

BIOFERTILIZER FROM ALGAE

(Project-Report)

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Biochemistry.

By

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

Accredited with Grade "A" by NAAC | 12B Status by UGC | Approved by AICTE

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of K.RUTHRA (38070003) who carried out the project entitled **"BIOFERTILIZER FROM ALGAE"** under our supervision from October 2020 to March 2021.

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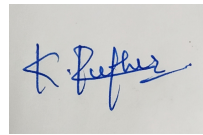
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DECLARATION

K.RUTHRA (38070003) hereby declare that the Project Report entitled **"BIOFERTILIZER FROM ALGAE"** done by me under the guidance of **Dr. RENUGADEVI** at **Sathyabama institute of Science and Technology, Chennai** is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biochemistry.

DATE: 07/04/2021



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ABSTRACT

Sustainable agriculture is advantageous over conventional agriculture for its capacity to accomplish food demand by utilizing environment resources without negatively affect .Fertilizer play a major role in agriculture crops. Algae are the kind of widespread photosynthetic organism which have a ability to enrich soil nutrients and enhance utilization of macro and micro nutrients. Algae biofertilizer presence of a large group of microorganism which have benefits to enhance the soil productivity.Algae have naturally nitrogen fixation which help to grow the plant fastly. Three types Cyanobacterial algae biofertilizer Azolla as dried plant, Spirulina as extracted powder and Geitlerinema as liquid were used to grown five different plants s(Trigonella foenum-graecum, Brassica, Sesamum indicum, Capsicum frutescens and Vigna radiata) for to known the early stage growth of the plant by measuring (cm). Algalization as been employed for observing the nitrogen ,potassium and phosphorus was taken by NPK test using NPK kit. Effect of Toxicity of algae biofertilizer on earthworm from there the length of the earth were observed for a week ,earthworm in normal soil it grown 0.5cm, in azolla biofertilizer its remain same length, in spirulina biofertilizer it grown 1m and in geitlerinema biofertilizer earthworm was shinkled and length were reduced. Apart from three algae fertilizer Azolla shown a major growth and high nitrogen fixation, Spirulina biofertilizer showed modrate growth and Geitlerinema was resulted expected result.



CHAPTER		PAGE
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NO.	TABLE OF CONTENT	PAGE NO
	TABLE	
	ABSTRACT LIST OF ABBREVIATION LIST OF TABLES LIST OF FIGURES	
1	INTRODUCTION	13
2	REVIEW OF LITERATURE	14
3	AIM AND SCOPE	18
3.1	AIM	18
3.2	OBJECTIVES	18
4	MATERIALS AND METHODOLOGY	19
4.1	MATERIALS	19
4.1.1	SELECTED SEEDS	19
4.1.2	SELECTED ALGAE	19
4.2	METHODOLOGY	19
4.2.1	SAMPLE COLLECTION	19
4.2.2	SAMPLE PREPARATION	19
4.2.3	METHODS OF PLANTING	20

4.2.4	NPK TEST	21
4.2.5	TOXICITY TEST OF ALGAE ON EARTHWORM	21
5	RESULTS AND DISCUSSION	22
5.1	EFFECT OF ALGAE ON PLANT GROWTH	22
5.2	READING OF PLANTS	25
5.3	RESULT OF NPK TEST	33
5.4	EFFECT OF ALGAE ON EARTHWORM	36
6	SUMMARY AND CONCLUSION	37



LIST OF ABBREVIATIONS AND SYMBOLS:

cm - Centimeter

g - Gram

mL - Milliliter

mg/kg - Milligram per kilogram

°C - Degree celcius

% - Percentage

BGA - Blue green algae

NPK - Nitrogen , phosphorous, potassium

CO₂ - Carbon-DI-oxide

N - Nitrogen

K - Potassium

P - Phosphorus

LIST OF FIGURES:

FIG NO:	TITLE	PAGE NO.
4.2.2.2	EXTRACTED SPIRULINA POWDER	20
4.2.2.2	AZOLLA BIOFERTILIZER	20
4.2.2.3	GEITLERINEMA LIQUID	20
5.1.1	EARLY GROWTH OF TRIGONELLA FOENUM- GRAECUM	24
5.1.2	EARLY GROWTH OF BRASSIA	24
5.1.3	EARLY GROWTH OF CAPSICUM FRUTESCEN	24
5.1.4	EARLY GROWTH OF VIGNA RADIATA	24
5.2.1.1	GRAPHICAL REPRESENTATION OF PLANT GROWTH TRIGONELLA FOENUM GRACUM IN NORMAL SOIL	26
5.2.1.2	GRAPHICAL REPRESENTATION OF PLANT GROWTH BRASSIA IN NORMAL SOIL	26
5.2.1.3	GRAPHICAL REPRESENTATION OF PLANT GROWTH SESAMUM INDICUM IN NORMAL SOIL	27
5.2.1.4	GRAPHICAL REPRESENTATION OF PLANT GROWTH CAPSICUM FRUTESCENS IN NORMAL SOIL	27
5.2.1.5	GRAPHICAL REPRESENTATION OF PLANT GROWTH VIGNA RADIATA IN NORMAL SOIL	27
5.2.2.1	GRAPHICAL REPRESENTATION OF PLANT GROWTH TRIGONELLA FOENUM GRACUM IN SPIRULINA BIOFERTILIZER	29
5.2.2.2	GRAPHICAL REPRESENTATION OF PLANT GROWTH BRASSIA IN SPIRULINA BIOFERTILIZER	29
5.2.2.3	GRAPHICAL REPRESENTATION OF PLANT	29

	GROWTH SESAMUM INDICUM IN SPIRULINA BIOFERTILIZER	
5.2.2.4	GRAPHICAL REPRESENTATION OF PLANT GROWTH CAPSICUM FRUTESCENS IN SPIRULINA BIOFERTILIZER	30
5.2.2.5	GRAPHICAL REPRESENTATION OF PLANT GROWTH VIGNA RADIATA IN SPIRULINA	30
5.2.3.1	GRAPHICAL REPRESENTATION OF PLANT GROWTH TRIGONALLA FOENUM-GRACUM IN AZOLLA BIOFERTILIZER	32
5.2.3.2	GRAPHICAL REPRESENTATION OF PLANT GROWTH BRASSIA IN AZOLLA BIOFERTILIZER	32
5.2.3.3	GRAPHICAL REPRESENTATION OF PLANT GROWTH SESAMUM INDICUM IN AZOLLA BIOFERTILIZER	32
5.2.3.4	GRAPHICAL REPRESENTATION OF PLANT GROWTH CAPSICUM FRUTESCENS IN AZOLLA BIOFERTILIZER	33
5.2.3.5	GRAPHICAL REPRESENTATION OF PLANT GROWTH VIGNA RADIATA IN AZOLLA BIOFERTILIZER	33

LIST OF TABLES:

TABLE NO.	TITLE	PAGE NO
5.1.1	SEED GROWN IN NORMAL SOIL	22
5.1.2	SEEDS GROWN IN AZOLLA BIOFERTILIZER	22
5.1.3	SEEDS GROWN IN SPIRULINA BIOFERTILIZER	23
5.1.4	SEEDS GROWN IN GEITLERINEMA BIOFERTILIZER	23
5.2.1	READING OF PLANT GROWN IN NORMAL PLANT	25
5.2.2	READING OF PLANT GROWN IN SPIRULINA BIOFERTILIZER	28
5.2.3	READING OF PLANT GROWN IN AZOLLA BIOFERTILIZER	30
5.3.1	NPK TEST VALUE OF NORMAL SOIL	34
5.3.2	NPK TEST VALUE OF SPIRULINA USED ON FEW DAYS	34
5.3.3	NPK TEST VALUE OF SPIRULINA USED ON MORE DAY	34
5.3.4	NPK TEST VALUE OF NORMAL SOIL	35
5.3.5	NPK TEST VALUE OF AZOLLA USED ON FEW DAY	35
5.3.6	NPK TEST VALUE OF AZOLLA USED ON MORE DAYS	35
5.3.7	NPK TEST VALUE OF GEITLERINEMA USED ON FEW DAYS	35
5.3.8	NPK TEST VALUE OF GEITLERINEMA USED ON MORE DAYS	36

5.4.1	LENGTH OF THE EARTHWORM	36
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CHAPTER 1: INTRODUCTION

An algal biofertilizer is a natural fertilizer. It helps to retain essential nutrients and water in the soil which is required for the proper growth of plants. Fertilizer plays a key role in agriculture crops. Biofertilizer is a substance which contains living microorganisms, when applied to plants which colonize the rhizosphere, the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizer adds nutrient through the natural process of nitrogen fixation, solubility phosphorus and stimulates plant growth through the synthesis of growth promoting substances. Biofertilizer can reduce the use of synthetic fertilizer and pesticide, but they are not yet able to replace their use. Since they play several roles a preferred scientific term for such beneficial bacteria is "plant - growth promoting rhizobacteria". Biofertilizer provides the "eco - friendly" organic agro - input. Biofertilizer such as rhizobium, azotobacter, azospirillum and BGA have been in use of long time. BGA synthesizes and liberates plant growth promoting substances such as auxins and amino compounds which stimulate plant growth.

Algae are a kind of widespread photosynthetic organism including eukaryotic green algae and prokaryotic blue algae. These fascinating organisms can be used in modern agriculture for their ability to enrich soil nutrient and enhance utilization of macro and micro nutrients. This was to assess the effect of marine algae fertilizer on growth of plants. The algae treatment increased growth performance at the early stage of growth and improved yield characteristics. Thus marine algae exhibited high growth and yield revealed the potential of the marine algae as fertilizer in plants. Algae extracts have been researched for several years to be used as a fertilizer. Beneficial effects of using algae have been reported in various parts. The simple presence of cyanobacteria in soil results in formation of soil aggregate, which reduces soil loss during rainy season and regulates aeration, soil temperature hence, improving physical and chemical properties of soil vis-à-vis physical environment of the plants. They have the potential to mobilize the insoluble forms of inorganic phosphates. Some beneficial activities of algae such as, it symbiotically associates with the plant roots. It maintains the natural habitat of the soil. It increases the crop yield by 20%-30% replaces chemical nitrogen and phosphorus by 30% and stimulates plant growth. Biofertilizer also promotes healthy soil, leading to greater farming sustainability. Cyanobacteria secrete growth promoting substances like IAA, IBA, IAA, Amino acids, proteins, vitamins etc.,. They add sufficient number of organic matter in soil. Azolla fixes N, increases organic matter and fertility in soil and also it shows tolerance against heavy metals. Algae was a renewable energy source.

Cyanobacteria are one of the major components of agricultural fields. The agricultural importance of BGA is related directly with their ability to fix nitrogen and other beneficial effects for plants and soil. Cyanobacteria have an emerging potential as biofertilizer. They have the ability to utilize CO₂, water, and nutrients to convert solar energy into biomass. Efficient application of cyanobacteria have been reported in agricultural practices to reduce global warming by decreasing CO₂ gas.



Spirulina is an organism that grows in both fresh and salt water. It is a type of cyanobacteria which is a family of single-celled microbes referred to as blue-green algae. Cyanobacteria can produce energy from sunlight through photosynthesis. Spirulina as a completely natural and healthy fertilizer. When compared to chemical fertilizer spirulina enhances the plant growth in terms of protein content. The spirulina fertilizer thus prepared makes a complete nutrition package for the plants that results in growth and higher yield. Gardeners have used spirulina as complete, effective foliar plant food. The spirulina fertilizer can be prepared in liquid and powder form to suit the requirements of the farmers and their application techniques.

Azolla is an aquatic fern consisting of a short, branched, floating stem, bearing roots, triangular or polygonal in shape and float on the surface of the water. The leaves are alternately arranged, each consisting of thick aerial dorsal lobe containing green chlorophyll. The most remarkable characteristics of azolla is its symbiotic relationship with the nitrogen-fixing blue-green algae (cyanobacteria) *Anabaena Azolla*. The fern provides nutrients and possibly other growth promoting substance. Symbiotic cyanobacteria azolla is responsible for nitrogen fixation which increases the fertility of the soil and in turn enhances the yield. Azolla is rich in proteins, amino acids, vitamins and minerals. By the presence of azolla crop or plant grows well due to its ability of giving nitrogen. Azolla is the most commonly used green manure on agriculture, due to its nitrogen fixing capacity, growth rate and ability to scavenge nutrients from soil and water.

Geitlerinema is the simple filamentous cyanobacteria genus geitlerinema is heterogeneous. Anoxygenic cyanobacteria that use sulphide as the electron donor for photosynthesis are a potentially influential. The thallus is thin, delicate, mostly bright blue green, rarely violet or brown, diffuse sometimes fascicle like, usually forming thin macroscopic mats.

Algae biofertilizer is a natural, organic and renewable energy source. They help to retain essential nutrients and water in the soil which is required for the proper growth of the plants. When chemical fertilizer is used they change the soil composition and causing contamination and pollution.

CHAPTER 2 : REVIEW OF LITERATURE

Deepali chittora et. al 2020, used cyanobacterium as a source of fertilizer for sustainable agriculture. Cyanobacteria are the most successful and sustained prokaryotic organism during the course of evolution. BGA are emerging candidates for efficient conversion of radiant energy into chemical energy. Cyanobacterium are used in ecofriendly sustainable agricultural practices for production of biomass of very high value. They have done the cyanobacterium under three cultivation methods.

1. Open system cultivation using sunlight.
2. Close system cultivation using sunlight.
3. Close system cultivation using artificial light

At the process of (CSCUC) raceway or circular type shallow open ponds are used



for the mass cultivation of cyanobacteria and microalgae. The advantage between this solar radiation is free of cost. Disadvantage is contamination by algae grazers and other microorganism are unpreventable which compromises net productivity. Contamination problem could conditions which generally prevent the growth of other organism, however this stratly limits th application of open system for cultivation of only selected organism.

In this system solar radiation is a source of energy transparent material such as plastic or glass is used for makind vessels which are placd outdoors in th natural light for illumination. Advantage of its shown as preventing contamination of grazres and comptitors provides a higher surface to volume ratio and cell densities obtaind often higher thn in opn system . cost of these system is increased it was appear as a diaadvantage on this system of cultivation for fertilizer .

In this system source of radiation is artifical light. Photobioreactor is used for the cultivation of various organism. It serve as an important tool for the production of high-value products such as stable-isotope-labeled biochemicals. The disadvantage is th utilization of plastic or glass material for making th vessel and power consumption is costly. In this method, small pits are prepared in the field and lifed with thick polythene shuts, for example : Anabaena , spirulinaem some other algal sample ete, have been used for inoculum preparation.

On this cyanobactrium have an emerged potential as biofertilizer. They have the ability to utilize CO_2 , water and nutrient to convert solar energy into biomass. Efficient application of cynobacterium have been reported in agricultural practices to reduce global warming by decreasing CO_2 gas. The overall study stated that the BGA biomass can be utilized for improving the quality of food products, physicochemical properties of soil. Controlling soil-borne disease added organic matter releas growth promoting substance, solubilize the insoluble phosphate, use as nutraceuticals and also apply in pharmaceuticals. Hence , biofert lizer propered from cynobacterium are economical and environmental- friendly.

Azolla biofertilizer substially improved NUE and yield for rice crops. The efficient use of (N) in crop production is critical for melting the challenges of food security and environment integrity. The objective of this study was to determine the efficiency of partially substituted azolla biofertilizer for symbiotic N fertilizer to improved NUE , reduce N loss and enhance rice cropping system , Min Zhang et al 2018.

Therefore , 3 year field expriement was conducted with 5 treatment by them; CK (control without urea). FN (farmers ' N Prattice) , FNA (the farmer N combined with azolla biofertilizer); RN (reducing farmers N by 25%) and RNA (substituting azolla biofertilizer for 25% farmers N). The NUE , ammonia (NH_3) volatilization , rice yield and net economic benefit were assessed.

The results showed that in the RNA and FNA treatment, azolla biofietilizer produced higher recovery efficiency of fertilizer N by 69% and 59%,provide higher agronomic N are efficiency by 52% and 31% and achieved higher partial factor

products of applied N by 43% and 13% then FN for 3 years, respectively. By these results it shows that fertilizer used field have more nitrogen than others. It was more helpful to understand the capacity of azolla and its working level on soil with plants. In addition, the RNA and FNA treatment achieved crop N recovery that was 64% and 49% higher than the FN treatment respectively. It has great power of converting soil nutrients and by promoting the atmospheric nitrogen to plant. The improved NUE under the azolla biofertilizer treatment were attributed to reduce N and enhance N uptake by rice plants.

The RNA & FNA treatment significantly reduced (N) loss by 48% and 26% as well as lowered NH₃ loss by 42% and 12% over FN, respectively. In this percentage of nitrogen and ammonia has easy to find the capacity of RNA on the field. In addition azolla could fix 52 and 4 kg N ha⁻¹ crop in the RNA and FNA treatment and thereby, azolla biofertilizer resulted in higher N uptake that was 17% and 33% higher in the RNA and FNA group than in FN, respectively. As a result the RNA and FNA treatment achieved higher rice by 8% and 14% over FN, respectively, but they attained similar and higher net economic benefit over FN for 3 years. Therefore, substituting azolla biofertilizer for 25% of urea N provides financially attractive option for farmer to substantially improved NUE and yield effectively reduce N loss in intensive rice cropping system.

Microalgal research has been an area of great interest as microalgae have higher productivity than land plants and can be used for the production of valuable commodities such as biofuel, animal feeds and agricultural fertilizer among others. The ability of spirulina-based fertilizer to enhance plant growth was demonstrated in leafy vegetable such as arugula (*Eruca sativa*), (Shy Chyi Wang et al, 2016).

Microalgae biorefinery system have been extensively studied from the perspective of resources, energy expenditure, biofuel production potential and high added value products. The genus spirulina (*Arthrospira*) stands out among the microalgae of commercial importance. It accounts for over 30% of biomass produced globally because of high protein content and carotenoid and phycocyanin content. It was the most useful algae which grown on water as well as waste water. Spirulina cultivation can be used to reduce greenhouse gases and for efficient treatment furthermore, its cellular morphology facilitates biomass recovery, which contributes to the process cost reduction. (Jorge Alberto Vieira Costa, et al, 2019).

Fertilizers can be expensive agricultural inputs for small-scale farmers, but there are technologies being developed which can offer farmers less expensive ways to ensure their soils are fertile and healthy.

Cyanobacteria, or blue-green algae, is one such example of a bio-fertilizer, a type of organic fertilizer which contains living organisms and harnesses naturally occurring inputs like solar energy, nitrogen, and water to ensure soil fertility and

plant growth. Small-scale farmers using bio-fertilizers have the potential to provide larger and more sustainable yields and healthier soils for themselves and their communities.

Currently, lack of soil fertility is the primary cause of declining per-capita food production in sub-Saharan Africa. Bio-fertilizers can help to maintain the natural soil habitat, while also improving fertility. More specifically, cyanobacteria fix nitrogen, which is an essential nutrient for supporting plant growth. The cyanobacteria tap the sun's energy captured during photosynthesis to fix nitrogen from the air and turn it into a form which plants can use. The cyanobacterial fertilizer production begins with cultures which are seeded in shallow ponds and are ready to use a few weeks later. According to researchers at the *Centre for Conservation and Utilization of Blue Green Algae* at the Indian Agricultural Research Institute, cyanobacteria can be useful for agricultural development because they "offer an economically attractive and ecologically sound alternative to chemical fertilizers for realizing the ultimate goal of increased .Thin Air Nitrogen Solutions, a company producing and marketing bio-fertilizers to small-scale farmers in the developing world, found that when cyanobacterial bio-fertilizer was applied to a variety of crops in Ethiopia, "yield are at par or exceeded outcomes from urea application and double those with zero fertilizer use." In addition, they found that plants which received these bio-fertilizer treatments had improved nutrition value; zinc and beta-carotene levels were more than doubled and iron increased by more than 50 percent compared to crops treated with nitrogen-rich urea chemical fertilizer(M. Upasana Mishra et al, 2014).

(A.karthikeyan et al,2008) The present study highlights the effect of interspecific competition between natural fertilizers, (cow dung), chemical fertilizer (urea), and biofertilizer. (Phormidium tenue, Bradyrhizobium sp.) on the overall growth performance and reproductive yield of *Vigna mungo* L. All the observations were done under the same experimental and environmental conditions. The differences in the growth parameters such as shoot length, root length, number of nodules, 100 grain weight, number of flowers, shoot and root weight were studied at different stages i.e., vegetative, flowering and pod forming. The biochemical constituents of leaf and seed were analyzed for chlorophyll, protein and total free amino acid content. By the study of this the BGA have shown increased growth rate and also increases the yield. It results that algal species was the best sample for the growth of rice field.

CHAPTER 3: AIM AND SCOPE

3.1 AIM:

To study the effects of seed germination by using Spirulina, Azolla and Geitlerinema of algae bio-fertilizer.

3.2 OBJECTIVE:

- 1.To assess the effect of Spirulina on seed germination of Trigonella foenum-graecum, Brassica, Sesamum indicum, Capsicum frutescens, Vigna radiata.
2. To assess the effect of Azolla on seed germination of Trigonella foenum-graecum, Brassica, Sesamum indicum, Capsicum frutescens, Vigna radiata.
- 3.To assess the effect of Geitlerinema on seed germination of Trigonella foenum-graecum, Brassica, Sesamum indicum, Capsicum frutescens, Vigna radiata.
- 4.To estimate the NPK value of the control soil and algae added soil.
- 5.To study the effect of algae on earthworm.



CHAPTER 4 : MATERIALS AND METHODOLOGY

4.1 MATERIALS

- 1.Pots
- 2.Soil
- 3.Water
- 4.Grating scale
- 5.Seeds (green gram , fenugreek, sesame , mustard and chilli)

4.1.1 SELECTED SEEDS:

Seeds are collected from Chennai market

- Trigonella frenum-graecum (fenugreek)
- Brassica (mustard)
- Sesamum indicum (sesame)
- Capsicum frutescens (chilli)
- Vigna radiata (green gram)

4.1.2 SELECTED ALGAE:

- 1.Spirulina
- 2.Azolla
- 3.Geitlerinema

4.2 METHODOLOGY

4.2.1 SAMPLE COLLECTION :

Spirulina : collected from online , Amazon.

Azolla: Collected from online , Amazon.

Geitlerinema:Collected from the department of Biotechnology,` Sathyabama university.

4.2.2 SAMPLE PREPARATION:

Spirulina : Directly collected as the extracted powder, given in fig 4.2.2.1.

Azolla: Collected as a plant and dried that plant at 21°C in the shadow region for 3 to 4 days.Then used to be a fertilizer, given in fig 4.2.2.2

Geitlerinema: Collects water from the sea in Chennai and boil the water at 100°C . Pour the water into the clean container and add that collected algae into it.Grown

the algae for 20 days , then squeeze the algae to extract its juice, given in fig 4.2.2.3.

**FIG 4.2.2.1 EXTRACTED SPIRULINA
POWDER**



**FIG 4.2.2.2 AZOLLA
BIOFERTILIZER**



FIG 4.2.2.3 GEITLERINEMA LIQUID



4.2.3 METHODS OF PLANTING :

Taken three types of fertilizer Spirulina as powder, Azolla as dried plant and Geitlerinema as liquid. From the day of seeding every 5 days measure the shoot and root of the plant using Grating scale. And every 7-8 days mentioned level of fertilizer were added

SPIRULINA: 250 g of soil was mixed with 10-20 g of extracted Spirulina powder , transfer to a pot filled with half of sand. Spread evenly over to that normal soil. Filled the base same as in 4 pots .30 - 40 Trigonella foenum-graecum seed, 50 -60 Brassica seeds, 20 - 30 Sesamum indicum seeds and 10 -15 Capsicum frutescens seeds are planted on this 4 pots. Then 10-20g of extracted spirulina powder is of water and this liquid mixed with 250g of soil and transfer to a pots like other seeded 40 - 60 Vigna radiata seeds .Finally poured sufficient water and leave to grow.

AZOLLA: 250g of soil mixed with 10g of Azolla plant and transfer to a pot filled half of soil . Spread evenly over to the normal soil..25-30 Trigonella foenum-

gracum seed, 40-60 Brassica seeds, 40-60 Sesamum indicum seeds and 10 -15 Capsicum frutescens seed and 30 - 40 Vigna radiata seeds are planted in five different pots . Poured the sufficient water and leave to grow.

GEITLERINEMA: Same 250g of soil mixed with 10-15ml of squeezed Geitlerinema liquid and transfer to a pot filled with half of soil. Planting the same amount of seeds as taken from the above mentioned Azolla. Poured the sufficient water and leave to grow.

4.2.4 NPK TEST:

Using the ready made kit for this this. Soil was collected from the planting pots.

Use the pipette to transfer 2.5 mL of the clear soil extract to the test tube. Add the entire contents of the Nitrogen (N) reagent to the test tube and replace the cap on the test tube , shake gently for 30 seconds to dissolved the reagent. Allow the test tube to stand for 30 seconds. Compare the colour of the solution in the test tube with colour screen given in the kit.Note down the Nitrogen content in the soil.

Use the pipette to taken 2-5 mL of the clear soil extract to the test tube. Add the entire content of the Phosphorus (P) reagent to the test tube and replace the cap on the test tube , shake gently for 30 seconds to dissolved the reagent. Allow the test tube to stand for 30 seconds.compare the colour of the solution in the test tube with colour screen given in the kit. Note down the Phosphorus content in the soil.

add 0.5 mL of the clean soil extract to the test tube.Add the Potassium (P) 1 reagent to the test tube until the mark. Add the entire content of the potassium (K) 2 reagent to the test tube and replace the cap on the test tube, shake gently for 30 seconds to dissolved the reagent . Allow the test tube to stand for 30 seconds. Compare the colour of the solution in the test tube with colour screen given in the kit. Note down the Potassium content in the soil. This NPK test taken in National Argo Foundation Laboratory Service Division in chennai.

4.2.5 TOXICITY TEST OF ALGAE ON EARTHWORM:

4 earthworm was taken and measured its length separately with grating scale. Taken 4 container first one contain normal soil used, second one contain soil with Azolla fertilizer, third one contain Spirulina with soil and last one contain soil with Geitlerinema. Add one-one earthworm to each container and leave the earthworm to eat that algae. Finally observed the size of the earthworm.

CHAPTR 5: RESULT AND DISCUSSION

5.1 EFFECT OF ALGAE ON PLANT GROWTH:

Three type of algae biofertilizer were used Spirulina as extracted powder, Azolla as dried plant and Geitlerinema as liquid. From this bio-fertilizer five different plants were used. The result and number of seed used in normal soil were given in table 5.1.1. The amount of seed used in biofertilizer were mention in 4.2.3. the result of seeds grown in normal were given in table 5.1.1. The result of seeds grown in Azolla biofertilizer were given in table 5.1.2 . The result of seeds grown in Spirulina were given in table 5.1.3 on this Brassica were grown and Sesamum indicum was affect by fungal formation after 8 days. The result of seeds grown in Geitlerinema were given in 5.1.4 .

TABLE 5.1.1 SEED GROWN IN NORMAL SOIL

S NO.	NAME OF SEEDS	NUMBER OF SEEDS USED	NUMBER OF SEEDS GROWN
1	Trigonella foenum-graecum (Fenugreek)	20 - 30	22
2	Brassica (Mustard)	20 - 25	6
3	Sesamum indicum (Sesame)	25 - 30	20
4	Capsicum frutescens (chilli)	5 - 10	3
5	Vigna radiata (Green gram)	20 - 30	24

Here, the sample seeds have been counted and applied with soil without the use of algal biofertilizer to know normal soil growth and bio fertilizer used soil growth level . And also to show the difference in normal soil and bio fertilizer used soil .

TABLE 5.1.2 SEEDS GROWN IN AZOLLA BIOFERTILIZER

This, seed samples have countedly applied to the soil and the growth level have been measured .

S NO.	NAME OF SEEDS	NUMBER OF SEEDS USED	NUMBER OF SEEDS GROWN
1	Trigonella foenum-graecum (Fenugreek)	25 - 30	27
2	Brassica (Mustard)	40 - 50	43
3	Sesamum indicum (Sesame)	40 - 60	49

4	Capsicum frutescens (Chilli)	10 - 15	12
5	Vigna radiata (Green gram)	30 - 40	37

TABLE 5.1.3 SEEDS GROWN IN SPIRULINA BIOFERTILIZER

Number of seeds growth have been measured in spirulina fertilizer applied seeds

S NO.	NAME OF SEEDS	NUMBER OF SEEDS USED	NUMBER OF SEEDS GROWN
1	Sesamum indicum (sesame)	30 - 40	35
2	Capsicum frutescens(chilli)	50 - 60	0
3	Trigonella foenum-graecum (Fenugreek)	20 - 30	23
4	Brassica (Mustard)	10 - 15	13
5	Vigna radiata (Green gram)	40 - 60	53

TABLE 5.1.4 SEEDS GROWN IN GEITLERINEMA BIOFERTILIZER

On the geitlerinema fertilizer sample used . It have not shown any growth on all seed samples .

S NO.	NAME OF SEEDS	NUMBER OF SEEDS USED	NUMBER OF SEEDS GROWN
1	Trigonella foenum-graecum (fenugreek)	25 - 30	0
2	Brassica (mustard)	40 - 50	0
3	Sesamum indicum (sesame)	40 - 60	0
4	Capsicum frutescens (chilli)	10 - 15	0
5	Vigna radiata (green gram)	30 - 40	0

FIG 5.1.1 EARLY GROWTH OF TRIGONELLA FOENUM-GRÆCUM



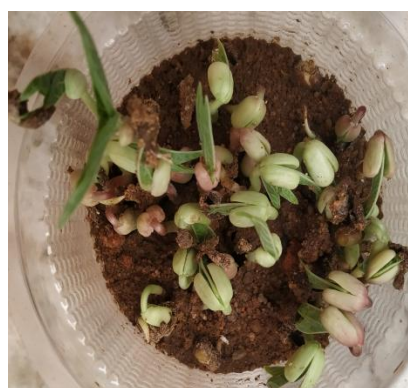
FIG 5.1.2 EARLY GROWTH OF BRASSIA



FIG 5.1.3 EARLY GROWTH OF CAPSICUM FRUTESCEN



FIG 5.1.4 EARLY GROWTH OF VIGNA RADIATA



5.2 READING OF PLANTS:

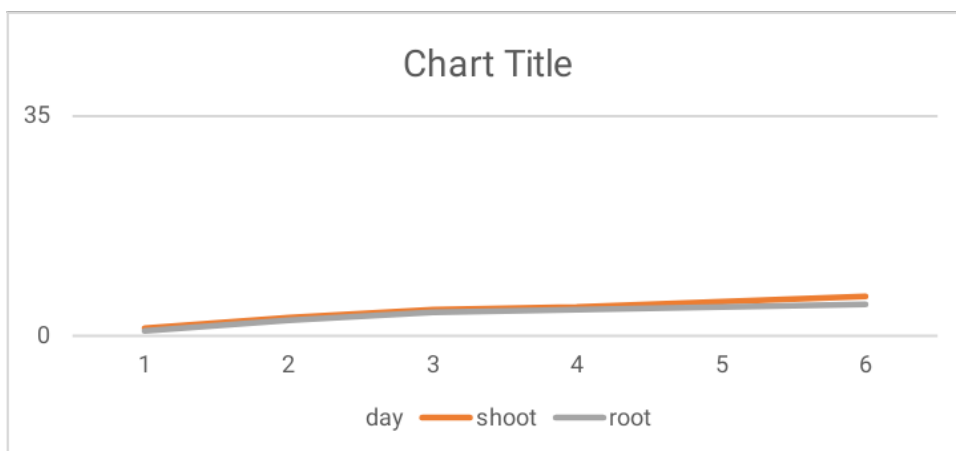
From the day of seeding every 5 days shoot and root size were measured with grating scale. The result of reading of the normal plant were given in table 5.2.1, Spirulina were given in table 5.2.2, Azolla were given in the table 5.2.3 and Geitlerinema were not grown so 0% of the result. over all Compared to normal plant growth azolla resulted more growth, spirulina resulted moderate and brassica and sesamum indicum were affected by fungi after 10 days and doesn't show grow further and geitlerinema resulted no growth.

TABLE 5.2.1 READING OF PLANT GROWN IN NORMAL PLANT

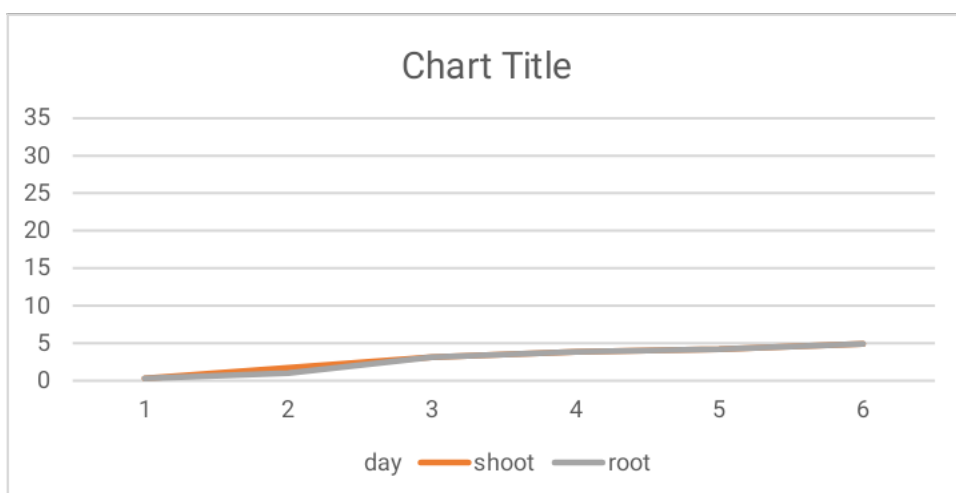
DAY	Trigonella frenum- graecum (fenugreek)	Brassica (mustard)	Sesamum indicum (sesame)	Capsicum frutescens (chilli)	Vigna radiata (green gram)
DAY 5	Shoot- 1cm Root-0.7cm	Shoot-0 Root-0	Shoot-1cm Root-1.8cm	Shoot- 0 Root-0	Shoot- 4.4cm Root-4cm
DAY 10	Shoot- 2.9cm Root-2.4cm	Shoot- 1.5cm Root-0.9cm	Shoot- 1.6cm Root-2.5cm	Shoot-0 Root-0	Shoot-5cm Root-4.9cm
DAY 15	Shoot-4cm Root-3.4cm	Shoot2.9cm Root-3cm	Shoot- 3.2cm Root-4cm	Shoot-2.2cm Root-1.3cm	Shoot- 5.7cm Root-5.3cm
DAY 20	Shoot-4.5cm Root-3.9cm	Shoot- 3.8cm Root-3.8cm	Shoot- 3.9cm Root-4.3cm	Shoot-2.7cm Root-1.9cm	Shoot- 6.1cm Root-5.7cm
DAY 25	Shoot-5.3cm Root-4.3cm	Shoot- 4.2cm Root-4cm	Shoot- 4.2cm Root-4.6cm	Shoot-3cm Root-2.3cm	Shoot- 6.5cm Root-5.9cm

DAY 30	Shoot-6cm Root-4.9cm	Shoot- 4.8cm Root-4.8cm	Shoot- 4.9cm Root-4.7cm	Shoot-3.3cm Root-2.5cm	Shoot- 6.9cm Root-6cm

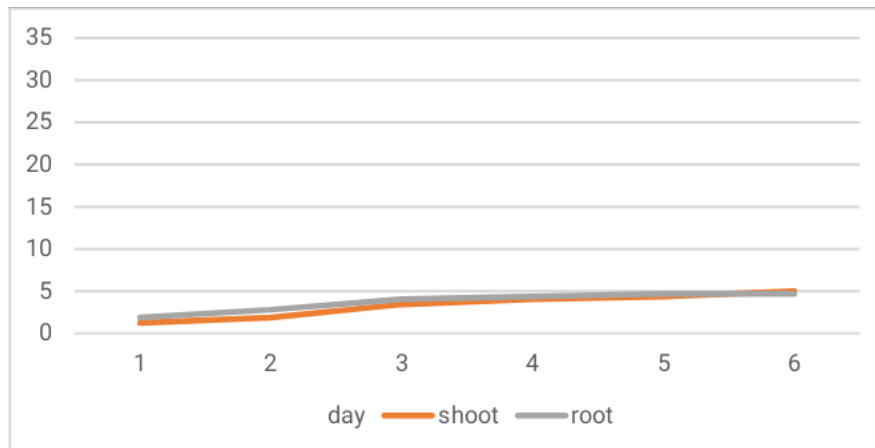
5.2.1.1 Graphical representation of plant growth of *Trigonella foenum-graecum* in normal soil



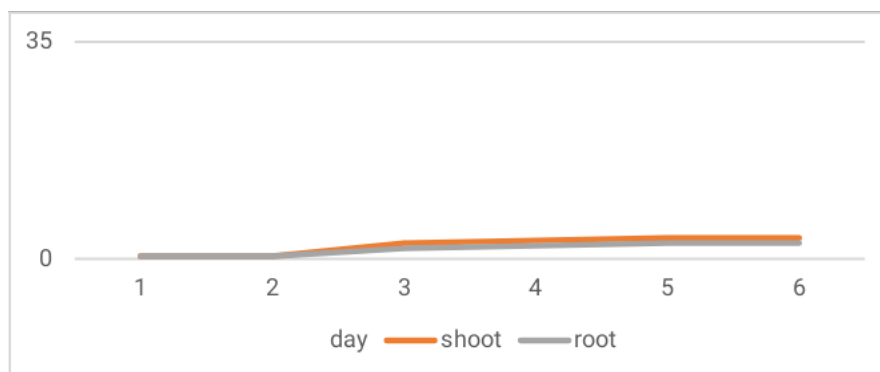
5.2.1.2 Graphical representation of plant growth of Brassia in normal soil



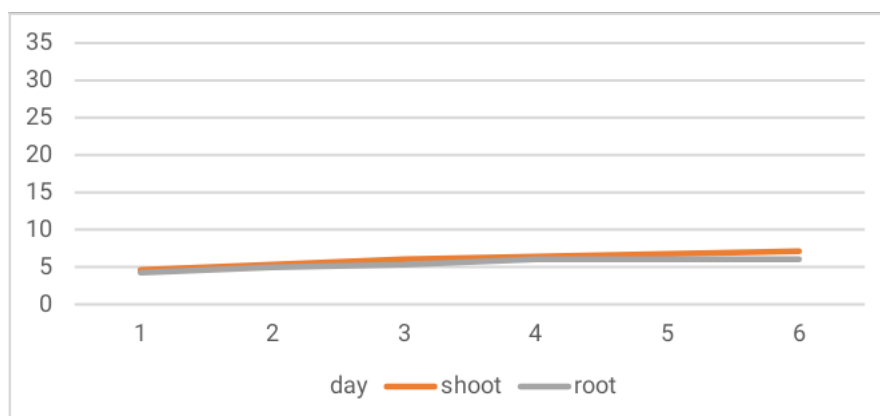
5.2.1.3 Graphical representation of plant growth of *Sesamum indicum* in normal soil



5.2.1.4 Graphical representation of plant growth of *Capsicum frutescens* in normal soil



5.2.1.5 Graphical representation of plant growth of *vigna radiata* in normal soil



Measurement of plant grown level has been noted and given in table 5.2.1 ;The seeds have been measure according to the calculated days of 5-30. Apart from all plants *Vigna radiata* (green gram) has grown well and showed maximum growth

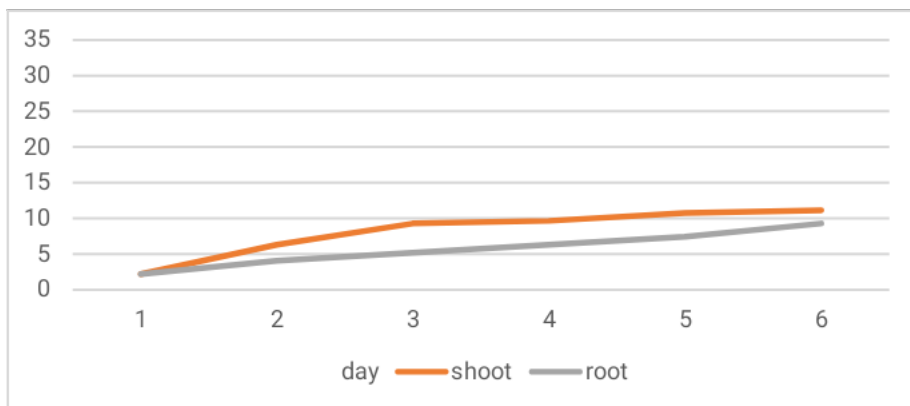
when compared to other seed samples, next level of high growth in initial stage was *Trigonella foenum-graecum* (fenugreek). *Brassica* (mustard) and *Sesamum indicum* (sesame) were almost shown similar growth . Finally *Capsicum frutescens* (chilli) were start to grow at 8th day and this was shown a minimum growth compared to all.And their graphical representation given above.

TABLE 5.2.2 READING OF PLANT GROWN IN SPIRULINA BIOFERTILIZER

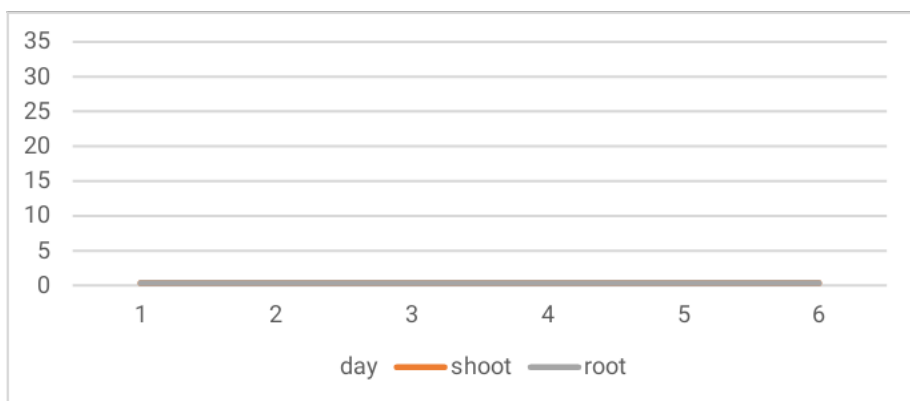
DAYS	Trigonella frenum- graecum (fenugreek)	Brassica (mustard)	Sesamum indicum (sesame)	Capsicum frutescens (chilli)	Vigna radiata (green gram)
DAY 5	Shoot- 1.9cm Root- 2cm	Shoot- 0 Root-0 No growth	Shoot- 1.5cm Root- 1cm	Shoot- 0.5cm Root-0.1cm	Shoot- 2.3cm Root-1cm
DAY 10	Shoot- 6cm Root- 1.7cm	Shoot- 0 Root- 0 Fungi formed	Shoot- 1.5cm Root- 1cm Fungi formed	Shoot- 1.2cm Root- 1cm	Shoot- 5.5cm Root-2cm
DAY 15	Shoot- 9cm Root-5cm	Shoot- 0 Root- 0	Shoot- 0 Root- 0	Shoot- 1.7cm Root- 1.3cm	Shoot-7cm Root-3.5cm
DAY 20	Shoot- 9.5cm Root- 6.1cm	Shoot- 0 Root- 0	Shoot- 0 Root- 0	Shoot- 2cm Root- 1.4cm	Shoot-8cm Root-4cm
DAY 25	Shoot- 10.5cm Root- 7.2cm	Shoot- 0 Root- 0	Shoot- 0 Root- 0	Shoot- 2.4cm Root- 1.6cm	Shoot- 8.4cm Root-4.3cm
DAY 30	Shoot- 11cm Root- 9cm	Shoot- 0 Root- 0	Shoot- 0 Root- 0	Shoot- 2.5cm	Shoot- 8.9cm

				Root- 1.6cm	Root-5cm
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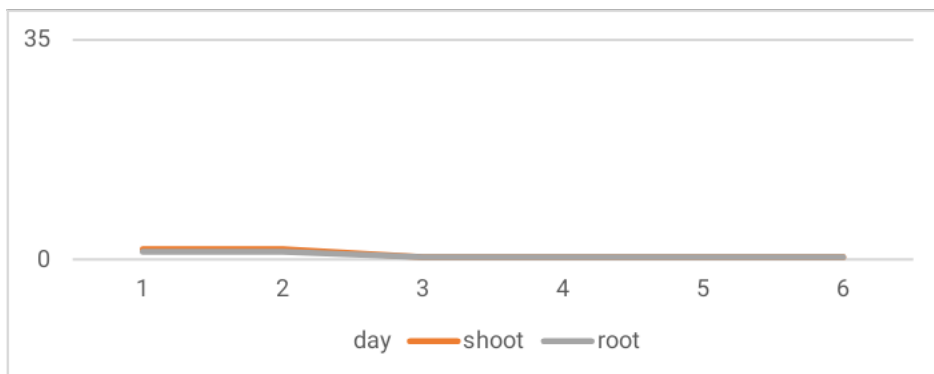
5.2.2.1 Graphical representation of plant growth of *Trigonella foenum-graecum* in spirulina biofertilizer



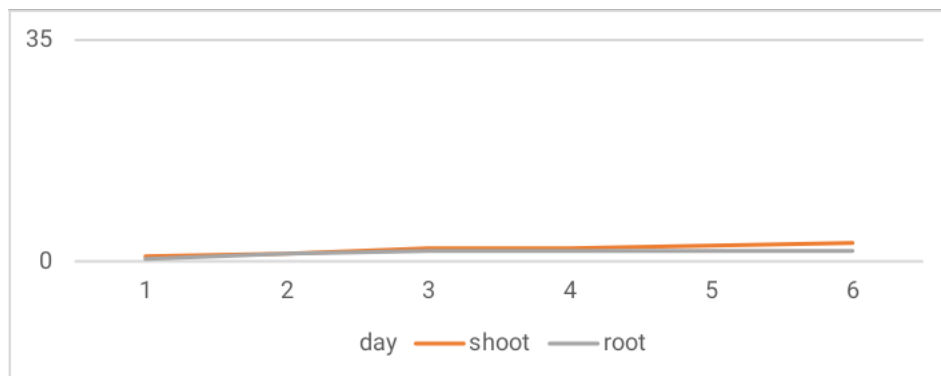
5.2.2.2 Graphical representation of plant growth of *Brassia* in spirulina biofertilizer



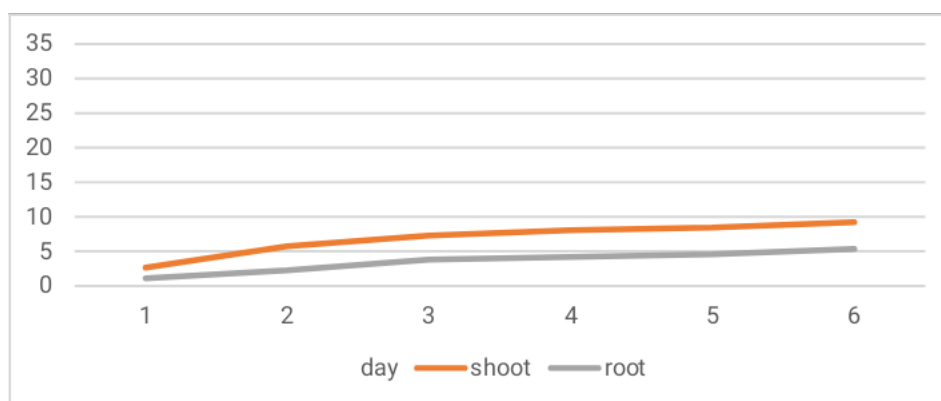
5.2.2.3 Graphical representation of plant growth of *Sesamum indicum* in spirulina biofertilizer



5.2.2.4 Graphical representation of plant growth of Capsicum frutescens in spirulina biofertilizer



5.2.2.5 Graphical representation of plant growth of vigna radiata in spirulina biofertilizer



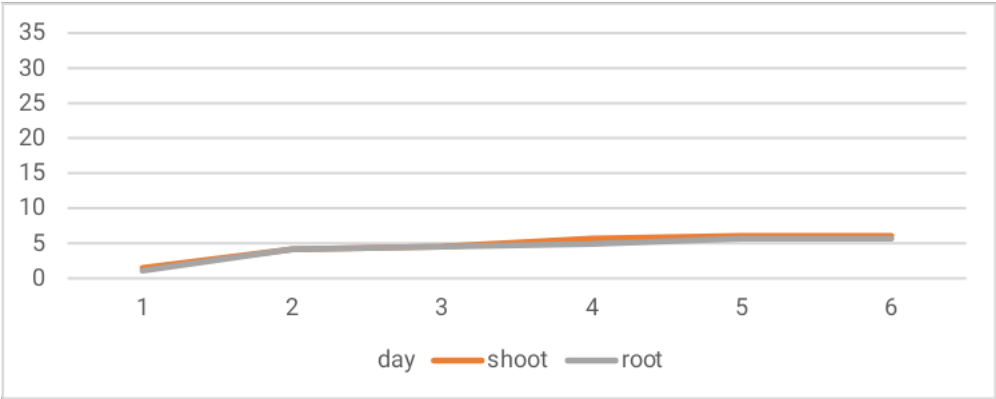
Here, the same seed samples have been used and growth of plant were measured every 5 days, given in the table 5.2.2. These five samples have been grown with the application of Spirulina biofertilizer. It has been applied as a powdery component with the amount of 10-20g for every 7-8 days. On this algae fertilizer sample, the Trigonella frenum-graecum (fenugreek) shown maximum growth compared to other four seed samples. Next level of high growth at initial stage was Vigna radiata (green gram). Capsicum frutescens (chilli) was shown minimum growth compared to others. Sesamum indicum (sesame) and Brassica (mustard) were not grown and affected by fungi at 9th and it lost its growth level. And their graphical representation were given above.

TABLE 5.2.3 READING OF PLANT GROWN IN AZOLLA BIOFERTILIZER

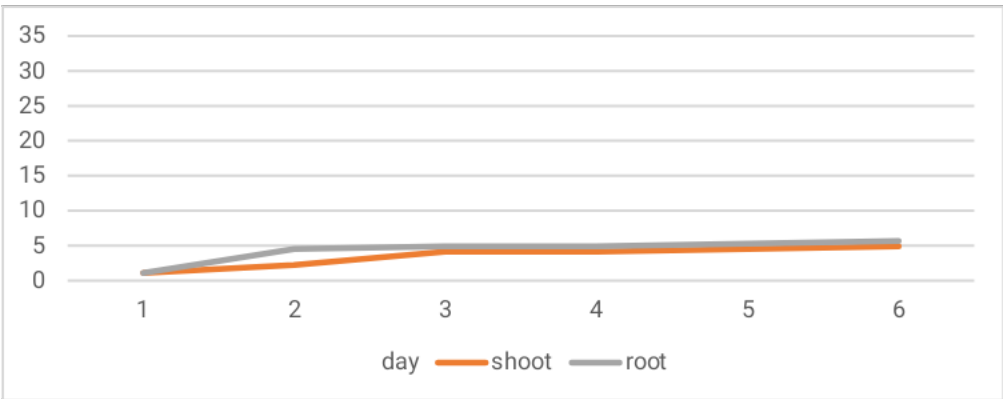
DAYS	Trigonella frenum-graecum (fenugreek)	Brassica (mustard)	Sesamum indicum (sesame)	Capsicum frutescens (chilli)	Vigna radiata (green gram)

DAY 5	Shoot- 1.3 cm Root- 0.9 cm	Shoot- 1.0cm Root- 1.1cm	Shoot- 1cm Root- 1.9cm	Shoot- 0 Root- 0	Shoot- 5.5cm Root-4.5cm
DAY10	Shoot- 4 cm Root- 4 cm	Shoot- 1.9cm Root-4.5cm	Shoot- 1.9cm Root- 4.5cm	Shoot- 2.0 cm Root- 1.9cm	Shoot- 6.3cm Root-4.9cm
DAY 15	Shoot- 4.3 cm Root- 4.5 cm	Shoot-4cm Root-4.7cm	Shoot- 4cm Root- 4.7cm	Shoot- 2.4cm Root- 2.4cm	Shoot- 7.1cm Root-5.5cm
DAY 20	Shoot- 5.3 cm Root- 4.9 cm	Shoot- 4.1cm Root-4.9cm	Shoot- 4.1cm Root- 4.9 cm	Shoot- 2.7cm Root- 2.6cm	Shoot-8cm Root-5.9cm
DAY 25	Shoot- 5.7 cm Root- 5.3 cm	Shoot- 4.3cm Root-5.1cm	Shoot- 4.3cm Root- 5cm	Shoot- 3.1cm Root- 2.9cm	Shoot- 8.5cm Root-6.2cm
DAY 30	Shoot- 5.9 cm Root- 5.4 cm	Shoot- 4.7cm Root-5.2cm	Shoot- 4.7cm Root- 5.3cm	Shoot- 3.5cm Root- 3.1cm	Shoot- 8.9cm Root-6.3cm

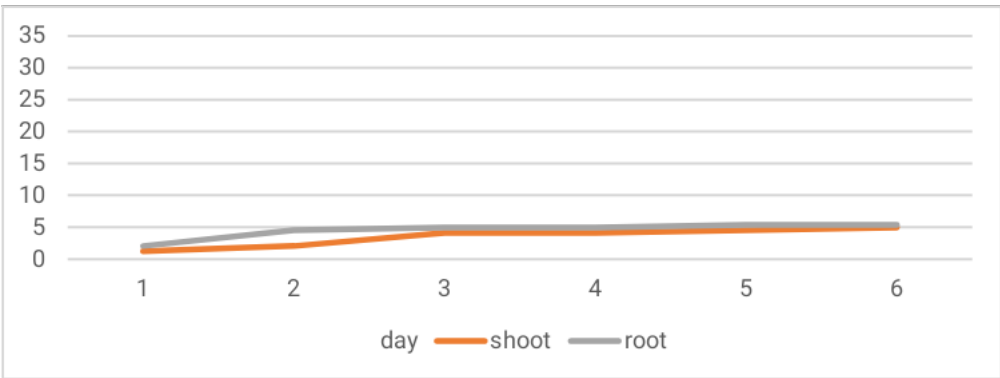
5.2.3.1 Graphical representation of plant growth of *Trigonella foenum-graecum* in azolla biofertilizer



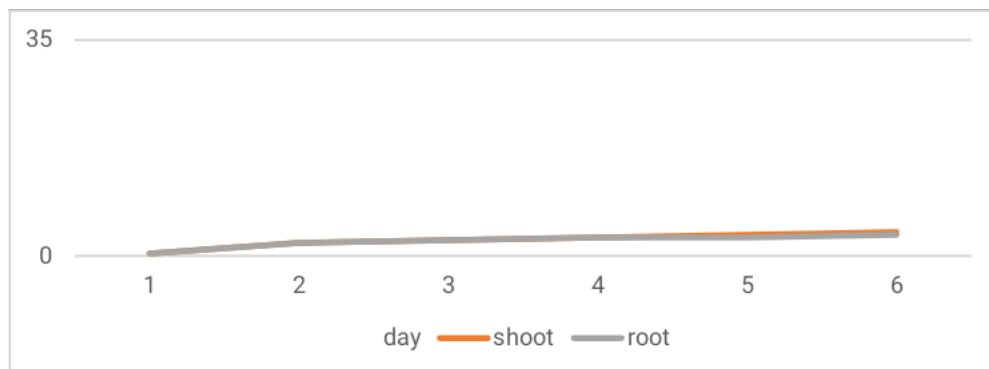
5.2.3.2 Graphical representation of plant growth of *Brassia* in azolla biofertilizer



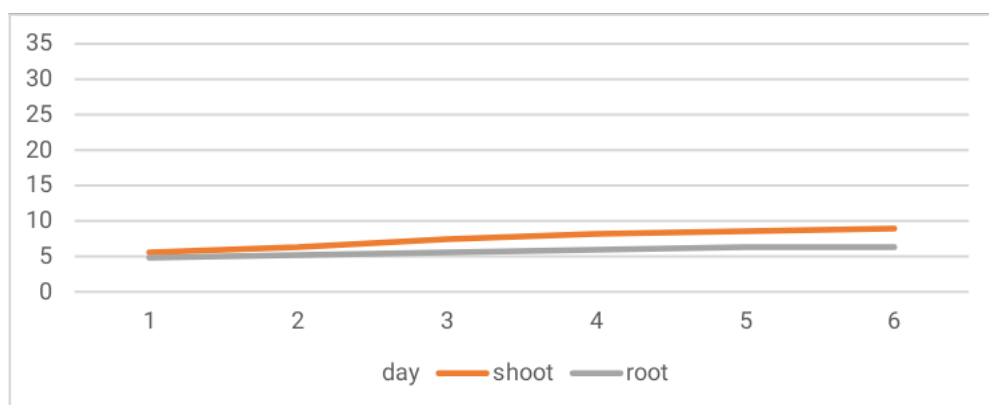
5.2.3.3 Graphical representation of plant growth of *Sesamum indicum* in azolla biofertilizer



5.2.3.4 Graphical representation of plant growth of *Capsicum frutescens* in azolla biofertilizer



5.2.3.5 Graphical representation of plant growth of *Vigna radiata* in azolla biofertilizer



Here also, same seed samples have been used and growth of plant were measured every 5 days, given in the table 5.2.3. These five samples have been grown with the application of Azolla biofertilizer. It has been applied as a dried plant of azolla with the amount of 10g for every 7-8 days. When compared to spirulina, geitlerinema even in normal soil, azolla biofertilizer shown major growth for all plants. In azolla biofertilizer maximum growth of the plant was *Vigna radiata* (green gram) and next high growth *Trigonella foenum-gracum* (fenugreek). *Brassica* (mustard) and *Sesamum indicum* (sesame) were shown similar growth and *Capsicum frutescens* (chilli) shown minimum growth compared to other. Graphical representation were given above.

5.3 RESULT OF NPK TEST

For NPK test two normal soil were used one was used for spirulina biofertilizer and second one used for azolla and geitlerinema biofertilizer. The amount of soil

sample were 80g for each sample.

TABLE 5.3.1 NPK TEST VALUE OF NORMAL SOIL

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	31.4
Available Phosphorous	mg/kg	26.29
Potassium exchangeable K	mg/kg	155

The normal soil have nitrogen , phosphorus and potassium content . On this the normal soil have high level of potassium content of about 155mg/kg and nitrogen have 31.4mg/kg.

TABLE 5.3.2 NPK TEST VALUE OF SPIRULINA USED ON FEW DAYS

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	5.8
Available Phosphorous	mg/kg	90.03
Potassium exchangeable K	mg/kg	259

Here, the few days of used spirulinum results, it shows high level of potassium of about 259mg/kg and nitrogen have 5.8kg/mg..

TABLE 5.3.3 NPK TEST VALUE OF SPIRULINA USED ON MORE DAY

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	35.4
Available Phosphorous	mg/kg	14.89
Potassium exchangeable K	mg/kg	99

Compared with normal soil, Spirulina got lower nitrogen in few days of biofertilizer application almost 25.4mg/kg was lower. This may be the seed were used to observed the nitrogen to grow. After in grown certain length the nitrogen level increased 4mg/kg.

TABLE 5.3.4 NPK TEST VALUE OF NORMAL SOIL

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	66.3
Available Phosphorous	mg/kg	71.73
Potassium exchangeable K	mg/kg	227

TABLE 5.3.5 NPK TEST VALUE OF AZOLLA USED ON FEW DAY

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	26.8
Available Phosphorous	mg/kg	61.63
Potassium exchangeable K	mg/kg	296

TABLE 5.3.6 NPK TEST VALUE OF AZOLLA USED ON MORE DAYS

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	40.5
Available Phosphorous	mg/kg	83.43
Potassium exchangeable K	mg/kg	312

The result of NPK test on azolla used soil on more days sample , it shows increasing rate of nitrogen , phosphorus and potassium on soil .

Compared with normal soil , azolla get lower nitrogen in few days of biofertilizer application almost 39.5mg/kg because the same like spirulina it may be seed observed nitrogen to grow. And then 13.7mg/kg nitrogen level increased than the few days of application. More azolla appilication Nitrogen level 40.5mg/kg which is lower than normal soil nitrogen level almost 25.8mg/kg. Most of the theory explained azolla have high nitrogen level but this studied got low nitrogen then normal . This may be happned the azolla plant were dried.

TABLE 5.3.7 NPK TEST VALUE OF GEITLERINEMA USED ON FEW DAYS

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	96.7
Available Phosphorous	mg/kg	51.25

Potassium exchangeable K	mg/kg	361
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TABLE 5.3.8 NPK TEST VALUE OF GEITLERINEMA USED ON MORE DAYS

PARAMETER	UNIT	RESULT
Nitrate Nitrogen	mg/kg	25.0
Available Phosphorous	mg/kg	72.19
Potassium exchangeable K	mg/kg	354

Compared with normal soil , geitlerinema get high nitrogen in initial stage of biofertilizer application almost 30.4mg/kg . And then nitrogen level decreased. In more days of biofertilizer application almost 41.3mg/kg were reduced and resulted no growth.

5.4 EFFECT OF ALGAE ON EARTHWORM:

Four earthworm were taken one in normal soil , second in spirulina ,third in azolla and fourth in geitlerinema. This algae were kept for one week to eat the algae. Earthworm measured before allow to eat the algae and after one week took and measured again , the length of the earthworm were given table 5.5.1.

TABLE 5.4.1 LENGTH OF THE EARTHWORM

EARTHWORM	BEFORE EATEN THE ALGAE	AFTER EATEN THE ALGAE
NORMAL	12	12.5
SPIRULINA	4	5
AZOLLA	13	13
GEITLERINEMA	13	8

Normal and spirulina showed earthworm length got increased 0.5cm and azolla have be in same length and finally earthworm in geitlerinema was shinkled and 5cm length reduced

CHAPTER 6 : SUMMARY AND CONCLUSION

The Azolla, spirulina, and spirulina, and geitlerinema of algae are belongs to the genus of cyanobacterium were used as sample. These samples are collected from online and from the department of biotechnology, sathyabama. These samples are used in three ways Azolla as dried at 21⁰ C and used as biofertilizer, spirulina was directly collected as extracted powder and geitlerinema as liquid. These biofertilizer were used to grow 5 different plants (Trigonella Foenum-graecum, Brassica, Sesamum indicum, capsicum frutescens, vigna radiata) for 30 days and measured the length of shoot and root of plant every 5 days. Also grown all this 5 plants in normal soil and measured its too. Compared to normal plants Azolla resulted more growth, spirulina resulted moderate growth and from this Brassica and Sesamum indicum were affected by fungi and geitlerinema resulted no growth. NPK test were taken for normal and sample used soil. Soil Sample has been collected few days of application and more days of application. The result of NPK shown Azolla and spirulina nitrogen level was lower than the normal in few days of application and get higher in more days of application. In geitlerinema in nitrogen level higher than the normal in few days of application and get lower in more days of application. The toxicity of algae on earthworm were observed by allowing the earthworm to eat the algae for one week. The observation shown in normal and spirulina the earthworm grown 0.5 cm, In azolla earthworm was in same length and geitlerinema the earthworm shrinkled and length reduced.

The conclusion of the study is azolla have more capacity to grown the plant, spirulina have moderate capacity and geitlerinema have no capacity to grown the plant. Azolla and spirulina have more nitrogen fixation than geitlerinema .

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