fig 1.2 paddy straw

CHAPTER 2 LITERATURE SURVEY

The residues of rotting plant and animal components, such as lignin, tannins, cellulose, and cutins, are known as humic substances (HS) (Tan *et al.*, 2000; Billingham, 2012; Hayes and Swift, 2020). After adding harvested residues to the soil, significant amounts of HS are still present (Wiesler *et al.*, 2016). Because of increased animal and biogas production, the number of harvested residues on most arable land has decreased, resulting in lower HS in the soil. Researchers have attempted to replenish the decreased HS with external applications over the last several decades (Rose *et al.*, 2014; Gerke, 2018). Soils, coal, lignite, and organic materials are the most common commercial sources of HS (Gollenbeek and Van Der Weide, 2020; Yang *et al.*, 2021).

Based on their solubility in water, acidic or alkaline solutions, HS are classified as humic acids (HA), fulvic acids (FA), and humins (De Melo *et al.*, 2016). Researchers have concentrated on the HA and FA fractions because they can quickly increase soil fertility and health and because the humin fraction in HS is non-degrading. Because of their chemical reactivity and ability to withstand microbial reactions, the HA and FA fractions of HS play advantageous roles in soils and plants (Billingham, 2012). The ability of HA to withstand degradation for extended periods of time, as well as its amphiphilic properties, allow it to form complex cations (Wood, 1996). The HA fraction contains approximately 60% organic carbon (C), which is essential for the growth of soil microorganisms (Sible *et al.*, 2021). Aside from C, HA contains nitrogen (N), oxygen (O), hydrogen (H), and sulfur (S).

Humic acids play several important roles, including increasing soil physical and biochemical activities by improving structure, texture, water holding capacity (WHC), and microbial population (Nardi *et al.*, 2017, 2021; Fuentes *et al.*, 2018; Shah *et al.*, 2018); increasing soil nutrients availability, particularly micronutrients, by chelating and co-transporting micronutrients to plants (Yang *et al.*, 2021); Humic acids boost crop growth by increasing plant growth hormones like auxin and cytokinin, which aid in stress resistance, nutrient metabolism, and photosynthesis (Billingham, 2012; Rose *et al.*, 2014; Canellas *et al.*, 2020; Laskosky *et al.*, 2020; Nardi *et al.*, 2021; van Tol de Castro *et al.*, 2021). Some studies have also found that HA has no effect on crop growth or soil health (Albiach *et al.*, 2001; Bybordi and Ebrahimian, 2013; El-

Bassiouny *et al.*, 2014; Mukherjee *et al.*, 2014; Kelapa and Banyuasin, 2016). Although high HA doses are associated with improved soil physical properties (Gollenbeek and Van Der Weide, 2020), their effects on soil chemical properties and crops remain unknown (Rose *et al.*, 2014). Rose *et al.* (2014) found that among the factors studied in mostly greenhouse experiments, HA source had a significant effect on both root and shoot growth, whereas application rate only had a significant effect on shoot growth. According to De Melo *et al.* (2016), carboxylic (COOH) and phenolic (OH) groups are the most common HA features that are responsible for their functions in the soil. According to a recent review by Nardi *et al.* (2021), the chemical and molecular structures of HA, as well as their sources and application rates, are critical in determining their effects on crops and soil. Importantly, HA application can result in inconsistent yield results, possibly due to different HA biological origins (Sible *et al.*, 2021).

According to several studies (Rupiasih, 2005; Garcíá *et al.*, 2016; Garca *et al.*, 2019; Nardi *et al.*, 2021; van Tol de Castro *et al.*, 2021), the functions of HA are linked to their source-dependent architectures. Although there are several functional groups in the HA structure, the phenolic (OH) and carboxylic (COOH) groups are the most prevalent (Nardi *et al.*, 2021). COOH and OH functional groups are primarily important for HA activities such as increasing soil physical and chemical characteristics and plant development (De Melo *et al.*, 2016; Nardi *et al.*, 2021). Separation of these functional groups results in the formation of polar and non-polar ends, which correspond to the hydrophilic and hydrophobic sections, respectively (Mirza *et al.*, 2011); both ends play roles in the mechanisms that bestow desirable HA properties. The hydrophilic end is associated with chelating capabilities, whereas the hydrophobic end is associated with repelling functions (Billingham, 2012). After the OH and COOH groups dissolve, the polar end of the anionic component forms complexes with cationic metals in the soil via electrostatic bonding, therefore maintaining these metals in the soil. The hydrophilic component, which is also water-loving, creates micelles, which boost soil WHC. The non-polar end, on the other hand, repels water molecules, limiting water penetration and increasing clay aggregate stability (Billingham, 2012). According to a recent study by van Tol de Castro *et al.*, (2021), the aromatic and aliphatic functional groups of HA were responsible for enhancing N absorption and soluble sugars, resulting in an increase in rice output. A previous study conducted by Garcíá *et al.*

(2016) found that aliphatic and aromatic functional groups in humic substances promoted the growth of roots in rice seedlings.

The practise of ploughing and planting on the same ground year after year has a deleterious impact on soil texture and structure. The use of HA has been shown to improve the texture and structure of deteriorated soils (Billingham, 2012; Yang *et al.*, 2021). Increased HA adsorption onto clay surfaces has been linked to soil structural stability (Chen *et al.*, 2017). Chelates with cationic metals are created when HA is added (Yamaguchi *et al.*, 2004; Billingham, 2012). These metals create complexes by serving as a bridge between the surfaces of HA and clay.

The effects of HA on soils and crops are dependent on the source of the HS (Rose *et al.*, 2014; Gollenbeek & Van Der Weide, 2020). The kind of HA used is determined by several aspects, including nutritional makeup, manner of manufacture, functional group composition, and intended use. Compost from manure, compost from green waste, soil, brown coal, and peat were the five main HA sources studied for their usefulness on crop agronomic parameters (reviewed by Rose *et al.*, 2014). The bioactivity potential of HA isolated from various organic sources varies (Martinez-balmori *et al.*, 2014). Commercially manufactured HA was shown to be less effective than HA synthesised from waste materials (Arancon *et al.*, 2006). Jindo *et al.* (2020) also discovered that HA made from composted materials was effective in raising plant physiological and agronomic activity. Khan *et al.*, (2018) did not discover any appreciable variations in wheat production between treatments using HA made from coal and plants. varying sources of HA have varying chemical and nutritional makeups, which can affect how well they operate in the soil. Garca *et al.*, (2019) discovered varying concentrations of functional groups in HA derived from Hill soil, Su Wanee River, peat, leonardite, and Elliot soil. Hamad and Tantawy (2018) conducted a pot experiment using three distinct sources of HA derived from clayey soil, podrite, and compost. They found that the amount of aromatic, aliphatic, and carboxyl functional groups contained in the various HA sources related to the growth of the sorghum root and shoots. Furthermore, high levels of the HA carboxylic functional group were favourably linked with N uptake (Piccolo *et al.*, 1992; Nardi *et al.*, 2000). But when different HA sources (such as lignite, soil, compost, coal, and peat) were investigated for their ability to kill fungus, HA sources with rich aromatic functional groups showed a negative correlation with this ability (Wei *et al.*, 2018).

This suggests that choosing HA sources should be done with a specific goal in mind. The results of a pot experiment by Laskosky *et al.* (2020) employing three distinct HA-containing sources with diverse chemical properties—humalite, peat, and biochar—resulted in barley plants with considerably higher N and P concentrations. There are few studies that assess and compare the impact of various HA sources on crop agronomic parameters in both lab and field settings, necessitating more study.

CHAPTER 3: AIM AND SCOPE

1. AIM:

- To develop novel fertilizer for crop growth using humic acid left out paddy straw-soaked water of mushroom production.
- To evaluate the effect of rice straw-soaked water on the growth of ground nut plant
- To evaluate the physical parameters by pot studies
- Nutrient analysis of the product

2. SCOPE:

- To formulate the fertilizer using humic acid enriched left out rice straw-soaked water of mushroom production.

CHAPTER 4: MATERIALS AND METHODS

1. MATERIALS:

- Paddy straw
- Ignite
- acetone
- Acetic acid
- Ethyl acetate
- Hexane
- Silica gel 60F254
- Bovine serum albumin
- Sodium carbonate
- Copper sulphate
- Sodium hydroxide
- Folin's phenol reagent
- Sodium potassium tartrate
- Potassium hydroxide
- Electronic balance
- Burette
- Distilled water
- Magnetic stirrer
- Centrifuge
- Sonicator
- Red soil
- Compost
- Petri plates
- Filter paper
- Cotton
- Spray bottle
- Petri plates
- Plant nursery bag
- Scissors
- Water

2. PADDY STRAW EXTRACTION

- Collection of paddy straw
- 150 grams of paddy straw was taken in a 2-litre conical flask mixed with 2 litres conical flask.
- Was kept for soaking for a whole day.
- After soaking it for 1 day the paddy straw extract was collected, a total of 1200ml paddy straw extract was collected.
- The paddy straw extract was boiled in heating mantle and ready.

3. HUMIC ACID PRODUCTION

- Collected the sample lignite 10 grams lignite was mixed with 150ml distilled water.
- The sample was sonicated with KOH was added dropwise.
- The sample were heated with constant stirring with the help of magnetic stirrer.
- Allowed to settle for day and collected the supernatant.
- Allow it settle for one day.
- Passed it through a through a filter paper and allowed it dry in the hot air oven.

4. FORMULATION OF RICE STRAW SOAKED WITH HUMIC ACID

- Paddy straw extract was mixed with humic acid.
- Paddy straw extract was taken in a conical flask 100ml and 1 gram of humic acid in powdered form and mixed.

5. CHARACTERIZATION STUDIES

1. *MACRO AND MICRONUTRIENT TEST*

- Paddy straw extract was given for macro and micronutrient test. This was done at the Chennai testing laboratory private limited.
- Paddy straw extract + humic acid was given for macro and micronutrient test. This was done at the Chennai testing laboratory private limited.

6. SEED GERMINATION STUDIES

- 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 ten concentration were prepared.
- Starting from 0.1 – 9.1 to 1.0 – 9.0. 0.1 ml of paddy straw extract was mixed with 9.1 ml of water like this a total of 10 concentration were taken for seed germination with all the three-sample paddy straw extract, humic acid and paddy straw extract + humic acid.
- The ground nut seeds were surface sterilized with 30% ethanol.
- The ground nut seed were soaked in for 1 hour. 0.1 - 9.9 10 seeds were soaked in this concentration likewise 10 seeds in every concentration.
- A total of 30 petri plates were used where 10 petri plates were used for paddy straw extract seed germination 10 petri plated were used for humic acid seed germination and 10 petri plates for paddy straw extract + humic acid seed germination.
- A separate petri plate was used for the control.
- The control seed were soaked in water for 1 hour.
- Each petri plate was layered with thin cotton layer in the bottom and filter paper on top of it.
- After soaking the ground nut seeds for one hour in the concentration the seeds were transferred to petri plates and observed for 1 week.
- Every day water was sprayed using spray bottle over 7 days and length was observed.

7. POT STUDIES

- 3 ground nut seeds were taken and soaked in each concentration for one hour in all the three-sample paddy straw extract, paddy straw extract + humic acid and humic acid.
- 3 ground nut seeds were soaked in plain water and kept as control.
- 93 plant bags were taken, 30 bags for each sample and 3 bags for concentration.
- A total of 25kg red soil was mixed with 2kg compost.
- All the plant bags were filled with approximately 250grams of pot mix.
- The soaked seeds were planted in plant bags, 3 seed per bag.
- The seeds were watered regularly about 12ml per bag, and its growth and length were observed.

8. BIOCHEMICAL ANALYSIS

The biochemical analysis is performed to find the protein concentration in the ground nut plant the biochemical test performed are protein estimation by lowry's method and TLC Thin Layer Chromatography

1. *TLC Thin Layer Chromatography*

- Prepare the plant extract with solvent (1 gm plant and 10 ml acetone)
- Solvent extract was subjected to thin layer chromatography one dimensional ascending method using silica gel 60F254.
- They were cut into 7x6 cm using ordinary household scissors.
- Plate marking were used to spot the sample for TLC applied sample volume 1 - micro litre with using capillary at 1 cm.
- Different solvent system was prepared.
- Solvent system 1: Hexane: Acetic acid (9:1)
- Solvent system 2: Hexane: Ethyl acetate: Acetic acid (5:4:1)
- Solvent system 3: Hexane: Ethyl acetate: Acetic acid (4:4:2)
- Solvent system 4: Hexane: Ethyl acetate: Acetic acid (3:6:1)
- Solvent system 5: Hexane: Ethyl acetate: Acetic acid (2:7:1)

- After pre-saturation with mobile phase for 20 minutes for development were used.
- The plate is visualized shining a UV light on the plate may visualize the location of the separated spot.
- The movement of the active compound was expressed by its retention factor (Rf), values.

2. ESTIMATION OF PROTEIN BY LOWRY'S METHOD

Reagents:

- Solution 1: 2 grams sodium carbonate + 0.4 grams NaOH + 100ml distilled water.
- Solution 2: 1 gram sodium potassium tartrate + 100mg Copper sulfate + 50 ml distilled water
- Alkaline copper sulfate solution: 2 ml of solution 2 is mixed with the solution 1, just prior to use.
- Folin's phenol reagent
- Standard protein solution: dissolve 100mg of BSA in 100ml distilled water.

Procedure:

- pipette 0.2, 0.4, 0.6, 0.8, and 1.0 millilitres of the protein solution into a series of test tubes
- Then add water to the mixture get up to 1 millilitre.
- Each test tube receives 5 ml of the Alkaline-copper sulfate solution, which is pipetted out, thoroughly mixed.
- After thoroughly mixed, they are allowed to settle for 10 to 15 minutes at room temperature.
- Each test tube receives 0.5ml of the reagent and incubate it for 30 minutes.
- Measure the absorbance at 620 nm and plot the standard graph.
- The test tube with 1ml distilled water serve as blank.
- Estimate the amount of protein present in given sample from the standard graph.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 PADDY STRAW EXTRACTION

The paddy straw was soaked in water in a conical flask for one day. 150grams paddy straw was soaked with 2 litres water and after soaking it for one day paddy straw extract is collected the next day as shown in fig 5.1 and fig 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 lignite was mixed with 150ml distilled water and then sonicated with KOH and the sample was stirred in magnetic stirrer and allowed to settle for a day and pass it through filter paper and allowed it dry in the hot air oven.



Fig 5.3: Humic acid

5.3 FORMULATION OF RICE STRAW SOAKED WITH HUMIC ACID

The paddy straw extract was mixed with humic acid 1ml of humic acid was mixed with 100ml of paddy straw extract.



Fig 5.4: formulation of rice straw soaked with humic acid

4. CHARACTERIZATION STUDIES

1. *macro and micronutrient test paddy straw extract and paddy straw extract + humic acid*

The Paddy straw extract macro and micronutrient test was done, and the results are tabulated.

Table 5.1: Result of macro and micronutrient of paddy straw extract

s.no	Parameters	Methods	Units	Results
1	Ph	4500-H+-B APHA 23rd.Edn.2017	-	8.9
2	Conductivity	2510-B-APHA 23rd.Edn.2017	µs/cm	6580
3	Nitrogen as N	4500-NH3 - B,C -APHA 23rd.Edn.2017	mg/l	940
4	Potassium as K	3500-K-B- APHA 23rd.Edn.2017	mg/l	522
5	Calcium as ca	3500-Ca-B- APHA 23rd.Edn.2017	mg/l	80
6	Magnesium as Mg	3500-Mg-B- APHA 23rd.Edn.2017	mg/l	146
7	Phosphorus as P	4500-P-D- APHA 23rd.Edn.2017	mg/l	104
8	Cation exchange capacity	FAO Method,2007 (Flame Photometric Method)	meq/l	27.6
9	Total organic carbon	By calculation	%	0.23
10	Sulphate as SO4	4500-SO4-B APHA 23rd.Edn.2017	mg/l	58
11	Boron as B	4500-B-A APHA 23rd.Edn.2017	mg/l	BDL(DL:0.5)
12	Iron ad Fe	3500-Fe-B APHA 23rd.Edn.2017	mg/l	3.84
13	Manganese as Mn	3111-B-APHA 23rd.Edn 2017	mg/l	10.8
14	Zinc as Zn	3111 C APHA 23rd.Edn.2017	mg/l	4.12
15	Copper as cu	3111-B-APHA 23rd.Edn.2017	mg/l	BDL(DL:0.12)

5.4.2 Macro and micronutrient test of paddy straw extract + humic acid

The Paddy straw extract + humic acid macro and micronutrient test was done, and the results are tabulated.

Table 5.2: Result of macro and micronutrient test of paddy straw extract + humic acid

s.no	Parameters	Methods	Units	Results
1	Ph	4500-H+-B APHA 23rd.Edn.2017	-	8.9
2	Conductivity	2510-B-APHA 23rd.Edn.2017	µs/cm	6589
3	Nitrogen as N	4500-NH3 - B,C -APHA 23rd.Edn.2017	mg/l	1020
4	Potassium as K	3500-K-B- APHA 23rd.Edn.2017	mg/l	580
5	Calcium as ca	3500-Ca-B- APHA 23rd.Edn.2017	mg/l	80
6	Magnesium as Mg	3500-Mg-B- APHA 23rd.Edn.2017	mg/l	170
7	Phosphorus as P	4500-P-D- APHA 23rd.Edn.2017	mg/l	126
8	Cation exchange capacity	FAO Method,2007 (Flame Photometric Method)	meq/l	25.9
9	Total organic carbon	By calculation	%	0.28
10	Sulphate as SO4	4500-SO4-B APHA 23rd.Edn.2017	mg/l	64
11	Boron as B	4500-B-A APHA 23rd.Edn.2017	mg/l	BDL(DL:0.5)
12	Iron ad Fe	3500-Fe-B APHA 23rd.Edn.2017	mg/l	4.32
13	Manganese as Mn	3111-B-APHA 23rd.Edn 2017	mg/l	11.7
14	Zinc as Zn	3111 C APHA 23rd.Edn.2017	mg/l	5.05
15	Copper as cu	3111-B-APHA 23rd.Edn.2017	mg/l	BDL(DL:0.12)

5. SEED GERMINATION

1. SEED GERMINATION USING PADDY STRAW EXTRACT

The seed germination was done using all the three-sample starting with paddy straw extract sample a total of 100 ground nut seeds were used and 10 seeds were used for each concentration that is 0.1 – 9.9 to 1.0 to 9.0 concentration all the seed were soaked in ten concentrations for 1 hour. The ground nut seeds were observed for seven days the seed growth was noted and water was sprayed using water sprayer.



Fig 5.5 ground nut seeds soaked in paddy straw extract for 1 hour

Table 5.3: Day 1 seed germination using paddy straw extract

Paddy straw extract (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	5	5
0.2	9.8	-	-	10
0.3	9.7	-	1	9
0.4	9.6	-	5	5
0.5	9.5	-	-	10
0.6	9.4	-	2	8
0.7	9.3	-	1	9
0.8	9.2	-	2	8
0.9	9.1	-	3	7
1.0	9.0	-	4	6
control		-	-	10



Fig 5.6: Day 1 seed germination using paddy straw extract

Table 5.4: Day 3 seed germination using paddy straw extract

Paddy straw extract (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	-	2	8
0.3	9.7	-	6	4
0.4	9.6	-	8	2
0.5	9.5	-	6	4
0.6	9.4	-	9	1
0.7	9.3	-	6	4
0.8	9.2	-	7	3
0.9	9.1	-	6	4
1.0	9.0	-	6	4
Control		-	-	10



Fig 5.7: Day 3 seed germination using paddy straw extract

Table 5.5: Day 5 seed germination using paddy straw extract

Paddy straw extract (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	-	3	7
0.3	9.7	-	8	2
0.4	9.6	-	9	1
0.5	9.5	-	8	2
0.6	9.4	-	10	-
0.7	9.3	-	7	3
0.8	9.2	-	9	1
0.9	9.1	-	8	2
1.0	9.0	-	8	2
control		-	-	10



Fig 5.8: Day 5 seed germination using paddy straw extract

Table 5.6: Day 7 seed germination using paddy straw extract

Paddy straw extract (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	1	5	4
0.3	9.7	-	10	-
0.4	9.6	-	10	-
0.5	9.5	-	9	1
0.6	9.4	-	10	-
0.7	9.3	-	9	1
0.8	9.2	-	10	-
0.9	9.1	6	4	-
1.0	9.0	1	8	1
control		-	-	10



Fig 5.9: Day 7 seed germination using paddy straw extract

Table 5.7: Length of the sprout of paddy straw extract seed germination

Paddy straw extract	Water	Length of the sprout
0.1	9.9	5cm
0.2	9.8	1cm
0.3	9.7	4cm
0.4	9.6	7cm
0.5	9.5	5cm
0.6	9.4	5.5cm
0.7	9.3	6cm
0.8	9.2	5cm
0.9	9.1	2.5cm
1	9	4cm
Control		No growth

From the table 5.6 and 5.7 we understand that the concentration 0.3 - 9.7, 0.6 - 9.4, 0.8 - 9.2 in paddy straw extract have a 100% seed growth rate and a longer sprout compared to other concentration.

5.5.2 SEED GERMINATION USING PADDY STRAW EXTRACT + HUMIC ACID

The seed germination was done was done using paddy straw extract + humic acid sample a total of 100 ground nut seed were used and 10 seeds for each concentration the seed growth was noted, for seven days water was sprayed every day using water sprayer.

Table 5.8: day 1 seed germination using paddy straw extract + humic acid

Paddy straw extract + Humic acid (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	-	10	-
0.3	9.7	-	8	2
0.4	9.6	-	10	-
0.5	9.5	-	9	-
0.6	9.4	-	5	5
0.7	9.3	1	8	1
0.8	9.2	-	5	5
0.9	9.1	-	6	4
1.0	9.0	-	8	2
control		-	-	10



Fig 5.10: Day 1 seed germination using paddy extract + humic acid

Table 5.9: Day 3 seed germination using paddy straw extract + humic acid

Paddy straw extract + humic acid(ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	-	10	-
0.3	9.7	-	10	-
0.4	9.6	1	9	-
0.5	9.5	-	10	-
0.6	9.4	1	7	2
0.7	9.3	1	8	-
0.8	9.2	1	6	3
0.9	9.1	-	8	2
1.0	9.0	3	7	-
control		-	-	10



Fig 5.11: Day 3 seed germination using paddy straw extract + humic acid

Table 5.10: Day 5 seed germination using paddy straw extract + humic acid

Paddy straw extract + humic acid (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	9	1	-
0.3	9.7	7	3	-
0.4	9.6	*	9	-
0.5	9.5	-	10	-
0.6	9.4	-	7	2
0.7	9.3	-	8	-
0.8	9.2	-	8	1
0.9	9.1	5	5	-
1.0	9.0	1	6	-
control				10

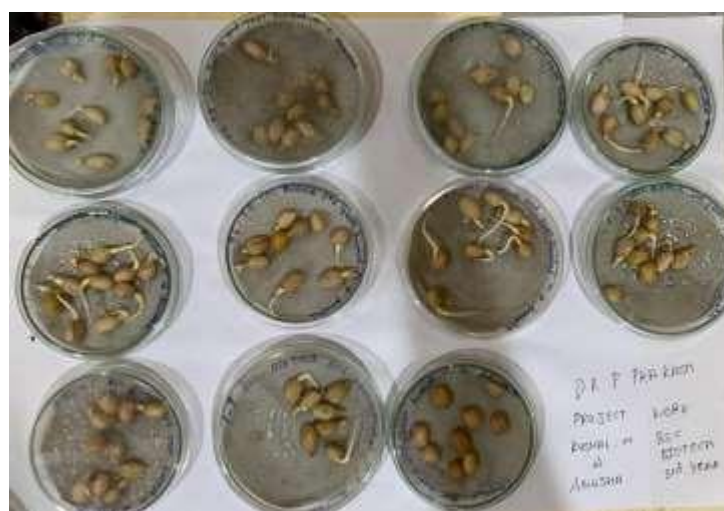


Fig 5.12: Day 5 seed germination using paddy straw extract + humic acid

Table 5.11: Day 7 seed germination using paddy straw extract + humic acid.

Paddy straw extract + humic acid (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	9	1	-
0.3	9.7	-	3	-
0.4	9.6	-	9	-
0.5	9.5	-	10	-
0.6	9.4	-	8	1
0.7	9.3	-	8	-
0.8	9.2	-	8	1
0.9	9.1	-	5	-
1.0	9.0	-	6	-
control		-	-	10



Fig 5.13: Day 7 seed germination using paddy straw extract + humic acid

Table 5.12: Length of the sprout of paddy straw extract + humic acid seed germination

Paddy straw extract + humic acid	Water	Length of the sprout
0.1	9.9	2cm
0.2	9.8	0.5cm
0.3	9.7	2cm
0.4	9.6	6cm
0.5	9.5	4.5cm
0.6	9.4	4.3cm
0.7	9.3	4.4cm
0.8	9.2	4cm
0.9	9.1	1cm
1	9	2.5cm
Control		Control

from the table 5.11 and 5.12 we understand that the concentration 0.4 - 9.6, 0.5 - 9.5, 0.6 - 9.4, 0.7 - 9.3 of the paddy straw extract + humic acid has a better seed growth rate and a better sprout length compared to other concentration.

5.5.3 SEED GERMINATION USING HUMIC ACID

The seed germination was done using humic acid sample a total of 100 ground nut seed were used and 10 seeds for each concentration the seed growth was noted, for seven days water was sprayed every day using water sprayer.

Table 5.13: Day 1 seed germination using humic acid

humic acid (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	5	5
0.2	9.8	-	5	5
0.3	9.7	-	5	5
0.4	9.6	-	4	6
0.5	9.5	-	3	7
0.6	9.4	-	4	6
0.7	9.3	-	2	8
0.8	9.2	-	5	5
0.9	9.1	-	2	8
1.0	9.0	-	1	9
control		-	3	7



Fig 5.14: Day 1 seed germination using humic acid

Table 5.14: Day 3 seed germination using humic acid

humic acid (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	-	10	-
0.2	9.8	-	10	-
0.3	9.7	-	10	-
0.4	9.6	-	10	-
0.5	9.5	-	9	1
0.6	9.4	-	7	3
0.7	9.3	-	10	-
0.8	9.2	-	6	4
0.9	9.1	-	10	-
1.0	9.0	-	7	3
control		-	3	7



Fig 5.15: Day 3 seed germination using humic acid

Table 5.15: Day 5 seed germination using humic acid

humic acid (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	7	3	-
0.2	9.8	-	7	3
0.3	9.7	-	7	3
0.4	9.6	-	6	4
0.5	9.5	2	3	5
0.6	9.4	-	5	5
0.7	9.3	6	-	4
0.8	9.2	3	5	2
0.9	9.1	1	8	1
1.0	9.0	2	6	2
control		1	2	7

**Fig 5.16: Day 5 seed germination using humic acid**

Table 5.16: Day 7 seed germination using humic acid

humic acid (ml)	Water (ml)	No of seed contaminated	No of seed grown	No of seed not grown
0.1	9.9	10	-	-
0.2	9.8	-	7	3
0.3	9.7	-	5	5
0.4	9.6	1	3	6
0.5	9.5	4	6	-
0.6	9.4	-	5	5
0.7	9.3	10	-	-
0.8	9.2	5	5	-
0.9	9.1	2	8	-
1.0	9.0	4	6	-
control		1	2	7



Fig 5.17: Day 7 seed germination using humic acid

Table 5.17: Length of sprout of humic acid seed germination

humic acid	Water	Length of the sprout
0.1	9.9	0.5cm
0.2	9.8	1cm
0.3	9.7	0.5cm
0.4	9.6	1cm
0.5	9.5	2cm
0.6	9.4	0.5cm
0.7	9.3	2cm
0.8	9.2	1cm
0.9	9.1	2.5cm
1	9	1cm
Control		1.5cm

From the table 5.16 and 5.17 we understand that the paddy straw extract and paddy straw extract + humic acid shows better result compared to humic acid the length of sprout is smaller compared to another sample.

6. POT STUDIES

The seeds were soaked in the 10 concentrations for 1 hour and were kept in the plant bag for growth.

1. *Day 1 pot studies*



Fig 5.18: day 1 paddy straw extract plant growth



Fig 5.19: day 1 humic acid plant growth



Fig 5.20: day 1 paddy straw extract + humic acid plant growth



Fig 5.21: day 1 control plant growth

5.6.2-week 1 pot studies



Fig 5.22: week 1 paddy straw extract plant growth



Fig 5.23: week 1 humic acid plant growth



Fig 5.24: week 1 paddy straw + humic acid plant growth



Fig 5.25: week 1 control plant growth

5.6.3-week 2 pot studies



Fig 5.26: week 2 paddy straw extract plant growth



Fig 5.27: week 2 humic acid plant growth



Fig 5.28: week 2 paddy straw extract + humic acid plant growth



Fig 5.29: week 2 control plant growth

Table 5.18: The length of the paddy straw extract plant

Paddy straw extract	Water	Length of the plant
0.2	9.8	18cm
0.3	9.7	17cm
0.6	9.4	17cm
0.7	9.3	18cm
0.8	9.2	11cm
0.8	9.2	14cm
1	9	23cm
Control		6cm

From the table 5.18 the concentration 1 - 9 of paddy has the highest length compared to other concentration.

Table 5.19: Length of the humic acid plant

Humic acid	Water	Length of the water
0.1	9.9	17cm
0.2	9.8	18cm
0.3	9.7	16cm
0.5	9.5	11cm
0.6	9.4	17cm
0.6	9.4	21cm
0.7	9.3	14cm
0.8	9.2	20cm
Control		6cm

From the table 5.19 we understand that the concentration 0.8 - 9.2 of humic acid has a highest plant length compared to other concentration.

Table 5.20: Length of the paddy straw extract + humic acid plant

Paddy straw extract + humic acid	Water	Length of the plant
0.2	9.8	13cm
0.4	9.6	17cm
0.6	9.4	10cm
0.8	9.2	18cm
0.8	9.2	17cm
0.9	9.1	16cm
0.9	9.1	19cm
control		6cm

From the table 5.20 we understand that the concentration 0.9 - 9.1 of paddy straw extract + humic acid has the highest length compared to other concentration.

7. BIOCHEMICAL ANALYSIS

1. THIN LAYER CHROMATOGRAPHY

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Table 5.21: Thin layer chromatography of paddy straw extract plant

No	Treatment	Rf value
Rf1	Hexane: acetic acid (9:1)	0.615
Rf2	Hexane: ethyl acetate: acetic acid (5:4:1)	0.9
Rf3	Hexane: ethyl acetate: acetic acid (4:4:2)	0.88
Rf4	Hexane: ethyl acetate: acetic acid (3:6:1)	0.905
Rf5	Hexane: ethyl acetate: acetic acid (2:7:1)	0.993

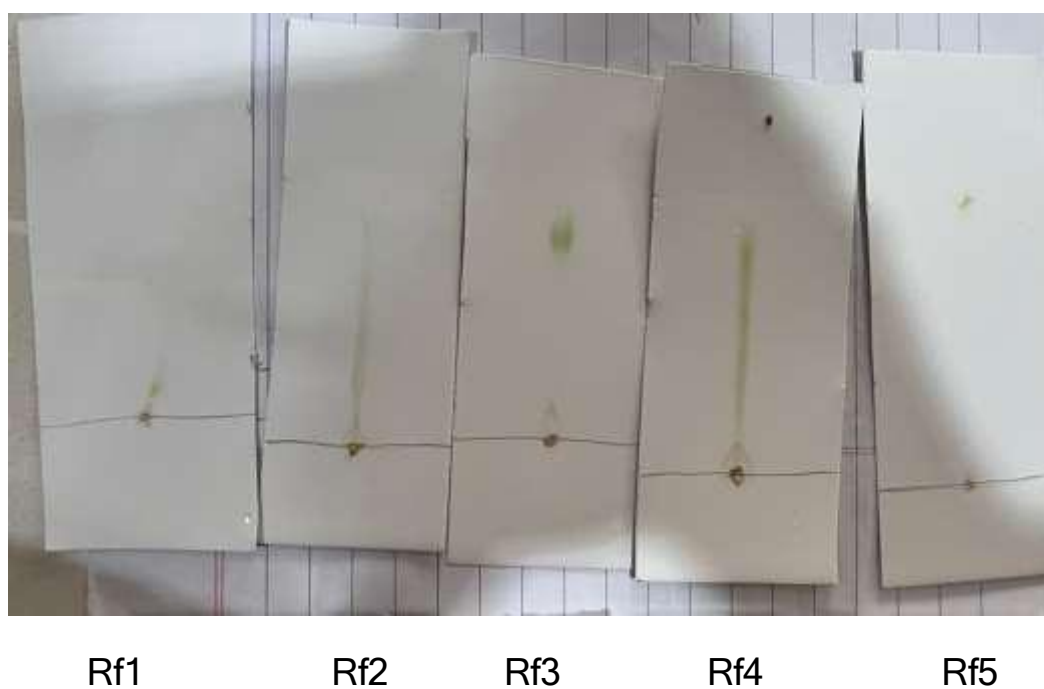
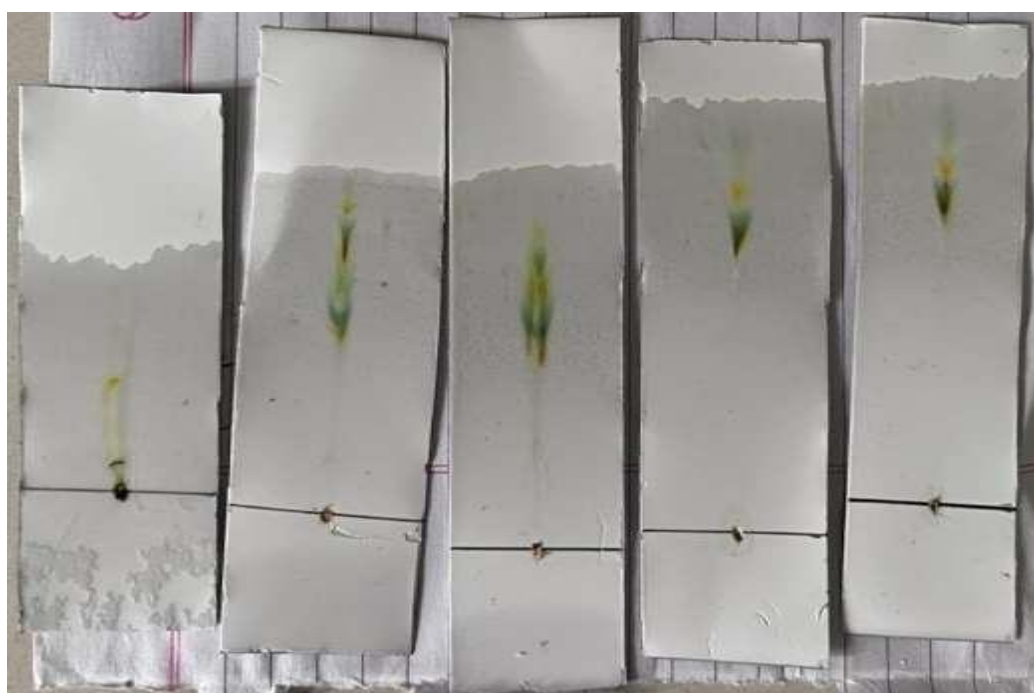


Fig 5.30: Thin layer chromatography of paddy straw extract plant

Table 5.22: Thin layer chromatography of humic acid plant

No	Treatment	Rf value
Rf1	Hexane: acetic acid (9:1)	0.526
Rf2	Hexane: ethyl acetate: acetic acid (5:4:1)	0.96
Rf3	Hexane: ethyl acetate: acetic acid (4:4:2)	0.818
Rf4	Hexane: ethyl acetate: acetic acid (3:6:1)	0.892
Rf5	Hexane: ethyl acetate: acetic acid (2:7:1)	0.859



Rf1

Rf2

Rf3

Rf4

Rf5

Fig 5.31: Thin layer chromatography of humic acid plant

Table 5.23: Thin layer chromatography of paddy straw extract + humic acid plant

No	Treatment	Rf value
Rf1	Hexane: acetic acid (9:1)	0.714
Rf2	Hexane: ethyl acetate: acetic acid (5:4:1)	0.786
Rf3	Hexane: ethyl acetate: acetic acid (4:4:2)	0.781
Rf4	Hexane: ethyl acetate: acetic acid (3:6:1)	0.808
Rf5	Hexane: ethyl acetate: acetic acid (2:7:1)	0.746



Rf1

Rf2

Rf3

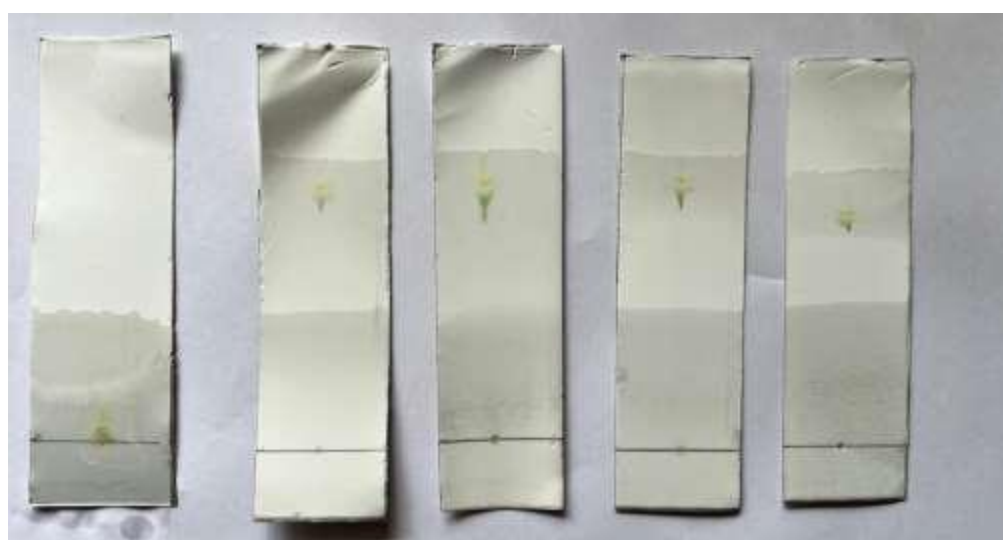
Rf4

Rf5

Fig 5.32: Thin layer chromatography of paddy straw + humic acid

Table 5.24: Thin layer chromatography of control plant

No	Treatment	Rf value
Rf1	Hexane: acetic acid (9:1)	0.363
Rf2	Hexane: ethyl acetate: acetic acid (5:4:1)	0.904
Rf3	Hexane: ethyl acetate: acetic acid (4:4:2)	0.937
Rf4	Hexane: ethyl acetate: acetic acid (3:6:1)	0.928
Rf5	Hexane: ethyl acetate: acetic acid (2:7:1)	0.870



Rf1

Rf2

Rf3

Rf4

Rf5

Fig 5.33: Thin layer chromatography control plant

5.7.2 ESTIMATION OF PROTEIN BY LOWRY'S METHOD

Table 5.25: Protein concentration of all the four sample

No	Treatment	Concentration
1	Paddy straw extract	850 μg
2	Humic acid	820 μg
3	Paddy straw extract + humic acid	830 μg
4	Control	530 μg

From the table 5.25 we understand that paddy straw extract, humic acid and paddy straw extract + humic acid have a better protein concentration than the control.

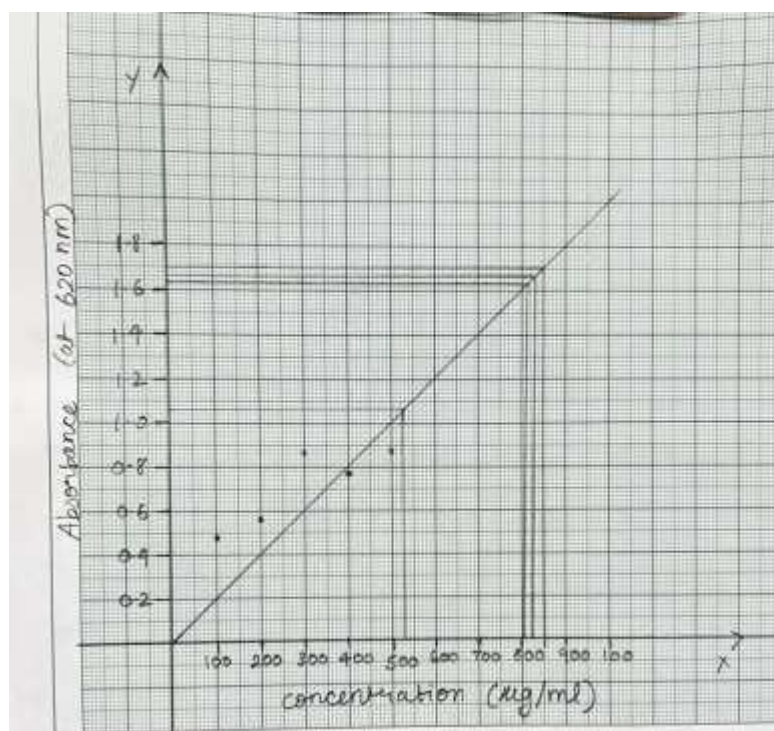


Fig 5.34: protein estimation standard graph

CHAPTER 6: SUMMARY AND CONCLUSION

6.1 SUMMARY

The paddy straw extract was prepared in our lab facility by soaking the paddy straw in water for one day. Humic acid was prepared in our lab facility with the steps involving the addition of KOH, sonication, centrifugation and drying. The formulation of paddy straw with humic acid was prepared by mixing the humic acid and paddy straw extract. Seed germination was done on ground nut seed using three different sample paddy straw extract, humic acid and paddy straw extract + humic acid with different concentration starting from 0.1 - 9.9 to 1.0 - 9.0 a total of 300 ground nut seeds were used 100 seeds for each sample and 10 ground nut seeds were kept for control was observed for a week and the length of sprout was observed. The paddy straw extract, humic acid and paddy straw extract + humic acid was further subjected to pot studies the plant bags were prepared with 250grams of red soil mixed compost and a total of 93 bags were prepared. Then the paddy straw extract and paddy straw extract + humic acid was given for macro and micronutrient test at Chennai testing laboratory private limited. The biochemical analysis was done for all the three sample and compared with the control. Thin layer chromatography and protein estimation by lowry's method to check the protein concentration of all the sample.

6.2 CONCLUSION

The study reveals that the formulated paddy straw extracts displayed certain significant nutritional components and effect on seed germination and pot studies. Since the ph of the fertilizer is high. Therefore, this study suggests that foliar spray could be applicable for crop development.

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