

Research Article



Individual and Combined Effect of Biofertilizer, Chemical Fertilizer and Vermicompost on *Amaranthus Tristis*

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ABSTRACT

Fertilizer is a very essential input in crop production. Biofertilization technology has taken a part to minimize production costs and at the same time avoid the environmental hazards. Organic agriculture is one of the ways that can produce high quality crops and increase yield. Hence this study is to investigate that how different combinations of biofertilizer (*Azospirillum*), chemical fertilizers and vermicompost would affect the growth of a green leafy vegetable, *Amaranthus tristis*. After the growth period of about 40 days the biometric and biochemical analysis was done and it shows that there is significant change in biometric parameters and increase in the biochemical constituents.

Keywords: *Azospirillum*, *Amaranthus tristis*, biofertilizer, chemical fertilizers, vermicompost.

INTRODUCTION

India is the second-largest producer and consumer of N fertilizer in the world, after China, with close to a 15 per cent share of the global total¹. Fertilizer use has been and will continue to be a major factor in the increasing agricultural production and productivity. Fertilization increases root growth, drought tolerance and resistant to frost. It helps plants to resist attack of pests and diseases and stalk lodging. The application of chemical fertilizers over a period has resulted in poor soil health, reduction in produce and increase in incidences of pest and disease and environmental pollution². Continuous imbalanced fertilization causes depletion in soil fertility, drop in crop yields, poor crop quality and diminishing profits.

Organic wastes and biofertilizers are the alternate sources to meet the nutrient requirement of crops and to bridge the future gaps³. Biofertilizer, organic manuring and bio-control have emerged as a promising component of integrating nutrient supply system in agriculture. Microbiological fertilizers are important to environment friendly sustainable agricultural practices⁴. They can be conveniently produced and partially substitute the chemical fertilizers to avoid environmental pollution. *Azospirillum* spp. contribute to increased yields of cereal and forage grasses by improving root development in properly colonized roots, increasing the rate of water and mineral uptake from the soil and by biological nitrogen fixation. It is one of the important biofertilizer, which is found to fix nitrogen in association with world's most staple food crops like rice, maize, sorghum, wheat and millets⁵.

Therefore organic farming helps to provide many advantages such as; eliminate the use of chemicals in the form of fertilizers/pesticides, recycle and regenerate waste into wealth; improve soil, plant, animal and

human health and creating an eco-friendly, sustainable and economical bio-system models.

Amaranth 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*, which is commonly known as "araikeerai" in Tamil. It is used to treat stomach flu, diarrhea, gastroenteritis, stop excessive menstruation and contraception. When applied externally, it can reduce tissue swelling from sprains and tick bites. It is gluten-free and hence is a good alternative to wheat. Amaranth can reduce total cholesterol and LDL and can increase HDL.

This study is conducted by growing the *Amaranthus tristis* plants in various treatments which include the individual and combined applications of biofertilizer (*Azospirillum*), chemical fertilizers and vermicompost, and finding the changes in its biometric and biochemical parameters. Hence the main purpose of the study is to find that which of the treatments would help in increasing the yield and nutritional content of the plant.

MATERIALS AND METHODS

The Biofertilizer (*Azospirillum*) and chemical fertilizer (urea and potash) were collected from Tamilnadu Agricultural University, Coimbatore. The vermicompost and seeds of *Amaranthus tristis* were collected from Super seeds and nursery, Coimbatore. A pot culture study was laid out in completely randomized design with four replications for each treatment. Each pot was uniformly filled with 8 kg of soil and treated with different combinations of *Azospirillum* (10g per pot), chemical fertilizer (0.2g urea and 0.08g potash per pot) and vermicompost (0.6kg per pot). The treatments were as follows;

T1- Control,

T2- Chemical fertilizer,



T3- *Azospirillum*,

T4- Vermicompost,

T5- *Azospirillum* + Vermicompost,

T6- *Azospirillum* + Chemical fertilizer,

T7- Chemical fertilizer + Vermicompost,

T8- Chemical fertilizer + *Azospirillum* + Vermicompost.

About 50 seeds were sown in each pot and allowed to germinate. They were then thinned to 15 plants per pot. The pots were watered daily and holes were made at the base of the pot to prevent water logging condition. The plants were uprooted on the 40th day without any damage. 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.

Biometric Observations

The fresh plants were used to determine the fresh weight, shoot length (measured from the point of first cotyledonary node to the tip of the longest leaves), root length (measured from the crown region of the plant to the tip of the root) and number of leaves. For dry weight, the plants were dried at 70°C overnight and weighed.

Biochemical Analysis

Chlorophyll estimation⁶

1g of the sample of leaf tissue was weighed and ground into a fine pulp with the addition of 20 ml of 80% acetone. The supernatant was centrifuged (5000 rpm for 5 minutes) and transferred to 100 ml volumetric flask. The residue was ground with 20 ml of 80% acetone; the supernatant was centrifuged and transferred to the same volumetric flask. This procedure was repeated until the residue turned colorless. The mortar and pestle were washed thoroughly with 80% acetone and the clear washings were collected in the volumetric flask. The volume was made up to 100 ml with 80% acetone. The absorbance value of the solution was read at 645 and 663 nm against the solvent (80% acetone) blank.

The amount of chlorophyll present in the extract as mg chlorophyll per g tissue was calculated using the following equations.

$$\text{mg chlorophyll } a/g \text{ tissue} = 12.7 (A_{662}) - 2.69 (A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll } b/g \text{ tissue} = 22.9 (A_{645}) - 2.69 (A_{662}) \times \frac{V}{1000 \times W}$$

$$\text{mg total chlorophyll } /g \text{ tissue} = 20.2 (A_{645}) + 8.02 (A_{662}) \times \frac{V}{1000 \times W}$$

Where,

A – Absorbance at specific wavelengths,

V – Final volume of chlorophyll extract in 80% acetone

W – Fresh weight of tissue extract

Carotenoid estimation⁷

5g of the sample was weighed and saponified for about 30 minutes in a shaking water bath at 37°C after extracting the sample in 12% alcoholic KOH. The saponified extract was transferred into a separating funnel packed with glass wool and calcium carbonate containing 10 to 15 ml of petroleum ether layer. The lower aqueous phase was transferred to another separating funnel, and the petroleum ether extract containing the carotenoids pigments to amber colored bottle. The extraction of the aqueous phase was repeated. A small quantity of anhydrous sodium sulphate was added to the petroleum ether extract to remove the turbidity. The final volume of the petroleum ether extract was noted and diluted by a known dilution factor. The absorbance of the extracts at 450nm and 503 nm was noted in spectrophotometer.

$$\text{Amount of total carotenoids present} = \frac{P \times 4 \times V \times 100}{W}$$

Where,

P – Optical density of the sample

V – Volume of the sample

W – Weight of the sample

Estimation of Total carbohydrates⁸

100mg of the sample was weighed into a boiling tube, was hydrolysed in a boiling water bath for 3 hours with 5ml of 2.5N hydrochloric acid and cooled to room temperature. This was neutralized with solid sodium carbonate until the effervescence ceases. The volume was made up to 100ml and centrifuged and the supernatant was collected. 0.5 to 1.0ml aliquots were used for analysis. The volume was made up to 1 ml in all tubes by adding distilled water. 4 ml of anthrone reagent was then added and heated for 8 minutes in a boiling water bath. Tubes were then cooled rapidly and read the absorbance at 630 nm.

Protein estimation⁹

500mg of the sample was ground well with a pestle and mortar in 5-10ml of the phosphate buffer, centrifuged and 0.1ml of the supernatant was used for the protein estimation.

The volume was made up to 1ml in all the test tubes. A tube with 1ml of water served as a blank. 5ml of reagent C was added to all the test tubes including the blank, mixed well and allowed to stand for 10minutes. 0.5ml of reagent D was then added, mixed well and was incubated at room temperature in the dark for 30minutes. The blue colour developed was read in a spectrophotometer at 660nm. A standard graph was drawn and the amount of protein in the sample was calculated.

Where, Reagent A is 2% sodium carbonate in 0.1N sodium hydroxide.

Reagent B is 0.5% copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1% potassium sodium tartarate.

Reagent C is alkaline copper sulphate solution: 50ml of reagent A was mixed with 1ml of reagent B, prior to use.

Reagent D is Folin-Ciocalteu Reagent

Vitamin A estimation¹⁰

2 μl of the sample in duplicates was used for this estimation. The volume in all the tubes was made upto 0.1 ml with chloroform. Using a fast delivery pipette, 2 ml of TCA reagent was added rapidly mixing the contents of the tube. The absorbance was recorded immediately at 620nm in a spectrophotometer. A standard graph was constructed and the concentration of vitamin A was calculated.

Vitamin C estimation¹¹

1g of the sample was homogenized in 10ml of 4% TCA and centrifuged (2000rpm for 10 minutes). To the supernatant obtained, a pinch of activated charcoal was added, shaken well and kept for 10 minutes. This was centrifuged once again and the charcoal residue was removed. The volume of the clear supernatants was then noted. 0.5 and 1.0 ml aliquots of this supernatant were taken for the assay. The assay volumes were made up 2.0ml with 4%TCA. 0.5ml of DNPH (2, 4-dinitrophenyl hydrazine) reagent was added to all the test tubes. 2 drops of 10% thiourea solution was then added and incubated at 37°C for 3 hours. The osazones formed were dissolved in 2.5ml of 85% sulphuric acid, in cold, drop by drop, with no appreciable rise in temperature. DNPH reagent and thiourea solution after the addition of H_2SO_4 served as the blank. The tubes were incubated for 30 minutes at room temperature, and the absorbance was read spectrophotometrically at 540nm. The content of ascorbic acid in the sample was calculated using the standard graph.

Phosphorus estimation¹²

The ash of 2g of vegetable sample was dissolved in 2 to 3 drops of concentrated hydrochloric acid and the volume was made up to 25 ml with distilled water. 0.1ml of the ash solution was taken, made up the volume to 4.7 ml with water and added 0.5 ml of molybdate I solution and 0.2 ml of ANSA (amino naphthol sulphonic acid). The color developed was read after 20 minutes in a colorimeter using red filter against a reagent blank.

Iron estimation

Iron estimation was carried out using wong's method. 2ml of the ash solution (sample) was taken and the volume was made up to 6.5 ml with distilled water. A blank using 6.5 ml of distilled water was used. 1ml of 30% H_2SO_4 , 1ml of 7% Potassium per sulphate and 1.5 ml of 40 % Potassium thiocyanate solution were added. The red colour developed was measured within 20 min at 540nm in a spectrophotometer. The concentration of iron in the sample solution was calculated using standard graph.

Statistical analysis like arithmetic mean and variance was employed to predict the results of the experiment.

RESULTS AND DISCUSSION

Biometric parameters

Root length: The application of vermicompost alone (T4) or in combination with *Azospirillum* and chemical fertilizer (T8) highly influenced the root length of *Amaranthus tristis* plants. This was followed by the treatment T5 (*Azospirillum* + vermicompost). The rest of the treatments recorded insignificant root length when compared to the control (shown in table 1). A study by Islam *et al* showed similar results, where the root length of the rice plants increased significantly on the combined application of *Azospirillum* and chemical fertilizers¹³.

Shoot length: All the treatments except T2 (chemical fertilizer) and T3 (*Azospirillum*) significantly ($p < 0.05$) enhanced the shoot length. Among the treatments, T8 with the tripartite application of *Azospirillum*, chemical fertilizer and vermicompost was found to be superior in registering the maximum shoot length, which was followed by T7 (chemical fertilizer + vermicompost), T5 (*Azospirillum* + vermicompost) and T4 (vermicompost) respectively. The shoot length of the treatment, T6 (*Azospirillum* + chemical fertilizer) was on par with the control. Thus the application of vermicompost along with *Azospirillum* and chemical fertilizer has highly influenced the growth of the shoot of *Amaranthus tristis* plants. Figure 1 shows the appearance of plants as on 40th day after sowing. The combined application of vermicompost with chemical fertilizer recorded significantly higher vine length in *Pisum sativum*¹⁴.

Fresh and dry weights: It is clear from the table 1 that the fresh and dry weights of the treatment T8 (*Azospirillum* + chemical fertilizer + vermicompost) was found to be maximum, which was followed by T4 (vermicompost), T5 (*Azospirillum* + vermicompost) and T7 (chemical fertilizer + vermicompost) respectively. Thus the application of vermicompost along with *Azospirillum* and chemical fertilizer influenced the fresh and dry weights of the *Amaranthus tristis* plants. A study by Zarei *et al* had also shown that the inoculation of *Azospirillum* increased fresh and dry weights of soybean¹⁵. The dry weight of sweet potato increased with the combined application of organic and inorganic fertilizers was also reported by Saikia *et al*¹⁶.

Number of leaves and moisture content: The maximum number of leaves was registered in T8 (*Azospirillum* + chemical fertilizer + vermicompost) which was followed by T7 (chemical fertilizer + vermicompost) and T5 (*Azospirillum* + vermicompost) respectively. The treatments T5 (*Azospirillum* + vermicompost) and T7 (chemical fertilizer + vermicompost) registered a comparable number of leaves. The rest of the treatments did not significantly influence the number of leaves of *Amaranthus tristis* plants. Similar results were reported by Jala-abadi *et al*, where the application of organic



manure and biofertilizer increased the grain yield and leaves in wheat¹⁷.

The application of vermicompost alone (T4) or in combination with *Azospirillum* (T5) and chemical fertilizer (T7 & T8) highly influenced the moisture content of the

Amaranthus tristis plants. The rest of the treatments recorded comparable moisture content. Yadav *et al.*, reported that the application of biofertilizers increased the moisture content in wheat crops¹⁸.

Table 1: Biometric observations in *Amaranthus tristis* on the 40th day of growth

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Number of leaves (Number/plant)	Moisture content (g)
T1	5.33	7.43	0.33	0.04	16.07	0.29
T2	4.93	6.93	0.37	0.03	7.06	0.33
T3	4.98	6.21	0.42	0.05	15.03	0.37
T4	8.08	11.45	1.75	0.19	24.01	1.56
T5	7.05	12.43	1.53	0.18	26.01	1.34
T6	3.43	7.42	0.36	0.06	16.04	0.30
T7	5.98	12.84	1.73	0.16	26.17	1.54
T8	8.25	13.13	1.76	0.25	30.02	1.51
CD(0.05)	0.009	0.095	0.009	0.031	0.154	0.006

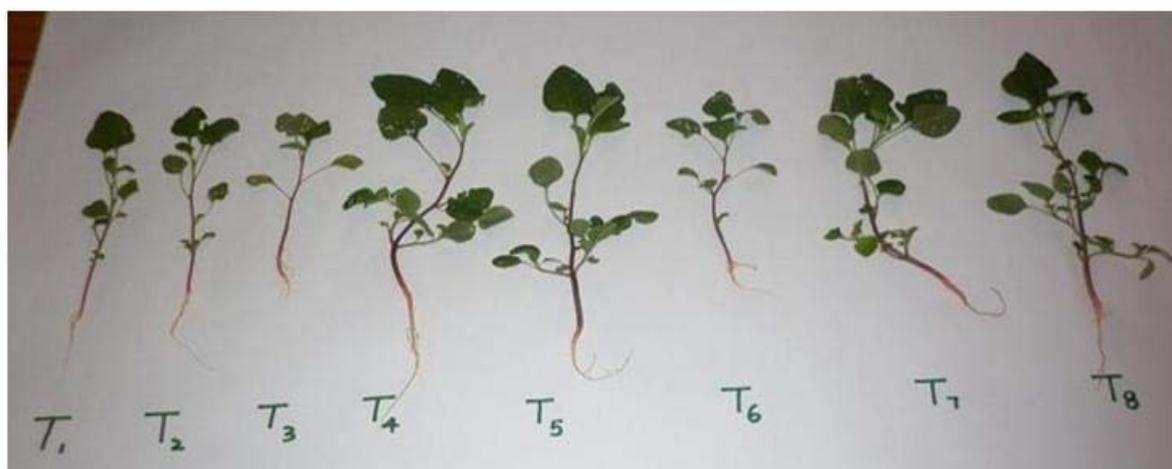


Figure 1: *Amaranthus tristis* plants after uprooting, on the 40th day of growth.

Table 2: Biochemical parameters in *Amaranthus tristis* at the 40th day of growth

Treatments	Chlorophyll (mg/g)	Carotenoids (mg/g)	Protein (mg/g)	Total carbohydrates (mg/g)	Vitamin A (mg/g)	Vitamin C (mg/g)	Phosphorus (mg/g)	Iron (mg/g)
T1	2.18	0.84	29.10	28.03	1.31	112.10	172.10	1.42
T2	2.50	1.05	36.10	36.03	1.45	144.07	252.03	2.21
T3	1.70	1.06	32.00	34.00	1.55	149.10	214.13	2.41
T4	2.20	1.34	46.10	42.03	1.70	160.10	265.23	2.67
T5	2.80	0.79	52.23	43.03	1.90	154.10	283.26	3.01
T6	2.12	0.74	39.23	33.03	1.40	118.03	153.30	1.31
T7	1.80	1.88	50.73	45.00	2.20	170.10	248.90	2.85
T8	1.81	1.54	61.17	48.03	2.80	191.07	294.10	3.85
CD(0.05)	0.015	0.005	0.283	0.173	0.017	0.172	0.240	0.008

Biochemical constituents

Chlorophyll: It is clear from table 2 that the chlorophyll content of the treatment T5 (*Azospirillum* + vermicompost) was found to be maximum when compared to the other treatments. This was followed by the treatments T2 (chemical fertilizer), T4 (vermicompost) and T6 (*Azospirillum* + chemical fertilizer) respectively. Thus the application of *Azospirillum* and vermicompost highly influenced the chlorophyll content of the *Amaranthus tristis* plants, which in turn reflects the growth. The application of biofertilizers in combination with other fertilizers greatly influenced the amount of chlorophyll in dill (*Anethum graveole*s) leaves¹⁹.

Carotenoids: Among the various treatments, T7 (chemical fertilizer + vermicompost) recorded the highest amount of carotenoids, which was followed by T8 (*Azospirillum* + chemical fertilizer + vermicompost) and T4 (vermicompost) respectively.

Protein: The protein content of all the treatments was found to be significantly higher when compared to the control. Among the treatments T8 (*Azospirillum* + chemical fertilizer + vermicompost) recorded the highest protein content, which was followed by T5 (*Azospirillum* + vermicompost) and T7 (chemical fertilizer + vermicompost) respectively. Thus the application of vermicompost along with the chemical fertilizer and *Azospirillum* highly influenced the protein content of *Amaranthus tristis*.

Total carbohydrates: The level of total carbohydrate content of the *Amaranthus tristis* was highly enhanced by the application of *Azospirillum*, chemical fertilizer and vermicompost (T8). This was followed by the application of vermicompost along with the chemical fertilizer (T7) and *Azospirillum* (T5) respectively. These findings are supported by the study led by Zaller *et al.*, who had found that the application of vermicompost highly enhanced the carbohydrate content in tomato plants²⁰.

Vitamin A: As depicted in Table 2, the vitamin A level was found to be higher in the treatment T8 (*Azospirillum* + chemical fertilizer + vermicompost) which was followed by T7 (chemical fertilizer + vermicompost) and T5 (*Azospirillum* + vermicompost) when compared to other treatments.

Vitamin C: All the treatments recorded significantly ($p < 0.05$) higher vitamin C content when compared to the control. Among the treatments, T8 (*Azospirillum* + chemical fertilizer + vermicompost) was found to be superior in registering the maximum amount of vitamin C, which was followed by T7 (chemical fertilizer + vermicompost), T4 (vermicompost) and T5 (*Azospirillum* + vermicompost) respectively.

Phosphorus: The combined application of *Azospirillum*, chemical fertilizer and vermicompost (T8) recorded the maximum phosphorus content followed by the dual application of *Azospirillum* and vermicompost (T5).

Iron: The combined application of *Azospirillum*, chemical fertilizer and vermicompost was found to be superior in registering the maximum iron content, which was followed by the treatment T5 (*Azospirillum* + vermicompost). The study by Densilin *et al.* also revealed that the combined application of biofertilizer, inorganic fertilizers and vermicompost increased the biochemical constituents in chilli fruits²¹.

CONCLUSION

Hence the conclusion of the study is that the combined application of *Azospirillum*, chemical fertilizer and vermicompost (T8) improved the biometric parameters (shoot length, fresh and dry weight and number of leaves). Also it increased the protein, total carbohydrates, vitamin A, vitamin C, phosphorus and iron content of the *Amaranthus tristis* plants. The application of vermicompost (T4) increased the root length and the moisture content of the *Amaranthus tristis*. The dual application of *Azospirillum* and vermicompost (T5) enhanced the chlorophyll content, whereas the application of chemical fertilizer and vermicompost (T7) improved the carotenoids content.

The study clearly indicates that the combined application of vermicompost, *Azospirillum* and chemical fertilizer is superior in enhancing the growth and development of the green leafy vegetable, *Amaranthus tristis*. Thus the increased growth and nutrient content of the *Amaranthus tritsis* plants was proved by the application of biofertilizers along with chemical fertilizers and vermicompost. Hence it is recommended that the use of biofertilizer and vermicompost along with the chemical fertilizer would be beneficial to the environment as it would reduce the use of inorganic fertilizers and promote sustainable agriculture.

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