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Research Article

Impact of individual and combined exposure of bio-fertilizer, chemical fertilizer and vermicompost on the biochemical constituents of *Amaranthus polygonoids* l

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ABSTRACT

An experiment was conducted to assess the impact of exposure of *Amaranths polygonoides* to biofertilizer, chemical fertilizer and vermicompost individually and in combination. The aim of this study was to screen the best combination and dose of bio-fertilizer, chemical fertilizer and vermicompost for getting favourable growth and nutritional characteristics. The treatment includes ten treatments with four replications. The plants samples were analysed with five days of intervals with morphological parameters and biochemical constituents such as total chlorophyll, carotenoids, total amino acid, protein, carbohydrates on 10th ,20th and 30th day old plants and cellular levels of calcium & iron on 20th and 30th day old plants. The greatest impact was exercised in organic fertilizer treatment (vermicompost) and NPK + Phosphobacteria + Azospirillum + Azolla. Soil analysis of both before and after treatment revealed increase in concentration of C, N, P and K when all the biofertilizers were combined with organic chemical fertilizers and applied to the soil. The result suggested that the Bio-fertilizers *Cyanobacteria, Phosphobacteria and Azospirillum* combined with half dose of inorganic fertilizer (NPK) can lead to enhancement in growth and nutritional status of leafy vegetable *Amaranthus polygonoids* compared to control (without fertilizer).This experiment specifically analysed the impact of cyanobacteria *Azolla* which serve as potential biofertilizer for rice fields but only few studies have shown their utilization as biofertilizer for vegetable crops..

Key words

Amaranths polygonoides, biofertilizers, chemical fertilizer, vermicompost,

1.INTRODUCTION

The major challenge affecting the globalization developing countries is to increase agricultural production without compromising on the quality of the product in such a way that that it can augur well in the world market. Agrochemicals responsible for making India self-sufficient through green revolution are no longer able to sustain the crop productivity to the expected level on account of their ill-effects of herbicides / pesticides on the agro system on whole. The use of biofertilizers and biopesticides is considered alternative to traditional use of chemical fertilizers and eco-friendly as is evidenced by reports of sustained high production, more efficient nutrient utilization by crops and restoration of balanced ecosystem. For instance, the high content of both micro and macro nutrients in organic manures along with the slow release of phosphorus could reduce the nutrient deficiency problems and lower the magnitude of phosphorus fixation.

Application of biofertilizers or the biological based products can be used as another advanced but simple biotechnological process considered as another alternative to develop organic, sustainable, green and pollution free agriculture (Koperuncholan and Ahmed John 2011b). There is a lot of interest generated in recent years to device alternate ways to collect, process, and compost, utilize organic manure as well as biofertilizers like *Azospirillum, Acetobacter, Rhizobium, Azolla,* and Phosphate solubilizing bacteria and enrich fertility status of the soil. Biological N2 fixing is most important source of nitrogen in soil and free-living nitrogen-fixing bacteria like *Azospirillium* have capacity to fix atmospheric nitrogen in form of ammonia in soil. Because of this property, *Azospirillium* is used as an important biofertilizer. Using the isolated strains of *Azospirillium*, biofertilizer is prepared on large scale and immobilized in to carriers like charcoal powder (Jakhar et al., 2011 and Koperuncholan and Ahmed John 2011a).

Cyanobacteria, gram negative bacteria, also play a remarkable role in the field of energy production, biofertilizer, human food, animal feed, polysaccharides, biochemical and pharmaceuticals and in cleaning up of the environment, etc. These oxygen evolving photosynthetic prokaryotes are found in varied aquatic and terrestrial habitats in nature as well as in association with Azolla biomass (Christopher et al., 2007 and Fazal Mohamed et al. 2011) serve as potential biofertilizers for rice fields but only few studies have shown their utilization as biofertilizer for vegetable crops. Vermicompost, a blackish–brown humus like coarse granular material having electrically charged particles is applied to soil for improved adsorption of plant nutrients from the soil. The organic carbon in vermicompost releases the nutrients slowly and steadily into the system and enables the plant to absorb these nutrients (Ahmed John and Koperuncholan, 2012a). Thus, vermicompost is a rich source of micronutrients, acts as chelating agent and regulates the availability of metallic micronutrients to the plants in absorbable form.

Hence the study was undertaken to analyse the effect of different commercial biofertilizer on morphological, biochemical parameters and soil quality of tropical *Amaranthus polygonoides.* This experiment specifically analysed the impact of cyanobacteria *Azolla* which serve as potential biofertilizers for rice fields but only few studies have shown their utilization as biofertilizer for vegetable crops.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL

Amaranthus polygonoids is a leafy vegetable is studied in the current experiment. It goes by the common name of Tropical Amaranth and belongs to the family *Amaranthaceae.* The seeds of tropical Amaranthus obtained from commercial agro shop in Tiruchirappalli were used in the experimental study.

2.2 BIOFERTILIZERS

The biofertilizers (carrier based packets) such as Cyanobacteria-Azolla, Phosphobacteria and Azospirillum were obtained from the Department of Microbiology, Anbil Dharmalingam Agriculture College & Research Institute, Tiruchirappalli. The chemical fertilizer (NPK), vermicompost obtained from commercial agro shop in Tiruchirappalli were used in experiments.

2.3 SOIL CHARACTERISTICS

The soil samples were taken from the field before stating the experiment and tested to in Government of Tamilnadu Soil Testing Laboratory Department of Agriculture, Tiruchirappalli for various physic-chemical characteristics.

2.4 TREATMENT AND EXPERIMENTAL DESIGN

The treatment includes ten treatments with four replications. The different treatment combinations and their dosage of fertilizers, biofertilizers and vermicompost to soil are presented. T1 - Control; T2 - Cyanobacteria (Azolla), [50g of carrier based Azolla]; T3 - Phosphobacteria [50g of carrier based Phosphobacteria]; T4 - Azospirillum, [50g of carrier based Azospirillum]; T5- Inorganic (N-P-K) [5g of N-P-K (19:19:19)]; T6 - Vermicompost [0.5 kg]; T8 - Inorganic (N-P-K) +Azospirillum, [5g of N-P-K (19:19:19) + 50 g of carrier based Azospirillum]; T7 - Inorganic (N-P-K) + Phosphobacteria [5g of N-P-K (19:19:19) + 50 g of carrier based Phosphobacteria]; T9 - Inorganic (N-P-K) + Cyanobacteria (Azolla); [5g of N-P-K (19:19:19) +50 g of carrier based Azolla]; T10 - Phosphobacteria + Azospirillum + cyanobacteria (Azolla) + Inorganic (N-P-K), [2.5 g of N-P-K (19:19:19) +12.5 g of carrier based Azolla + Phosphobacteria + Azospirillum].

Earthen pots of 15 cm diameter and 18 cm depth were filled with 5 kg soil were used for all treatments. Soil was sterilized in order to obtain the individualistic impact of different treatments. The sterilized soil for all nine pots are mixed with the respective components. The application of biofertilizers, inorganic fertilizer or vermicompost were done a day prior to seed sowing.

3. ANALYSIS OF MORPHOLOGICAL PARAMETERS

The morphological measurements of the plants such as height of the shoot and length of the root were measured at 5 days of intervals (i.e.5th, 10th, 15th, 20th, 25th and 30th day) for all ten treatments.

3.1 QUANTITATIVE ANALYSIS OF PHYTOCONSTITUENTS

The chlorophyll pigments in the leaves were estimated following the method of Arnon 1949. The amount of total chlorophyll, carotenoids were measured on 10th ,20th and 30th day of treatment. After pre-cleaning, weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was made up to a known one and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated and is expressed in mg/g fresh weight. Likewise, the biochemical parameters, such as, total amino acid, protein, carbohydrates, and cellular levels of calcium and iron were quantified in 20 and 30 day old plants. The amino acids were estimated by Koperuncholan and Ahmed John 2011) method which is calorimetrically measured at 570nm. Proteins were estimated by Bradford method and the absorbance was measured at 595 nm against blank/ sample. Carbohydrates were estimated by anthrone method which can be measured by using calorimetrically at 620nm (or) by using a red filter. All the trials were performed thrice and the mean values were presented.

3.2 TRACE METAL ANALYSIS

The plant sample such as *A. polygonoids* was collected from the Tiruchirappalli district, Tamil Nadu. The plant leaves were carefully removed and washed with sterile distilled water, separately. The cleaned leaves were dried in shadow area and were grinned with mortar and pestle. The powered plant samples were stored in sterile plastic container. The 1 g of powdered plant sample was treated with aqua-regia mixture in Teflon bomb and was incubated at 140 °C for 2-3 days. After incubation, the reaction mixture was filtered with whatman No.1 filter paper. Then, the extraction was tested for trace metals (Fe, and Ca) analysis by the 797 VA Computrace voltametry, Metrohm. To avoid the contamination, the devices were rinsed with acidified water (10% HNO_3) and weighted to dissolve metals before analysis. And, all the equipment's and containers were soaked in 10% NHO_3 for 24 h then rinsed thoroughly in de-ionized water before use. Also find the below detectable limit of the instruments.

4. RESULTS AND DISCUSSION

4.1 PHYTOCHEMICAL CONSTITUENTS OF SECONDARY METABOLITES

In terms of morphological evaluation, the results revealed that there were significant differences in Amaranthus polygonoids. The height was found to increase to a significant level with the application of organic manure vermicompost (T6) and then in T10 (Phosphobacteria+ Azospirillum +Azolla+ inorganic fertilizer) Table 1. The photosynthetic pigments chlorophyll (chlorophyll 'a ', 'b' and the total) and carotenoid content of leaves on 10th, 20th and 30th day old plant increased in all treatments. (Graph 1-3).

The total amino acid, protein and carbohydrate contents increased at all treatments compared to the control. T2 (Azolla treatment) caused minimum levels of all the above parameters. (Graph 1-3). Amino acid, protein and carbohydrate content continued to go up in 30 day old plants. The leaves were assayed for total Fe and Ca contents on 20^{th} and 30^{th} days of treatment. (Graph 4-6). The soil testing report shows its pH was not significantly altered upon various treatment of soil. T10 (Phosphobacteria+ Azospirillum + Azolla + N-P-K) treatments had a great impact in terms of organic C content and NPK levels) (Table 2).

Densilin et al (2009) and Ahmed John and Koperuncholan (2012) reported that the vegetative growth parameters increased in the biofertilizers treated plants compared to control. Vermicompost is rich in plant macro nutrients (N, P2O5 and K2O), secondary elements (Ca, Mg) and vital micronutrients like Fe, B, Zn and Mo (Aher and Khapke, 2009; Ramesh et al. 2014). Therefore, there is a positive relationship between the application of vermicompost and enhancement in growth of Amaranthus polygonoids observed in the present study. Treatment with biofertilizers enhanced the chlorophyll and carotenoid content of Amaranthus polygonoids. This is evident from the data shown in Graph. 1-3. Such an increase in the levels of pigments on biofertilizer treatment was reported earlier by Koperuncholan and Manogaran, (2015). The increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency. It can be used as one of the criteria for quantifying photosynthetic rate and there is a synergistic interaction between applied biofertilizers and chlorophyll content (Koperuncholan, 2015 and Sinthiya and Koperuncholan 2015). The presence of higher amount of total carbohydrates in biofertilizer treated plants indicate that the operation of synergistic effect.

There was significant enhancement in Ca and Fe content of the experimental plant exposed to biofertilizers, NPK and its combination. The increase in their concentration was significant over control (T1) but the combined exposure of biofertilizers and NPK (T10) caused the highest values. These results are in conformity with the results obtained by Mishra et al (2011) and Koperuncholan et al. (2010). who reported high Ca and Fe content in seed of Fenugreek after exposure to combination of biofertilizers. From the above findings it is concluded that, application of different biofertilizers, organic manures and biofertilizers along with lower dose of chemical fertilizer plays a significant role in enhancing soil fertility. An integrated nutrient management (INM) is the best available option and depends on the judicious use of biofertilizers and organic manures in suitable combination with chemical fertilizers. A sound management of fertilization ensures an enhanced and safeguarded environment.

Table 1. Shoot length, root length and shoot-root ratio of *Amaranthus polygonoids*

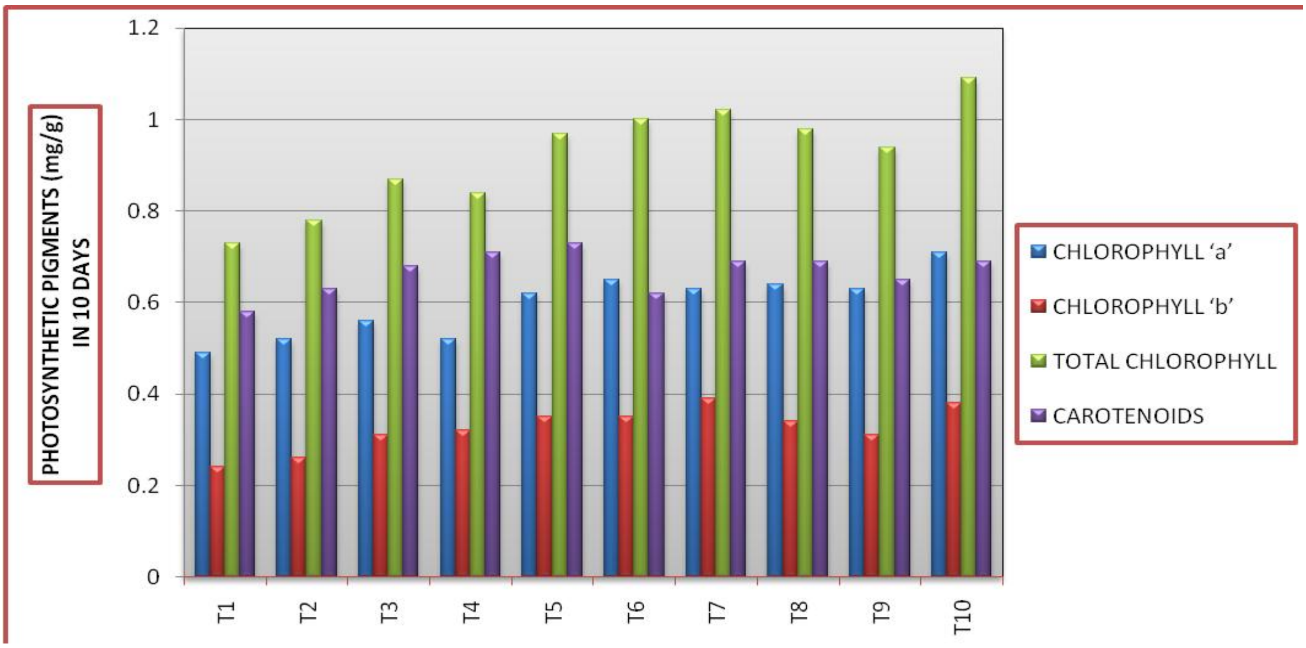
No of Days	Part of the plant	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
IN 5 DAYS	SHOOT	2.2	2.1	2.5	2.4	2.4	3	2.5	2.4	2.4	2.6
	ROOT(cm)	0.5	0.5	0.6	0.5	0.6	1.6	0.6	0.5	0.6	0.7
	ROOT SHOOT RATIO	1:4.4	1:4.2	1:4.2	1:4.8	1:4	1:1.9	1:4.2	1:4.8	1:4	1:3.8
IN 10 DAYS	SHOOT	3.1	3.2	3.4	3.4	3.6	4.6	3.8	3.7	3.6	4.2
	ROOT(cm)	1.5	1.4	1.7	1.6	1.8	2.3	1.8	1.7	1.8	2
	ROOT SHOOT RATIO	1:2	1:2.3	1:2	1:2.1	1:2	1:2	1:2.1	1:2.2	1:2	1:2.1
IN 15 DAYS	SHOOT	4.4	4.3	5.6	5.6	5.7	7.1	5	5.8	5.7	6.6
	ROOT(cm)	2	2.2	2.5	2.5	2.8	3.6	2.9	2.8	2.5	3.3
	ROOT SHOOT RATIO	1:2.2	1:1.9	1:2.2	1:2.2	1:2.04	1:1.9	1:1.7	1:2.1	1:2.3	1:2
IN 20 DAYS	SHOOT	6.5	6.5	7.9	7.8	8	9.8	8.4	8.2	8.1	8.9
	ROOT(cm)	3.6	3.5	4.1	4.1	4	4.8	4.3	4.2	4.2	4.5
	ROOT SHOOT RATIO	1:1.8	1:1.8	1:1.9	1:1.9	1:2	1:2.04	1:1.9	1:1.96	1:1.9	1:2
IN 25 DAYS	SHOOT	8.1	8	9.3	9.2	9.4	11.7	9.8	9.6	9.5	10.3
	ROOT(cm)	4.2	4	5.2	5.2	5.3	5.9	5.4	5.4	5.3	5.5
	ROOT SHOOT RATIO	1:1.7	1:2	1:1.8	1:1.8	1:1.8	1:2	1:1.8	1:1.8	1:1.8	1:1.2
IN 30 DAYS	SHOOT	10.2	10	11.6	11.5	11.6	14.5	11.8	11.7	11.6	12.8
	ROOT(cm)	5.3	5.2	5.8	5.6	5.8	6.3	5.8	5.7	5.7	6
	ROOT SHOOT RATIO	1:1.9	1:1.9	1:2	1:2.04	1:2.04	1:2.3	1:2.04	1:2.08	1:2.04	1:2.2

T1 - Control; T2- Azolla treatment ; T3- PSB treatment ; T4 - Azospirillum treatment ; T5 - NPK treatment ; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum+Azolla +NPK

Table 3. Properties of soil before and after its treatment

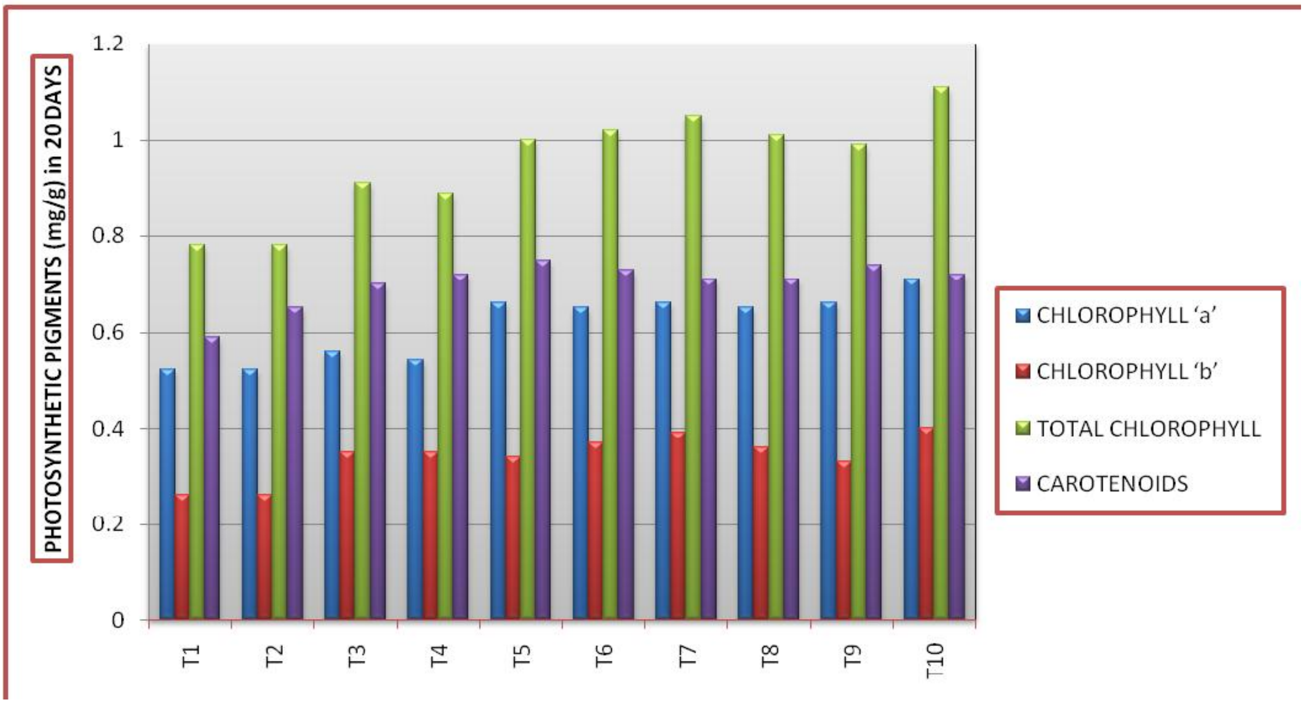
Treatment	pH	Organic - C (%)	Total - N (%)	Total - P (%)	Total - K (%)
T1	7.24	0.23	0.58	0.21	0.73
T2	7.24	0.24	0.60	0.20	0.69
T3	7.25	0.29	0.66	0.28	0.74
T4	7.25	0.30	0.65	0.19	0.76
T5	7.13	0.18	0.72	0.33	0.88
T6	7.25	0.32	0.68	0.30	0.91
T7	7.20	0.26	0.75	0.32	0.90
T8	7.19	0.26	0.74	0.29	0.88
T9	7.16	0.18	0.72	0.34	0.89
T10	7.25	0.34	0.78	0.35	0.90

T1 - Control; T2- Azolla treatment; T3- PSB treatment; T4 - Azospirillum treatment; T5 - NPK treatment; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum + Azolla +NPK



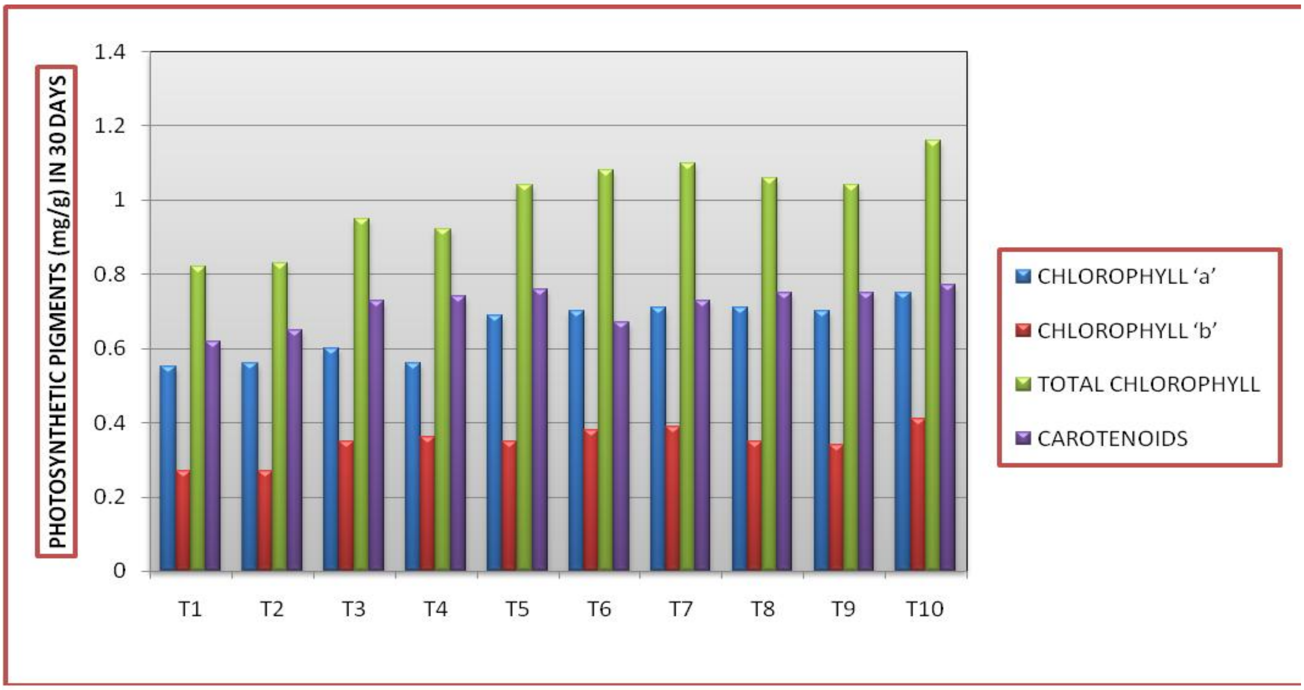
T1 - Control; T2- Azolla treatment; T3- PSB treatment; T4 - Azospirillum treatment; T5 - NPK treatment; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum Azolla +NPK

Graph-1. Photosynthetic pigment contents of leaves in 10-day old *Amaranthus polygonoids*



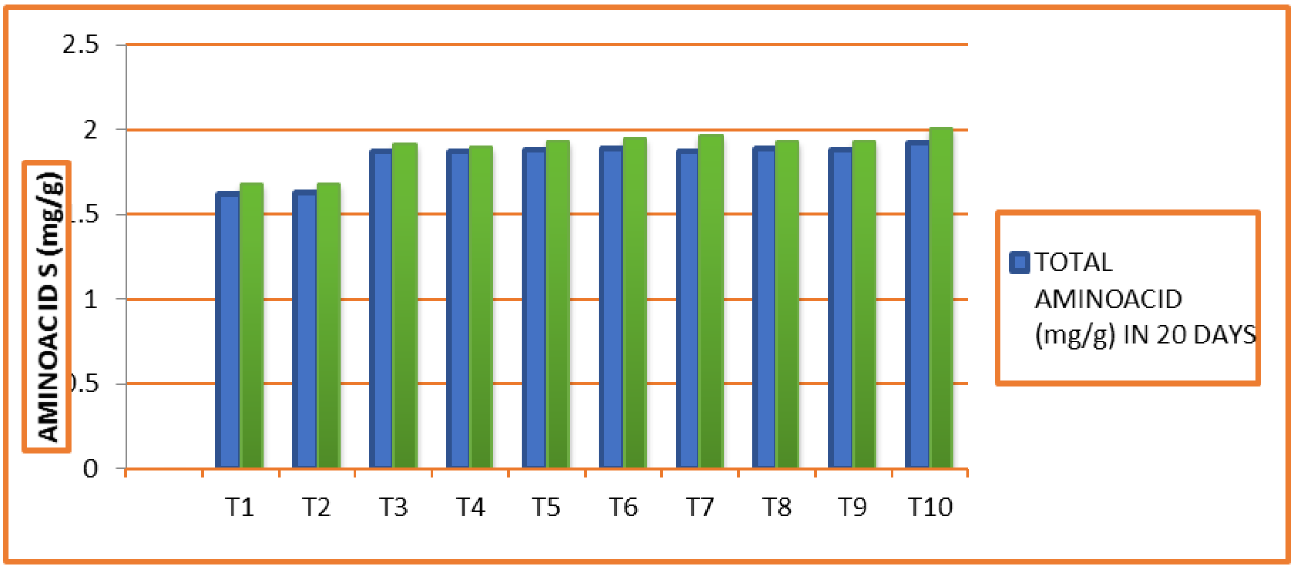
T1 - Control; T2- Azolla treatment; T3- PSB treatment; T4 - Azospirillum treatment; T5 - NPK treatment; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum Azolla +NPK

Graph 2. Photosynthetic pigment contents of leaves in 20-day old *Amaranthus polygonoids*



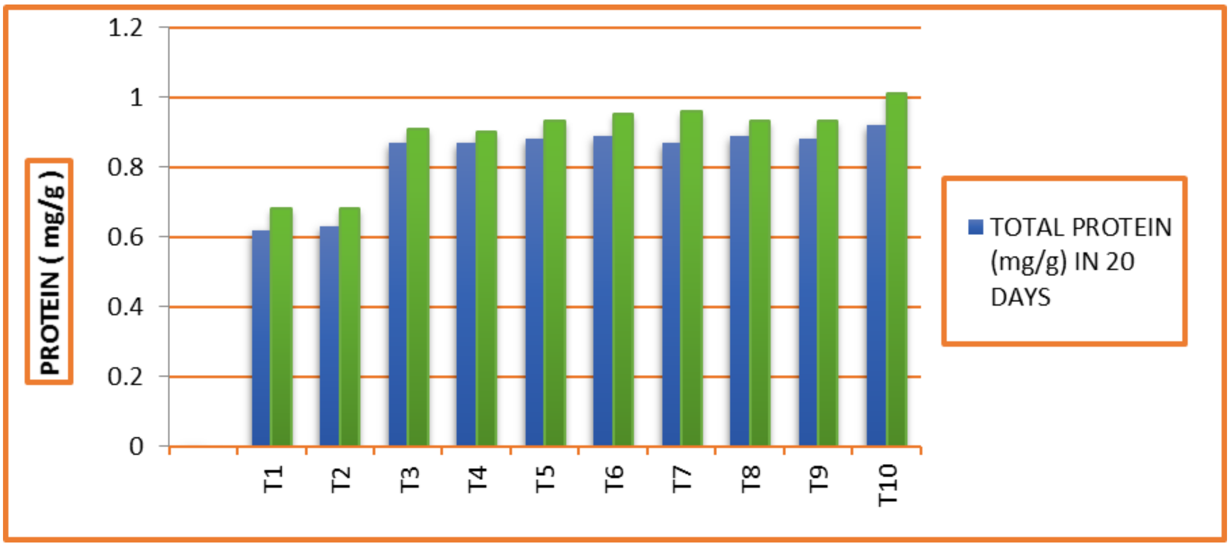
T1 - Control; T2- Azolla treatment; T3- PSB treatment; T4 - Azospirillum treatment; T5 - NPK treatment; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum Azolla +NPK

Graph 3. Photosynthetic pigment contents of leaves in 30-day old *Amaranthus polygonoids*



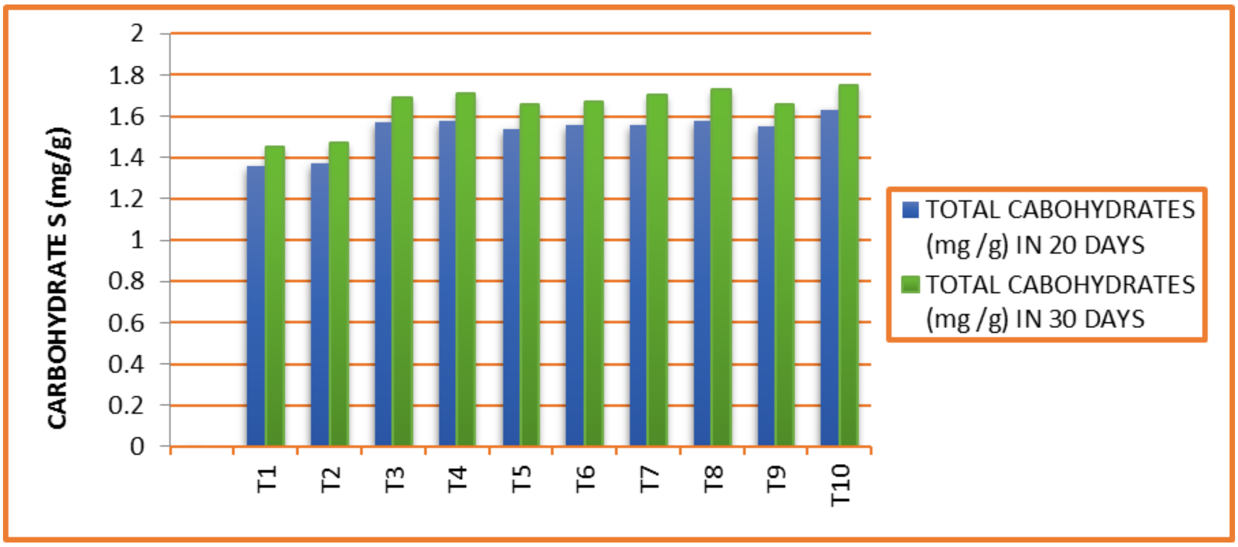
T1 - Control; T2- Azolla treatment; T3- PSB treatment; T4 - Azospirillum treatment; T5 - NPK treatment; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum Azolla +NPK

Graph 4. Total amino acid content of leaves of *Amaranthus polygonoids*



T1 - Control; T2- Azolla treatment; T3- PSB treatment; T4 - Azospirillum treatment; T5 - NPK treatment; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum Azolla +NPK

Graph 5. Total protein content of leaves of *Amaranthus polygonoids*



T1 - Control; T2- Azolla treatment; T3- PSB treatment; T4 - Azospirillum treatment; T5 - NPK treatment; T6 - Vermicompost treatment; T7- NPK+PSB; T8 - NPK + Azospirillum; T9- NPK +Azolla; T10 – PSB +Azospirillum Azolla +NPK

Graph 6. Total carbohydrate content of leaves of *Amaranthus polygonoids*

5. CONCLUSION

In order to overcome the adverse effects of chemical cultivation and the deficit in nutrient supply, it is suggested that efforts should be made to exploit all the available resources of nutrients under the 'integrated nutrient management (INM)'. This is the best available option and depends on the judicious use of Biofertilizers and organic manures in suitable combination with chemical fertilizers. INM only ensures higher productivity but ensures the clean environment. In this context, the present study is considered important and it is suggested that in the presence of all the biofertilizers, only half the dose of NPK was enough to promote growth and all nutrient parameters. Biofertilizers are cheaper, easy to be produced, pollution free and cuts down at least by 50% that is incurred towards the use of chemical fertilizers.

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