Production of Biofertilizer using *Rhizobium* and *Azotobacter* And its Effect on *Amaranthus Tristis* and *Vigna Acontifolia*

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Abstract- Rhizobium and Azotobacterare the plant growth promoting bacteria which are gram negative and mostly used as biofertilizers.The main aim of this project was the mass production of Rhizobium and Azotobacter. Initially the organismswere isolated from the root nodules of Vigna radiata and soil sample respectively and conformation was done using the biochemical tests. This study was to investigate how different combinations of biofertilizer (Rhizobium and Azotobacter), chemical fertilizers would affect the growth of a green leafy vegetable, Amaranthus tristis and Vigna acontifolia. After the growth period of about 40 days the biometric and biochemical analysis was carried out and it was observed that there was significant change in biometric parameters and increase in the biochemical constituents. From the N,P,K test performed it was observed that these nutrients are present in correct proportion in biofertilizer added soil than chemical fertilizer added soil.

Keywords- Amaramthus tristis,Azotobacter, Biofertilizers, Biometric observations, Chemical fertilizers, N,P,K test, Rhizobium,Vigna acontifolia.

I. INTRODUCTION

Biofertilizer is a substance which contains living microorganisms which, when applied to the seed, plant surfaces promote growth by increasing the supply or availability of primary nutrients to the host plant. The nitrogen cycle is a series of processes that converts nitrogen gas to organic substances and back to nitrogen in nature[**1**].

Rhizobium is the most well-known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place. *Rhizobium* is a soil habitat Gram-negative bacterium, which is able to colonize the legume roots and fixes atmospheric nitrogen symbiotically [**2**]. *Azotobacter* spp. is free-living aerobic bacteria dominantly found in soils. They are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year.[**3**].

Amaranthus is widely cultivated in various regions of the world as well as in India as a food and leafy vegetable. The plant chosen for the study is *Amaranthus tristis.*The plantcan reduce total cholesterol and LDL and can increase HDL.*Vignaacontifolia* is a drought resistant legume, commonly grown in arid and semi-arid region of India[**4**].
N, P and K test determines the Nitrogen, phosphorus and potassium level. It is not only an essential part of carbohydrates, fats and oils but also an essential ingredient of proteins. N, P and K test determines the quantity of that factor which is essential for that plant. If the factors are not getting in sufficient forms they cause deficiency.[**5**].

II. MATERIALS

Root nodules of *Vigna radiata* and *Amaranthus tristis*, Seeds of *Amaranthus tristis* and *Vigna acontifolia*,Yeast extract mannitol broth, Ashby's broth and agar, Chemical fertilizer (urea+ potash), 2.5N hydrochloric acid.

III. METHODOLOGY

The isolation of Rhizobium was done using the root nodules of *Vigna radiata*and YEM medium.

Plates was kept incubator at 37^{o}C for 24 hrs. After 24 hours well isolated colonies was observed. Following tests were carried out for confirmation of organism.

a) Growth in 2% sodium chloride concentration
b) Growth at 37- 40^{o}C
c) PRODUCTION OF BIOFERTILIZER

For the production of biofertilizer liquid media were used. The 2-4 isolated colonies of Rhizobium species added in the YEM broth(100ml) and Azotobacter species in the Ashby's broth (100ml) respectively. Flasks incubated for 24 hours. Also a chemical fertilizer was prepared using the 0.0125gm urea and 0.005gm of potash for 500gm of soil. For experimental trials bags with 1/2 kg soil capacity were used.

Biofertilizer applied to seed, root and soil. Six polythene bags each containing the 500gm sterile soil were taken for test. They were grouped as follows:
Group 1. Control = Soil + Seed (without biofertilizer)
Group 2. Soil +Rhizobium biofertilizer (50ml) + Seed
Group 3. Soil+Azotobacter biofertilizer (50ml) +Seed
Group 4. Soil+ Chemical fertilizer (50ml) +Seed
Group 5.Soil+Rhizobium fertilizer (25ml) +Azotobacter fertilizer (25ml) + Seed
Group 6.Soil+Rhizobium fertilizer (16.5ml) + Azotobacter fertilizer (16.5ml) + Chemical fertilizer (16.5ml) + Seed.

The same procedure was done for the *Amaranthus tristis* seeds and *Vigna acontifolia* seeds. About 20 seeds were sown in each pot and allowed to germinate. The pots were watered daily. The adhering soil particles were removed by washing gently with water and the water droplets were removed by blotting with the filter paper. Then these plants were used for the biometric observation and biochemical analysis.

d) Biochemical Analysis:

a)Estimation of Total carbohydrates by Anthrone method
b)Protein estimation by Folin- Lowery method(4)

IV. RESULTS

Colony characteristics of Azotobacter and Rhizobium were noted down.

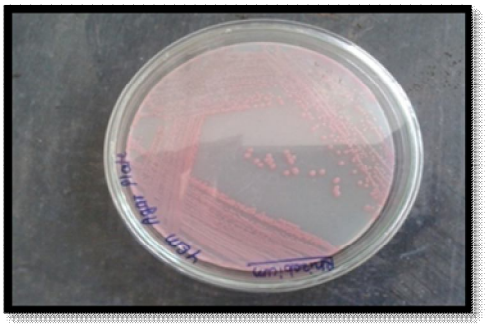

Figure 1: Isolated bacterial Colonies of *Rhizobium* on YEMA media

Rhizobium forms pink, translucent, flat,elevated and small colonies on YEMA medium. From the biochemical test, it was seen that the organism does not grow in 2% NaCl concentration and at 37-40^{0}C on YEMA plate. Hence the species was identified as *R. meliloti.* It was used further for biofertilizer production.

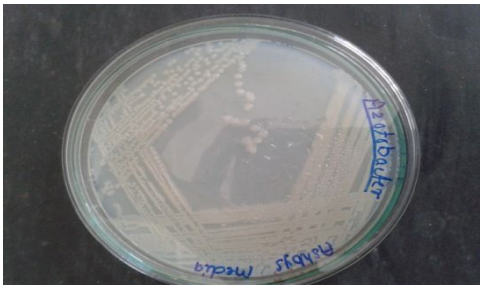

Figure 2: Isolated colonies of *Azotobacter* on Ashby's media.

Azotobacter, shows the yellow green fluorescence on Burks plate after 4 days' incubation period due to pigment production. Carbon utilization test using mannitol was positive. From the gram staining and biochemicals the organism was identified as *A.vinelandii*. This species was used further for the production of biofertilizer.

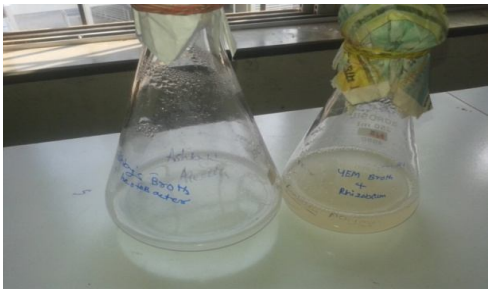

Figure 3: The liquid biofertilizer using *Rhizobium* species and *Azotobacter* species

Figure 4: *Vigna acontifolia* plant before uprooting on 15th day

Figure 5: *Amaranthus tristis* plants before uprooting on the 15th day of growth

BIOMETRIC PARAMETERS

Biometric observations of *Amaranthus tristis* and *Vigna acontifolia*

1. Root length,
2. Shoot length,
3. Fresh and dry weight,
4. Number of leaves and moisture content.

Table 1: Biometric Observation for Amaranthus tristis

Group	Mean no of leaves	Mean Root length (cm)	Mean shoot length (cm)	Mean fresh weight (g)	Mean Dry Weight (g)	Mean Moisture (g)
1	2.55	1.15	2.01	2.00	0.34	1.66
2	2.65	1.69	1.38	2.34	0.60	1.74
3	3.5	1.85	2.20	2.94	0.68	2.26
4	2.1	1.21	2.44	2.84	0.72	2.12
5	3.75	2.0	2.39	3.01	0.83	2.18
6	3.95	2.26	2.25	3.33	0.98	2.43

Table 2: Biometric observation for Vigna acontifolia

Group	Mean no of leaves	Mean root length (cm)	Mean shoot length (cm)	Mean fresh weight (g)	Mean dry Weight (g)	Mean moisture (g)
1	1.8	1.25	4.55	3.27	0.31	3.06
2	2.05	1.31	4.89	4.02	0.38	3.64
3	1.7	1.07	4.36	3.97	0.27	3.37
4	2.1	1.3	4.74	4.00	0.22	3.78
5	2.25	1.48	6.31	4.32	0.28	4.04
6	2.7	1.69	7.36	5.30	0.32	4.98

5. Total carbohydrate estimation:

Standard graph was prepared for glucose using anthrone method.

Table 3: Estimation of carbohydrate test for Amaranthus tristis.

Group	Absorbance 630nm	Value by standard graph (µg/ml)
1	0.52	520
2	0.58	580
3	0.65	650
4	0.60	600
5	0.65	620
6	0.67	660

Table 4:Estimation of total carbohydrate for Vigna acontifolia.

Group	Absorbance 630nm	Value by standard graph (µg/ml)
1	0.52	520
2	0.62	620
3	0.58	580
4	0.57	510
5	0.62	620
6	0.66	660

6. Protein estimation:

Standard graph of protein by Folin Lowry method was prepared

Table 5: Protein estimation forAmaranthus tristis.

Group	Absorbance 660nm	Value by standard graph (µg/ml)
1	0.04	180
2	0.06	270
3	0.08	360
4	0.07	310
5	0.12	480
6	0.13	580

Table 6: Protein estimation for Vigna acontifolia

Group	Absorbance 660nm	Value by standard graph (µg/ml)
1	0.08	360
2	0.10	450
3	0.07	310
4	0.09	400
5	0.12	540
6	0.20	880

7. N,P,K TEST:

Table 7: N,P, K test for Amaranthus tristis

Group	pH	Electrical conductivity	Organic matter (%)	N %	P %	K %
1	6.93	0.42	0.30	2.10	0.10	7.77
2	6.86	0.41	0.54	2.78	0.12	7.37
3	7.04	0.57	0.42	2.93	0.18	6.34
4	7.84	0.55	0.12	1.84	0.10	6.57
5	6.82	0.47	0.41	3.63	0.18	6.30
6	7.0	0.55	0.45	3.84	0.19	6.57

Table 8: N, P, K test for Vigna acontifolia

Group	pH	Electrical conductivity	Organic matter(%)	N %	P %	K %
1	6.0	0.46	0.47	2.20	0.12	4.43
2	6.76	0.51	0.61	3.57	0.11	7.24
3	7.04	0.49	0.45	3.10	0.11	6.64
4	7.11	0.88	0.69	1.84	0.08	7.31
5	6.90	0.54	0.45	4.10	0.12	2.33
6	7.02	0.60	0.46	4.15	0.12	3.98

V. DISCUSSION

The biometric observation of *Amaranthus* plant showed increase in root length, shoot length, number of leaves, fresh weight, dry weight and Moisture contents. The effect of *Azotobacter* fertilizer alone showed high effect on each parameter than the combined effect of rhizobium and chemical fertilizer. But the combined effect of *Rhizobium+ Azotobacter+* chemical fertilizer showed the highest record in each parameter than anyother treatments.

In the *Vigna acontifolia* plant showed the individual effect and combined effect on biometric parameters. In the *Vigna acontifolia* plant the *rhizobium* fertilizer showed more effective results than individual effect on biometric parameters but in the combined effect of all fertilizers showed highest effect than individual fertilizer. It indicated that in *Amaranthus plant, Azotobacter* fertilizer showed the individual effective result and rhizobium fertilizer showed the most individual effect on *Vigna acontifolia* plant, but combined effect of *rhizobium,Azotobacter* and chemical fertilizer shows major effect on both plants.

VI. CONCLUSION

Hence the conclusion of the study is that the combined application of *Azotobacter, Rhizobium and* chemical fertilizer improved that biometric parameters (shoot length, fresh and dry weight and number of leaves). Also, it increased the protein and total carbohydrates in the *Amaranthus tristis* plants as well as *Vigna acontifolia* plant. The application of *Azotobacter* increased the root length of the *Amaranthus tristis.* The application of rhizobium fertilizer increases the shoot length in the *Vigna acontifolia* plants.Thus, the combined effect increased growth and nutrient content of the *Amaranthustritsis* plants and *Vigna acontifolia* plant was proved by the application of biofertilizers along with chemical fertilizer. Hence it is recommended that the use of biofertilizer, along with the chemical fertilizer would be beneficial to the environment. In this research, other important parameter considered for physical and chemical characteristics profile of soil showed at start of experiment inadequate NPK for plantation and after the use of appropriate biofertilizer profile was potentially improved. From this study, it can be concluded that application of biofertilizer and chemical fertilizer either singly or in combination could improve production in agriculture.

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