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Review Article

STUDY ON THE EFFECT OF DIFFERENT BIO-FERTILIZERS ON THE GROWTH OF *AMARANTHUS VIRIDIS* L.

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A B S T R A C T

Bio-fertilizers are a suitable supplement to chemical fertilizers to meet the integrated nutrient demand of the crops. In *Amaranthusviridis* L. the growth parameters such as root length, shoot length, fresh weight and dry weight was estimated to be higher in plants treated with *Azospirillum* on the 30th day. On the 45th day, the root length and shoot length was found to be higher in *Azospirillum*treated plants, but the fresh weight and dry weight were found to be more in plants treated with *Azospirillum*, VAM fungi and Phosphobacteria.

INTRODUCTION

Bio-fertilizers such as *Rhizobium, Azospirillum* and Phosphobacteria provide nitrogen and phosphorus nutrients to crop plants through nitrogen fixation and phosphorous solubilization processes. These Bio-fertilizers could be effectively utilized for rice, pulses, millets, cotton, sugarcane, ladies finger, tomato, vegetable and other horticulture crops. Bio-fertilizers is one of the prime input in organic farming that not only enhances the crop growth and yield but also improves the soil health and sustain soil fertility. A field experiment carried out to study the effect of organic manures and bio-fertilizers on the quality parameters of black night shade have shown an increased yield due to the application of Vermicompost (Ammaan and Subramanian, 2017).

Mycorrhiza is the mutualistic association between plant roots and fungal mycelia. The mycorrhizal fungi mobilize phosphates and other micronutrients like zinc, boron and molybdenum from adjacent soil to the root system through hyphal network. Enhanced uptake of phosphorus and increased plant growth due to inoculation of soil with VAM fungi in horticultural crops such as chilli, tomato, etc has been earlier studied. Soil moisture plays a significant role on mycorrhizal development and colonization. The importance of bio-fertilizers are:

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- They increase the yield of plants by 15-35%.
- They are effective even under semi-arid conditions
- Farmers can prepare the inoculum themselves
- They improve soil texture and do not allow pathogens to flourish,
- They produce vitamins and growth promoting bio-chemicals and are non-polluting.

In recent years, bio-fertilizers have emerged as a promising component of agriculture. Our whole ecosystem of agriculture depends in many important ways, on microbial activities and there appears to be a tremendous potential for making use of microorganisms in increasing the production of many plants. Bio-fertilizers stimulate the plant growth, protecting the soil, act against drought and soil diseases. Using bio-fertilizers that contain different microbial strains has led to a decrease in the use of chemical fertilizers and has provided high quality products free from agrochemicals for human safety. Bio-fertilizers directly or indirectly improve the nutrient level to the soil. Bio-fertilizers contain different types of microorganisms, it also has the ability to convert non nutritional soil to nutritional and it makes the soil rich in minerals and phosphorus and other nutrients. At the same time, it increases the primary and secondary metabolites.
Bio-fertilizers increase the fresh and dry weight of plants.

Bio-fertilizers mostly increases the biomass and root weight of the plants by their increasing the metabolic activity of the plant. Many developing countries are using bio-fertilizers as an alternate to chemical fertilizers. These microorganisms affect the host plant by one or more mechanism. These

microorganisms increase the root rhizosphere and increase the plant growth. 98% of the Indian soil contain insufficient amount of available phosphate usage and therefore green manures help to improve the phosphate level of the soil.

Vegetables play an important role in human nutrition. Most are low in fat and calories but are bulky and filling. They supply dietary fiber and are important sources of essential vitamins, minerals, and trace elements. Particularly important are the antioxidant vitamins A, C, and E. When vegetables are included in the diet, there is found to be a reduction in the incidence of cancer, stroke, cardiovascular disease, and other chronic ailments. Fruit and vegetables, particularly leafy vegetables, have been implicated in nearly half the gastrointestinal infections caused by norovirus in the United States. These foods are commonly eaten raw.

Amaranthus viridis L. is a vigorous, erect, branched, annual plant growing 10 - 100cm tall. Occasionally the plant may become a short-lived perennial. The plant is often harvested from the wild as a source of food and medicine for local use. It is sometimes cultivated in the Tropics for its edible leaves. No members of this genus are known to be poisonous, but when grown on nitrogen-rich soils they are known to concentrate nitrates in the leaves. This is especially noticeable on land where chemical fertilizers are used. Nitrates are implicated in stomach cancers, blue babies and some other health problems. It is inadvisable, therefore, to eat this plant if it is grown inorganically. *Amaranthus viridis* is a cosmopolitan species in the botanical family Amaranthaceae and is commonly known as slender amaranth or green amaranth.

Amaranthus viridis L. is an annual herb with an upright, light green stem that grows to about 60-80 cm in height. Numerous branches emerge from the base, and the leaves are ovate, 3-6 cm long, 2-4 cm wide, with long petioles of about 5 cm. The plant has terminal panicles with few branches, and small green flowers with 3 stamens. *Amaranthus viridis* is eaten in North eastern Indian state Manipur where it is known as *Cheng-kruk* and eaten traditionally as a vegetable in South India, especially in Kerala, where it is known as *"Kuppacheera"*. It is a common vegetable in Bengali cuisine and is called "note shak" ("shak" means leafy vegetable).

It is also eaten as a vegetable in parts of Africa. In the 19th Century, *A. viridis*, or green amaranth was an item of food in Australia. The botanist Joseph Maiden wrote in 1889: "It is an excellent substitute for spinach", being far superior too much of the leaves of the white beet sold for spinach in Sydney.

Green amaranth also has clusters of nutty edible seeds, which can be eaten as snacks or used in biscuits. Porridge can be made by boiling the seeds in water. Unlike other amaranths, the seeds can be easily harvested by scraping the ripe spikes of seeds between the fingers. *Amaranthus viridis* L. is used as a medicinal herb in traditional Ayurvedic medicine, under the Sanskrit name *Tanduliya*.

The main objective of the current work is to study the efficiency of three different bio-fertilizers namely *Azosprillum,* Phosphobacteria and Vesicular Arbuscular Mycorrhizal fungi on the growth of *Amaranthus viridis* L. in pot culture study.

MATERIALS AND METHODS

Amaranthus viridis L. belongs to the family Amaranthaceae. Growth studies were carried out under different treatments of biofertilizers namely *Azospirillum*, Phosphobacteria and Vesicular Arbuscular Mycorhizaatdifferent stages of growth.

Collection of the seeds

Seeds of *Amaranthusviridis* L. was obtained from Tamil Nadu Agricultural University Coimbatore.

Collection of biofertilizers

The bio-fertilizers such as *Azosprillum*, VAM and Phosphobacteria were collected from TNAU, Coimbatore.

Morphology of the plant

***Amaranthus viridis* L.**

Systematic Position

Order : Caryophyllaes
Family : Amaranthaceae
Genus : *Amaranthus*
Species : *A.viridis*L.

Plate 1 Habit of *Amaranthus virids* L.

Description

- *Amaranthus viridis* L. is an annual herb with an upright, light green stem that grows to about 60–100 cm in height (Plate 1)
- Numerous branches emerge from the base.
- The leaves are ovate, 3–6 cm long, 2–4 cm wide, with long petioles of about 5 cm.
- The plant has terminal panicles with few branches, and small green flowers with 3 stamens.
- In the 19th Century *A. viridis*, or green amaranth was an item of food in Australia.
- Green amaranth also has clusters of nutty edible seeds, which can be eaten as snacks or used in biscuits.
- Porridge can be made by boiling the seeds in water. Unlike other amaranths, the seeds can be easily harvested by scraping the ripe spikes of seeds between the fingers.

Medicinal uses

- *Amaranthus* leaves are rich incarbohydrates, proteins, vitamin K, folate, riboflavin, vitamin A, vitamin B6 and vitamin C. It is used as an allopathic medicine.
- Amaranth leaves are terrific source of manganese, iron, copper, calcium, magnesium, potassium and phosphorus necessary for maintaining proper mineral balance in the body. It fights against the cancer.

- Vitamin K is known to reduce excessive bleeding by improving blood clotting. This type of vitamin also improves the functioning of the kidney.
- Vitamin C improves the rate at which a wound on the body heals hence preventing body cells from damage. It also improves the health of teeth and strengthens the gums. Vitamin C increases the rate at which iron is absorbed into the body. It also reduces aging and increases one's immunity hence making them more resistant to diseases.
- It improves visual acuity hence supporting good vision. Vitamin A also serves to protect human from viral infections. It also fights and clears viruses that have already infected the body. Vitamin A keeps the body moist and protects the body from free radicles.
- It supports and strengthens weak bones. Manganese reduces the occurrence of rashes on the skin and maintains the normal color and health of the hair. This nutrient is also used to regulate the cholesterol levels in the body.

Bio-Fertilizers

Azospirillum

They are called as associative endosymbiont on roots of grasses and similar types of plants. They are known to fix atmospheric nitrogen and benefit host plants by supplying growth hormones and vitamins. *Azospirillum* is considered to be more efficient and it has been reported that *Azospirillum* inoculation increases the growth, nitrogen uptake and yield in number of crops (Mallikarjuna Rao *et al.,* 2014).

Vesicular Arbuscular Mycorrhiza (VAM)

Mycorrhiza is a mutualistic association between plant roots and fungal mycelia. Many graminaceous plants, legumes and horticultural crops are highly susceptible to VAM colonization. The transfer of nutrients mainly phosphorus from the soil to the cells of the root cortex is mediated by intracellular obligate fungal endosymbiont of the genera *Glomus, Gigaspora, Endosone*, etc. which possess vesicles for storage of nutrients and arbuscules for funneling these nutrients into the root system.

The mycorrhizal fungi mobilize phosphates and other micronutrients like zinc, boron and molybdenum from adjacent soil to the root system through hyphal network (Mallikarjuna Rao *et al.*, 2014)

Phosphobacteria

Microorganisms are also involved in the availability of phosphorus, the second most important nutrient required by crop plants. The phosphate solubilizing bacteria (PSB) solubilize the insoluble phosphates and make them available for crop plants in the rhizosphere region (Mallikarjura Rao *et al*., 2014)

METHODS

Pot Culture Experiment

The seeds obtained from TNAU, Coimbatore were soaked in different organic fertilizers overnight. Later, the seeds were sown in pots (30cm×24cm×30cm sized pots) containing red soil and sandy soil in the ratio 1:1. The treated pots were maintained in triplicates. The effect of different bio-fertilizers on the growth parameters of *Amaranthus viridis* L.were assessed. Thulasi extract was sprayed at intervals to control the growth of insects. The different bio-fertilizer treatments given were:

T_0-Control
T_1-*Azosprillum*
T_2-Vesicular Arbuscular mycorrhiza
T_3-Phosphobacteria
T_4-Azosprillum + VAM + Phosphobacteria

Growth Parameters

In *Amaranthus viridis* L., the study was carried out up to 45th day only because, the plant started losing its vigour after that period. Plant samples were uprooted carefully on 30th and 45thday and the following growth parameters were measured and recorded for all the treatments.

1. Root length (cm)
2. Shoot length (cm)
3. Number of leaves
4. Fresh weight (gm)
5. Dry weight (gm)

Root Length

The plants were taken from control pot and other treatment pots and washed to get rid of adhering soil particles. Then, the length of the roots was measured with the help of a scale from root collar point to root tip and expressed in centimeter. Ten seedlings were randomly selected from each treatment and their root length was measured using cm scale and recorded in cm/seedling.

Shoot Length

The shoot length of the plants was measured with the help of scale from the shoot collar point to shoot apex and expressed in centimeter. Ten seedlings were randomly selected from each treatment and their root length was measured using cm scale and recorded in cm/seedling. Three readings were taken for statistical analysis.

Number of leaves

The number of leaves present in the uprooted plants was calculated.

Fresh Weight

Fresh weight of the plants was measured with the help of an electronic digital balance and expressed in grams.

Dry weight

The collected plant materials were kept in hot air oven at 55°C for 24 hours. Then, the dry weight of the plants was measured using an electronic digital balance and expressed in grams.

Statistical Analysis

The data obtained from various biometric observations were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978). The significance and critical differences of various treatments were analyzed.

RESULTS AND DISCUSSION

The study conducted in *Amaranthus viridis* L. using different bio-fertilizer treatments showed the following results.

Growth parameters

Amaranthus viridis L.

The green leafy vegetable selected for the present study showed growth only up to 45 days. Therefore, the growth parameters were calculated for 30th day and 45th day.

The growth parameters such as shoot length and root length were observed to be more in T_1 (55.00±5.30cm) and T_4 (15.60±2.48cm) respectively. The number of leaves was found to be higher on 30th day in T_4 and the value was 40.33±4.93.

The fresh weight and dry weight of the plant on the 30th day was found to be higher in plants treated with *Azospirillum* (T_1) and the values were 39.57±1.77g and 4.74±0.42g respectively (Table 1). Earlier, Ahmed and Jha (1997) and Mohammed *et al*. (1989) have reported increased phosphrous uptake by wheat and gram with the inoculation of Phosphobacteria. Recent work on application of PGPR in crop development implies the use of such PGPR strains that work well in both pot and field studies (Datta *et al*., 2011).

Table 1 Growth Parameters of *Amaranthus viridis* L. using different bio-fertilizers on 30th day

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T_0	43.00 ± 1.48	9.33 ± 0.21	19.67 ± 2.08	26.24 ± 2.77	3.56 ± 0.59
T_1	55.00 ± 5.30	12.07 ± 1.99	27.67 ± 2.89	39.57 ± 1.77	4.74 ± 0.42
T_2	53.33 ± 2.73	11.43 ± 0.31	27.33 ± 6.66	33.45 ± 1.64	4.61 ± 0.65
T_3	54.23 ± 3.37	11.77 ± 0.90	26.00 ± 1.00	28.83 ± 1.12	4.04 ± 0.18
T_4	50.87 ± 4.13	15.60 ± 2.48	40.33 ± 4.93	32.29 ± 3.32	4.00 ± 0.94
SEd	2.9694	1.2123	3.3133	1.8536	0.4988
Cd (p<0.05)	6.6163	2.7013	7.3825	4.1302	1.1113

Values are mean ± SD of three samples in each group

Plate 2 Growth of *Amaranthus viridis*L. on 45th day

Table 2 Growth Parameters of *Amaranthus Viridis* L. using different bio-fertilizers on 45th day

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T_0	76.17 ± 4.29	11.10 ± 2.62	35.33 ± 1.15	56.55 ± 11.44	18.89 ± 3.78
T1	99.20 ± 4.35	17.30 ± 0.56	51.67 ± 5.51	82.16 ± 21.40	33.08 ± 0.80
T_2	95.53 ± 5.84	23.27 ± 1.11	66.33 ± 17.01	77.21 ± 17.07	27.49 ± 7.02
T_3	93.07 ± 1.70	20.37 ± 6.56	47.33 ± 8.50	79.40 ± 18.48	26.24 ± 6.53
T_4	90.17 ± 4.94	20.13 ± 3.85	61.00 ± 12.12	73.21 ± 8.20	25.63 ± 4.07
SEd	3.6272	3.3005	8.4879	13.1.98	2.2054
Cd (p<0.05)	8.0819	4.5140	18.9123	29.2106	6.1389

Values are mean ± SD of three samples in each group

The growth of *Amaranthus viridis* L. was measured on the 45th day (Table 2; Plate 2). The shoot length and root length was found to be higher in T_1 (99.20 ± 4.35 cm) and T_2 (23.27 ± 1.11 cm) respectively. Variations were observed in the growth parameters based on the treatments. The number of leaves present in the plant on the 45th day was 66.33 ± 17.01 which was higher and observed in T_2 (Table 2).

The bio-fertilizers have high potential of supplementary nitrogen and also convert the unavailable P as well as S into available forms as in Ashwagandha (Ramesh Babu, 1996), Senna (Vyas and Purbey, 2005).

The fresh weight and the dry weight of the plant was measured on the 45th day and found to be higher in T_4 i.e., combination of different bio-fertilizers (Table 2).

Application of higher dosage of inorganic fertilizers along with the bio-fertilizers influenced the growth and yield of onion significantly (Singh *et al*., 2017).

In the study carried out by Sridevi and Ramakrishnan (2010). On the plant growth and yield of cotton, AM inoculation significantly increased the plant growth and yield of cotton at all the levels of NPK. The statistical analysis of various growth parameters showed significance at 5% level.

Studies on the effect of PSB, *Azosprillum* and *Azotobacter* by Choudhary *et al.* (2017) have indicated that the application of bio-fertilizers not only improves the quality of Knol-Khol, but also gives a maximum monitory benefit. They have concluded that the use of PSB, *Azosprillum* and *Azotobacter* could significantly increase the yield of Knol-Khol and also the net return of the crop. Bio-fertilizers are natural fertilizers containing microorganisms that enhance crop productivity through nitrogen fixation, solublizing of plant nutrients and produce plant growth regulators. Work done by Kumar *et al*. (2002) has proved that the potato yield could be significantly increased by the application of bio-fertilizer. This study is in accordance with the present study of tomato and *Amaranthus* that showed high yield by the treatment of bio-fertilizer.

Green leafy vegetables found in South India, used as a source of food have many health benefits like protection from eye problems, iron deficiency and oxidative damage. They are the most inexpensive sources of several phytonutrients like pro-vitamin A, vitamin C, folic acid and minerals like calcium, iron, phosphorus, sodium and potassium.

Green leafy vegetables contain several chemical constituents which are pharmacologically importantas they are proved to be beneficial in many specific diseases like cancer, diabetes, hepatotoxicity, nephrotoxicity and many microbial attacks. Biological fertilizer can be recommended for the sake of achieving higher quality production. Use of VAM fungi is being encouraged in agriculture. Use of VAM fungi can protect the crop from seed borne pathogens. Bio-fertilizers could be a good replacement of chemical fertilizer for improving the growth of vegetable crops.

On the basis of the results obtained and the discussion made so far, it may be concluded that application of the organic fertilizer orbio-fertilizer is the most effective way for higher growth of the leafy vegetable taken for the study. Hence, the use of and management of natural resource in sustainable agriculture, the microbial fertilizers hold vast potential for future. The conclusion is based on only pot culture experiments. Further studies in the field are required to strongly support the current investigation.

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