

**Study on the effect of different bio-fertilizers on the Growth parameters,  
Biochemical and Phytochemical constituents of *Solanum lycopersicum* L.  
and *Amaranthus viridis* L.**

By

**MALATHI, R.  
(16PBO008)**

**Thesis Submitted to the  
Avinashilingam Institute for Home Science and Higher Education  
for Women, Coimbatore - 641 043**

**In Partial Fulfillment of the Requirements for the  
Degree of Master of Science in Botany**

**APRIL 2018**

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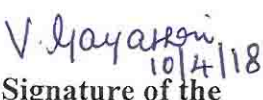
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Signature of the  
Head of the Department

  
Signature of the  
Supervisor

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## I. INTRODUCTION

Bio-fertilizers have shown great potential as supplementary, renewable and environmental friendly sources of plant nutrients and are an important component of Integrated Nutrient Management and Integrated Plant Nutrition System. The bio-fertilizer is based on renewable source of energy which does not pollute the environment. The current demand of sustainable agriculture has paved the way for bio-fertilizers usage and its advantage over chemical fertilizers has raised the awareness among the farmers. The bio-fertilizers play a major role in organic farming. In addition to nitrogen, bio-fertilizers provide certain growth promoting substances like hormones, vitamins, amino acids, etc. Clean agriculture depends on using bio-fertilizers as well as organic fertilizers in order to produce high yields and quality vegetable.

India is an agriculture based country. In order to feed the ever growing populations, India has to increase the per unit area productivity. Bio-fertilizers are one of the best modern tools for agriculture. It is a gift of our modern agriculture science. Bio-fertilizers are applied in the agriculture field as a replacement of our conventional fertilizers consisting of compost, household wastes and green manure. Those are not as effective as chemical fertilizers, so farmers often try to use chemical fertilizers in the agriculture field for crop development. Bio-fertilizers contain micro organisms which promote the adequate supply of nutrients to the host plants to ensure their proper development of growth and regulation in their physiology. Living micro organisms are used in the preparations of bio-fertilizers. Shelf life is the first and foremost problem of the carrier based bio-fertilizers which are up to three months and it does not retain throughout the crops cycle. Liquid bio-fertilizer is increasingly available in the market as one of the alternatives to chemical fertilizers and pesticide. One of the benefits from bio-fertilizers is a, contribution from population of microorganism available.

The beneficial use of nitrogen fixing microorganism viz. *Azotobacter* and *Azospirillum*, as a supplementary source of N-nutrition to crops is well documented. *Azospirillum* synthesizes considerable amount of biologically active substances like vitamins, nicotinic acid, indole acetic acids and gibberellins. All these hormones help the plant for better germination, early emergence and better root development.

Phosphobacteria means microbial inoculants capable of solubilizing phosphate. Commonly used Phosphobacteria is *Bacillus megaterium*. Around 95-99% of the total soil

phosphorus is insoluble which is directly not available to plants. They multiply fast in the root zone. The P-solubilizers containing bacteria or fungi may convert insoluble form of phosphate to soluble form by producing organic acids. About 15-25% of insoluble phosphate can be solubilized, saving chemical fertilizers significantly. Phosphobacteria can solubilize about 30 kg of insoluble source of phosphorus and make it available to plants. Application of phosphobacteria along with nitrogen fixing bacteria promotes growth and yield of the crops by 30% example maize, rice, tomato, bitter gourd etc.

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.

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:

- 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 micro organisms 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. VAM fungi increases the fresh and dry weight of tomato plant. *Azospirillum* is a free living nitrogen fixing bacteria. It fixes the nitrogen in plants by low oxygen tension.

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.

The tomato edible, often red vegetable of the plant *Solanum lycopersicum* commonly known as a tomato plant belongs to the nightshade family, *Solanaceae*. The species originated in Western South America. Its use as a cultivated food may have originated with the indigenous people of México. The Spanish discovered the tomato from their contact with the Aztec people during the Spanish colonization of the Americas and then brought it to Europe, and, from there, to other parts of the European colonized world during the 16th century.

Tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While tomatoes are botanically berry-type fruits, they are

considered culinary vegetables as an ingredient or side dish for savoury meals. Numerous varieties of tomato are widely grown in temperate climates across the world, with greenhouses allowing its production throughout the year. The plants typically grow to 1–3 meters (3–10 ft) in height and have a weak stem that sprawls. It is a perennial in its native habitat, and cultivated as an annual. Fruit size varies according to cultivar, with a width range of 0.5–4 inches (1.3–10.2 cm).

Tomatoes are considered a fruit or vegetable depending on context. According to *Encyclopedia Britannica*, tomatoes are a fruit labeled in grocery stores as a vegetable due to the taste and nutritional purposes. Botanically, a tomato is a fruit, a berry, consisting of the ovary, together with its seeds of a flowering plant. However, the tomato has much lower sugar content than other edible fruits, and is therefore not as sweet. Typically served as part of a salad or main course of a meal, rather than at dessert, it is, in the US, considered a "culinary vegetable". One exception is that tomatoes are treated as a fruit in home canning practices: they are acidic enough to process in a water bath rather than a pressure cooker as vegetables require. Tomatoes are not the only food source with this ambiguity: bell peppers, cucumbers, green beans, eggplants, avocados, and squashes of all kinds are all botanically fruits, yet cooked as vegetables.

Tomato plants are vines, initially decumbent, typically growing 180 cm (6 ft) or more above the ground if supported, although erect bush varieties have been bred, generally 100 cm (3 ft) tall or shorter. Indeterminate types are "tender" perennials, dying annually in temperate climates (they are originally native to tropical highlands), although they can live up to three years in a greenhouse in some cases. Determinate types are annual in all climates.

Tomato plants are dicots, and grow as a series of branching stems, with a terminal bud at the tip that does the actual growing. When that tip eventually stops growing, whether because of pruning or flowering, lateral buds take over and grow into other, fully functional, vines. Tomato vines are typically pubescent, meaning covered with fine short hairs. These hairs facilitate the veining process, turning into roots wherever the plant is in contact with the ground and moisture, especially if the vine's connection to its original root has been damaged or severed.

Most tomato plants have compound leaves, and are called regular leaf plants, but some cultivars have simple leaves known as potato leaf style because of their resemblance to that particular relative. The leaves are 10–25 cm (4–10 in) long, odd pinnate, with five to 9

leaflets on petioles (Acquaah, 2002), each leaflet up to 8 cm (3 in) long, with a serrated margin; both the stem and leaves are densely glandular-hairy. Their flowers, appearing on the apical meristem, have the anthers fused along the edges, forming a column surrounding the pistil's style. Flowers in domestic cultivars can be self-fertilizing. The flowers are 1–2 cm (0.4–0.8 inch) across, yellow, with five pointed lobes on the corolla; they are borne in a cyme of three to 12 together.

Tomato fruit is classified as a berry. The tomato is grown worldwide for its edible fruits, with thousands of cultivars. Most modern tomato cultivars are smooth surfaced. While virtually all commercial tomato varieties are red, some cultivars – especially heirlooms – produce fruit in blue, green, yellow, orange, pink, black, brown, ivory, white, and purple. Such fruits are not widely available in grocery stores, nor are their seedlings available in typical nurseries, but they can be bought as seed. Variations include multicolored fruit with stripes (Green Zebra), fuzzy skin on the fruit (Fuzzy Peach, Red Boar), multiple colors (Hillbilly, Burracker's Favorite, Lucky Cross), etc.

The tomato is now grown and eaten around the world. It is used in diverse ways, including raw in salads, and processed into ketchup or tomato soup. Unripe green tomatoes can also be breaded and fried, used to make salsa, or pickled. Tomato juices sold as a drink, and is used in cocktails such as the Bloody Mary.

Tomatoes are acidic, making them especially easy to preserve in home canning whole, in pieces, as tomato sauce or paste. The fruit is also preserved by drying, often in the sun, and sold either in bags or in jars with oil. Although tomatoes originated in the Americas, they have become extensively used in Mediterranean cuisine. They are a key ingredient in pizza, and are commonly used in pasta sauces.

### **Nutrition**

A tomato is 95% water, contains 4% carbohydrates and less than 1% each of fat and protein. In a 100 gram amount, raw tomatoes supply 18 calories and are a moderate source of vitamin C (17% of the Daily Value), but otherwise are absent of significant nutrient content.

*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*.

## Objectives

- The main objective of the current research project is to study the efficiency of three different bio-fertilizers namely *Azospirillum*, Phosphobacteria and Vesicular Arbuscular Mycorrhizal fungi on the growth and yield of *Solanum lycopersicum* L. and *Amaranthus viridis* L. in pot culture study.
- Preliminary Phytochemical Screening to study the presence of secondary metabolites in *Solanum lycopersicum* L. and *Amaranthus viridis* L.

## II. REVIEW OF LITERATURE

The literature available on the use of bio-fertilizer on the growth parameters, biochemical parameters and phytochemical constituents relevant to the current investigation has been reviewed and presented in this chapter.

Earlier the effect of biofertilizers on the growth, yield of quality of Knol-Khol has been studied by Choudhary *et al.* (2017).

Angadi *et al.* (2017) have reported higher yield of tomato in bio-fertilizer treated plant when compare to control plants. They studied the effect of organic manures and bio-fertilizers on plant growth, seed yield and seedling characteristics in tomato plant.

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 in yield due to the application of Vermicompost (Ammaan and Subramanian, 2017).

Saeed *et al.* (2015) have reported a significant increase in fruit yield of tomato when treated with different levels of phosphate and bio-fertilizer.

A significant improvement in growth and biochemical parameters of *Vigna unguiculate* has been reported by Badar *et al.*, (2015). They studied the influence of organic, inorganic and bio-fertilizes on physical and biochemical parameters of *Vigna unguiculata*.

Bandopadhyay (2015) had carried out studies on the effect of dual inoculation of studies on the effect of dual inoculation of plant growth promoting Rhizobacteria on different non-leguminous plants under pot condition. He has reported that all parameters than the control plants.

A review on the Arbuscular mycorrhizal fungias a biofertilizer has been studied by Sadhana (2014).

A study by Kaur (2014) on the effect of nitrogen-fixing bacteria *Azotobacter* and *Azospirillum* on the growth of *Rosa polyantha* have shown a significant increase in yield by the application of mixed inoculants.

Aadeoluwa and Akinyemi (2014) studied the, dry mater and soil qualities of *Amaranthus* under organic and inorganic fertilizer treatments.

Earlier Paramanik and Chikkaswamy (2014) have reported a maximum growth and yield of some medicinal plants treated with VAM and biofertilizers.

The study carried out by Hemavani and Thippeswamy (2014) have reported a marked increase in shoot length, root length, fresh and dry weight of ground nut due to AMF colonization.

Kavitha *et al.* (2013) investigated different combinations of bio-fertilizer (*Azospirillum*), the growth of a green leafy vegetable *Amaranthus tristis* and found a significant change in biometric parameters and increase in biochemical constituents.

Aggani (2013) have done a broad spectrum of analysis on the development of bio-fertilizers and its future perspective.

Bio-fertilizers are eco-friendly and enhance germination. They also reduce the chemical inputs and enhance the yield both qualitatively and quantitatively (Darzi, 2012).

Abbasniyare *et al.*, (2012) have studied the effect of bio-fertilizer on the growth of *Spathiphyllum illusion*. The effect of inorganic, organic and bio-fertilizers on the growth and yield of bitter gourd have been carried out by Thriveni *et al.* (2015). In their study, they have reported that application of NPK integrated with vermicompost and bio-fertilizers turned to be the best treatment for increasing growth and yield of bitter gourd.

Ramakrishnan and Selvakumar (2012) studied the effect of bio-fertilizers on enhancement of growth and yield of tomato. They observed that there was significant increase in growth and yield parameters in bio-fertilizer treated plants rather than the control plants.

The positive effect on the growth and yield of local ecotypes of chilli peppers due to mycorrhizal inoculation has been studied under field conditions by Marihal *et al.*, (2011).

Earlier studies on the nutritional value of *Amaranthus* spp. (Schonfeldt and Pretorius, 2011) have proved that raw leaves of *A.tricolor*, *Cleome gynandra* L. and *Corchorus olitorius* L. contain higher concentration of iron, zinc as well as phosphorous and calcium.

Thenmozhi *et al.* (2010) have studied the effect of various bio-fertilizers on the growth and biomass production of selected vegetables. In their study, they have shown that a combined application of bio-fertilizers and bio manures is an essential requirement for growth and biomass production of leafy vegetables like *Amaranthus* and Garden pea.

Wajhmode *et al.* (2010) studied the effect of bio-fertilizer and gibberellic acid on the yield of onion. Kumar *et al.* (2002) have done studies on the effect of bio-fertilizers and their methods of inoculation on growth and yield of potato.

The study on the effect of mineral Vs bio-fertilizer on the growth, yield and essential oil content of coriander revealed a significant increase in plant height, number of branches, fruit yield etc. (Rahimi *et al.* 2009).

Ghanti and Sharanji (2009) have studied the effect of bio-fertilizers on the growth, yield and quality of onion. Their study was based on the use of different combinations of bio-fertilizer to the crop.

Addition of organic fertilizer to bio-fertilizer to bio-fertilizers has shown an enhanced growth in potato (Awad, 2002) and rice (Naseer and Bali, 2007).

Studies on the effect of bio-fertilizers on the quality parameters of potato have been studied by Mahendran and Kumar (1998). The association of VAM fungi with the root system of vascular plants improves the nutrient uptake. The fungi withdraw the glucose from plant roots act as a significant sink for carbohydrates (Kottke, 2002).

AM fungi (Arbuscular mycorrhizae) is the most important supplement for the increasing high cost of NPK fertilizer input, into cropping system without substantial loss in yield. An AM fungus is reported to be involved in improvement of plant growth and enhance accumulation of plant nutrients (mainly phosphorus, zinc, copper and sulphur) through greater soil exploration by mycorrhizal hyphae. AM association is known to help in the growth of various crops including horticultural plants like carrot or tomato. The beneficial uses of AM inoculum in agriculture and raising nurseries have been reported by Muthukumar *et al.* (2004).

Bio-fertilizers are carried-based preparations containing beneficial microorganisms in viable state in viable state intended for seed or soil application. In recent years, they have emerged as a promising component of integrated nutrient supply system. They are likely to assume greater significance as a complements or supplements to the chemical fertilizers because of high nutrient turnover, exorbitant cost of fertilizers, soil and environmental protection. Bio-fertilizers are less expensive, eco-friendly, providing plant hormones and help in sustainable crop production through maintenance of soil productivity.

### III. MATERIALS AND METHODS

The plants taken for the present study were *Solanumlyopersicum* L. belonging to the family Solanaceae and *Amaranthus viridis* L. belonging to the family Amaranthaceae. Growth studies were carried out and bio-chemical parameters were carried out under different treatments of biofertilizers namely *Azospirillum*, Phosphobacteria and Vesicular Arbuscular Mycorrhiza during different stages of growth of the plants.

#### Collection of the seeds

Seeds of *Solanum lycopersicum* L. and *Amaranthusviridis* L. were obtained from Tamil Nadu Agricultural University Coimbatore.

#### Collection of biofertilizers

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

#### Morphology of the plant

##### *Solanum lycopersicum* L.

##### *Systematic position*

Order	: Solanales
Family	: Solanaceae
Genus	: <i>Solanum</i>
Species	: <i>S.lycopersicum</i> L.



**Plate: 1 Habit of *Solanum lycopersicum* L.**

## **A. Description of the plant**

- The tomato is native to South America, but, grows in temperate climates worldwide.
- Tomato is an easily grown vine plant that belongs to the night shade family
- The tomato (*Lycopersicon esculentum*) is a short-lived perennial plant, grown as an annual plant, typically growing to 1-3 m tall, with a weakly woody stem that usually scrambles over other plants.
- The fruit is an edible, brightly colored (usually red, from the pigment lycopene) berry, 1-2 cm diameter in wild plants, commonly much larger in cultivated forms.
- The tomato begins its colorful and varied history upon the coastal highlands of Western South America, where it was being enjoyed by the native people for a long time.
- Tomatoes are consumed raw, or in salads, sauces and drinks. Tomatoes are rich in Vitamin A and are a kitchen- favourite throughout the world.
- Fruits can be harvested within 60-70 days' time.

## **Medicinal uses**

- Tomato is good for liver health. Tomato has detoxification effect in the body.
- People eating tomatoes regularly have a reduced risk of cancer diseases such as lung, prostate, stomach, cervical, breast, oral, colorectal, esophageal, pancreatic and many other types of cancer.
- It reduces the risk of cardiovascular diseases because of lycopene in it.
- Maintain healthy blood pressure and reduce blood glucose in people with diabetes.
- Tomatoes contain key carotenoids such as lutein and lycopene. These can protect the eye against light-induced damage.

## **B. *Amaranthus viridis* L.**

### **Systematic Position**

Order : Caryophyllales

Family : Amaranthaceae

Genus : *Amaranthus*

Species : *A. viridis* L.



**Plate 2: Habit of *Amaranthus viridis* L.**

## **Description**

- *Amaranthus viridis* L. is an annual herb with an upright, light green stem that grows to about 60–100 cm in height.
- 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 in carbohydrates, proteins, vitamin K, folate, riboflavin, vitamin A, vitamin B6 and vitamin C. It is used as an allopathic medicine.
- Amaranth leaves are a terrific source of manganese, iron, copper, calcium, magnesium, potassium and phosphorus necessary for maintaining proper mineral balance in the body. It fights against 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 ones 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 radicals.
- 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 (Mallikarjuna 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 organic fertilizers on the growth, biochemical and yield parameters of *Solanum lycopersicum* L. and *Amaranthus viridis* L.were assessed. A preliminary phytochemical screening was also carried out for both the plants. The growth and biochemical parameters at different stages of growth of the plants were analysed. Thulasi extract was sprayed at intervals to control the growth of insects. The different organic fertilizer treatments given were:

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

### **Growth Parameters**

Plant samples were uprooted carefully on 30<sup>th</sup>, 45<sup>th</sup>day and 60<sup>th</sup> day 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 were 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.

### **Biochemical parameters**

The estimation of Chlorophyll, Protein and Carbohydrate were be done using standard procedures

Chlorophyll (Arnon, 1949)

Protein (Lowry *et al.*, 1951)

Carbohydrate (Hedge and Hofreiter, 1962)

### **Phytochemical parameters**

The estimation of Alkaloids, Tannins, Flavonoids, Quinones, Phlobatannins, Phenol, Carbohydrates, Amino acids, Steroids, Terpenoids, fats and oil will be done by using

preliminary phytochemical analysis of various primary and secondary plant metabolites . It was carried out using the method of Harborne (1984).

## II. Biochemical Parameters

The following biochemical parameters studied were Chlorophyll, Protein and Carbohydrate. In *Solanum lycopersicum* L. the biochemical parameters were estimated on 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day and in *Amaranthus viridis* L. it was estimated on 30<sup>th</sup> and 45<sup>th</sup> day.

### METHODS

#### 1. Estimation of Chlorophyll Content

Chlorophyll 'a', 'b' and total chlorophyll were analyzed following the method of Arnon (1949).

#### Materials Required

Analytical grade acetone was diluted to 80 % acetone.

#### Procedure

- One gram of freshly cut sample of leaf was taken into a clean mortar.
- The leaf bits were ground to a fine pulp with the addition of 20 ml of 80 % (w/v) acetone.
- The mixture thus obtained was centrifuged at 5000 rpm for 5 minutes.
- The supernatant was transferred to 100ml volumetric flask. This procedure was repeated until the residue became colorless.
- The washing was collected and the volume was made up to 100ml in the flask with acetone.
- The absorbance of the solution was read in a spectrophotometer at 645 and 663 nm against the solvent blank (80% acetone).

#### Calculation

The amount of chlorophyll present in the extract was calculated (mg chlorophyll / gm tissue) using the formula,

- $\text{mg chlorophyll 'a' / gm tissue} = 12.7 A_{663} - 2.69 A_{645} \times V / (1000 \times W)$

- $\text{mg chlorophyll 'b' / gm tissue} = 22.9A_{645} - 4.68A_{663} \times V / (1000 \times W)$
- $\text{mg total chlorophyll / gm tissue} = 20.2A_{645} + 8.02A_{663} \times V / (1000 \times W)$

Where

A = Absorbance at specific wave length

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of the tissue

## 2. Estimation of Protein (Lowry *et al.*, 1951)

### Principle

The blue colour developed by phosphomolybdic phosphotungstic components in the Folin- ciocalteau reagent by the amino acids, tyrosine and tryptophan present in the protein and the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured by Lowry's method.

### Materials Required

- Two percent sodium carbonate in 0.1N sodium hydroxide (Reagent A).
- 0.5 percent copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in 1 percent potassium sodium tartarate (Reagent B).
- Alkaline copper solution: mix 50ml of reagent A and 1ml of reagent B prior to use (Reagent C).
- Folin- Ciocalteau reagent (Reagent D)

Protein solution (stock standard): Accurately 50mg of bovine serum albumin (fraction V) was weighed and dissolved in distilled water and made up to 50ml in a standard flask.

Working standard: 10 ml of stock solution was diluted to 50ml with distilled water in a standard flask. One ml of this solution contains 200mg protein.

### Procedure

#### Extraction of protein from samples

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

### **Estimation of Protein**

- About 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard were pipetted out into a series of test tubes.
- About 0.1 and 0.2 ml of the sample was pipette out in two other test tubes.
- The volume was made up to 1ml using distilled water in all the test tubes. A tube with one ml of water served as the blank.
- About 5ml of reagent C was added to each tube including the blank. Mixed well and allowed to stand for 10 min.
- To this, 0.5ml of reagent D was added, mixed well and incubated in dark for 30 minutes. Blue colour developed was read at 660nm.
- A standard graph was drawn and the amount of protein present in the sample was calculated.

### **Calculation**

The amount of protein present in the sample was expressed in  $\text{mg} / \text{gm} = \text{mg of protein/volume of test standard} \times \text{concentration of the standard}$ .

## **3. Estimation of Carbohydrate Content (Hedge and Hofreiter, 1962)**

### **Anthrone Method**

#### **Principle**

Concentrated sulphuric acid hydrolyses the glycoside bond of carbohydrate to the given monosaccharides which were then dehydrated to furfural. The furfural reacted with anthrone (10-Keto 9, 10-dihydro anthracene) to give the blue coloured complex which was measured calorimetrically at 630 nm.

### **Materials Required**

- 2.5 N HCl
- Anthrone reagent was prepared by dissolving 200mg anthrone in 100ml of ice cold 95% H<sub>2</sub>SO<sub>4</sub>. Prepared freshly before use.

Stock standard: 100mg of glucose was dissolved in 100ml of water.

Working standard: 5ml of stock standard solution was diluted to 100ml using distilled water (50mg/ml).

### **Procedure**

- About 100mg of the sample was taken in a boiling tube and was hydrolyzed by keeping it in boiling water bath for three hours with 5ml of 2.5 N HCl and cooled at room temperature.
- Then it was neutralized with solid sodium carbonate until the effervescence ceases.
- The volume was made up to 100ml and centrifuged.
- The supernatant was collected and 0.1 ml and 0.2 ml aliquot was taken for analysis.
- The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard and a blank was maintained.
- The volume was made up to 1ml in all the tube including the sample tube by adding distilled water.
- Then, 4ml of anthrone reagent was added and heated for eight minutes in a boiling water bath.
- Then, it was cooled rapidly and blue green colour developed was read at 630nm.
- A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- From the graph, the amount of carbohydrate present in the sample was calculated.

### **Calculation**

Amount of carbohydrate present in 100 mg of the sample  
= mg of glucose/volume of test sample x100

## **III. Yield parameters**

### **Number of fruits**

The number of fruits obtained on 45<sup>th</sup> day and 60<sup>th</sup> day were calculated for *Solanum lycopersicum* L. For *Amaranthus viridis* L., the study was carried out up to 45<sup>th</sup> day only because, the plant started losing its vigour after that period.

## **IV. Preliminary Phytochemical Screening**

The following Phytochemical parameters were observed in the leaves of the two vegetable crops taken for study.

### **Preparation of plant extract**

The leaves of both the plants taken for the present study were collected, cleaned and air dried under shade for almost three weeks. After drying, the leaves were then blended using a household electric blender. This fine powder was analysed for phytochemical constituents present in it. The plant sample was soaked in water, ethanol and chloroform for overnight extraction and filtered through Whatman No.1. filter paper. Qualitative tests were conducted on these extracts.

### **Test for Alkaloids**

#### **Mayer's test**

To 1ml of extract, 2ml of Conc. HCl was added. Then, a few drops of Mayer's reagent were added. Green colour or white precipitate indicates the presence of alkaloids.

### **Test for Tannins**

To 1ml of extract, 2ml of 0.1% Ferric chloride was added. Brownish green or blue black colouration indicates the presence of tannins.

### **Test for Flavonoids**

To 1ml of extract, 1ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids.

### **Test for Quinones**

A small amount of the extract was treated with Conc. HCl and observed for the formation of yellow precipitate.

### **Test for Phlobatannins**

To 1ml of extract, few drops of 1% aqueous hydrochloric acid was added. A red precipitate formed indicates the presence of phlobatannins.

### **Test for Phenol**

To 1ml of extract 5ml of Folin-ciocalteau reagent and 4ml of sodium carbonate was added. Appearance of blue colour showed the presence of phenol.

### **Test for Carbohydrates**

- a) To 1ml of extract, 5ml of Benedict's reagent was added and boiled for 5 minutes. Bluish green colour indicated the presence of carbohydrates.
- b) Purple colour is seen with the addition of few drops of Molisch'reagent and Conc. H<sub>2</sub>SO<sub>4</sub>.

### **Test for Amino acids**

To 1ml of filtrate, few drops of 0.2% ninhydrin was added and heated for 5 minutes. Formation of blue colour indicated the presence of amino acid.

### **Test for Steroids**

To 1ml of the filtrate, 10ml of Chloroform and 10ml of sulphuric acid was added slowly by the sides of the test tube. Upper layer turning red and the sulphuric acid layer turning yellow with green fluorescent indicates the presence of steroids.

### **Test for Terpenoids**

To 1ml of filtrate, 2ml of chloroform was added and few drops of concentrated sulphuric acid were added carefully. An interface with a reddish brown colouration is formed showing the presence of terpenoids.

### **Test for fats and oil**

To 1 ml of extract, a few drops of Sudan III solution was added . A shining orange colour showed the presence of fixed oil and fat.

### **Statistical analysis**

The data obtained from various biochemical observations were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978).

## IV. RESULTS AND DISCUSSION

The study was conducted in *Solanum lycopersicum* L. and *Amaranthus viridis* L. using different biofertilizer treatments. The result of the study is as follows:

### I. Growth parameters

#### 1. *Solanum lycopersicum* L.

Growth parameters such as shoot length, root length, number of leaves, fresh weight and dry weight of *Solanum lycopersicum* L. was calculated on the 30<sup>th</sup> day, 45<sup>th</sup> day and 60<sup>th</sup> (Plate 3,4 and 5). The shoot length and root length on the 30<sup>th</sup> day was higher in T<sub>4</sub> i.e., the use of combination of bio-fertilizers such as *Azospirillum*, VAM and Phosphobacteria. The values were  $29.9 \pm 0.70$  cm and  $8.87 \pm 0.57$  cm respectively (Table 1). The number of leaves on the 30<sup>th</sup> day was estimated to be  $55.00 \pm 9.54$  (Table 1) in T<sub>4</sub>.

The fresh weight and the dry weight was also found to be higher in T<sub>4</sub> and the values were  $15.20 \pm 1.17$  g and  $1.36 \pm 0.52$ g respectively (Table 1); (Fig. 1)

Improvement in growth and yield parameters in plants treated with bio-fertilizers were due to enhanced uptake of nutrients by the plants (Borea, 1991).

On the 45<sup>th</sup> day, similar to 30<sup>th</sup> day, the shoot length and root length showed its higher value in T<sub>4</sub> (combination of all three bio-fertilizers). The values obtained were  $63.73 \pm 2.81$  cm and  $21.67 \pm 3.01$  cm respectively (Table 2); (Fig. 2).

The higher number of leaves present in the plant on the 45<sup>th</sup> day was  $171.67 \pm 2.31$  (Table 2) in plant treated with VAM (T<sub>2</sub>).

The fresh weight and dry weight was observed to be higher in plants treated with *Azospirillum*, VAM and Phosphobacteria. The readings obtained were  $76.65 \pm 4.16$  g and  $19.16 \pm 1.04$ g respectively.

The growth parameters on the 60<sup>th</sup> day were also estimated and tabulated (Table 3); (Fig. 1). The shoot length and root length was estimated to be higher in T<sub>3</sub> ( $74.20 \pm 5.5$ cm) and T<sub>4</sub> ( $27.47 \pm 1.27$ ) respectively on the 60<sup>th</sup> day. The number of leaves in the plant was found to be higher in T<sub>2</sub> ( $201.67 \pm 3.21$ ).



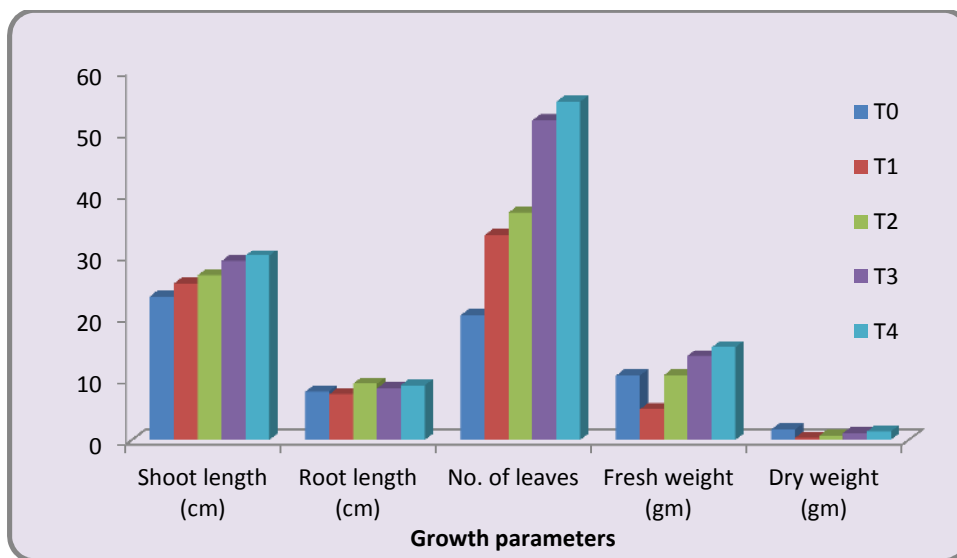
**Plate 3- Growth of *Solanum lycopersicum* L. on 30<sup>th</sup> day**

**Table 1**

**Growth Parameters of *Solanum lycopersicum* L. using Different  
biofertilizers on 30<sup>th</sup> day**

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T <sub>0</sub>	23.37 ± 0.95	7.87 ± 0.95	20.33 ± 1.53	10.48 ± 0.30	1.70 ± 0.01
T <sub>1</sub>	25.40 ± 3.50	7.43 ± 1.17	33.33 ± 1.53	4.96 ± 0.67	0.33 ± 0.30
T <sub>2</sub>	26.73 ± 1.91	9.13 ± 1.14	37.00 ± 2.00	10.52 ± 0.82	0.67 ± 0.15
T <sub>3</sub>	29.03 ± 0.99	8.37 ± 0.75	52.00 ± 11.53	13.69 ± 1.31	1.13 ± 0.23
T <sub>4</sub>	<b>29.90 ± 0.70</b>	<b>8.87 ± 0.57</b>	<b>55.00 ± 9.54</b>	<b>15.20 ± 1.17</b>	<b>1.36 ± 0.52</b>
SEd	1.5608	0.7709	5.5698	0.7571	0.2393
Cd (p<0.05)	3.4776	1.7176	12.4103	1.6869	0.5332

Values are mean ± SD of three samples in each group



**Fig.1 Growth Parameters of *Solanum lycopersicum* L. using different biofertilizers on 30<sup>th</sup> day**

#### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria



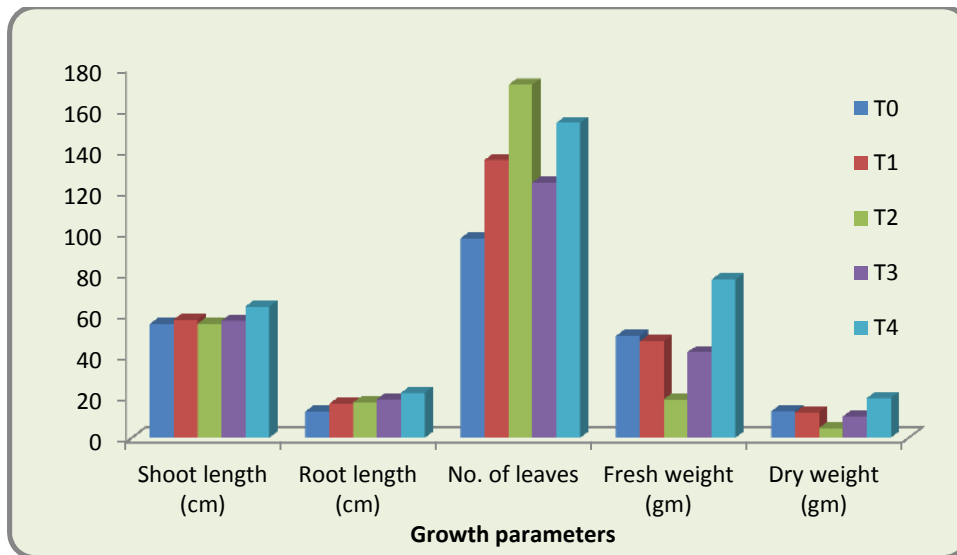
**Plate 4- Growth of *Solanum lycopersicum* L. on 45<sup>th</sup> day**

**Table 2**

**Growth Parameters of *Solanum lycopersicum* L. using different  
Bio-fertilizers on 45<sup>th</sup> Day**

<b>Treatments</b>	<b>Shoot length (cm)</b>	<b>Root length (cm)</b>	<b>No. of leaves</b>	<b>Fresh weight (gm)</b>	<b>Dry weight (gm)</b>
T <sub>0</sub>	55.53 ± 3.85	12.70 ± 0.75	97.00 ± 5.29	49.71 ± 13.86	12.85 ± 4.00
T <sub>1</sub>	57.50 ± 2.13	16.37 ± 1.45	135.00 ± 18.36	47.06 ± 7.17	12.25 ± 1.37
T <sub>2</sub>	55.57 ± 2.36	16.97 ± 0.83	<b>171.67 ± 2.31</b>	18.55 ± 3.42	4.64 ± 0.85
T <sub>3</sub>	56.97 ± 1.12	18.60 ± 1.20	124.00 ± 10.44	41.42 ± 16.37	10.35 ± 4.09
T <sub>4</sub>	<b>63.73 ± 2.81</b>	<b>21.67 ± 3.01</b>	153.33 ± 26.63	<b>76.65 ± 4.16</b>	<b>19.16 ± 1.04</b>
SEd	2.1330	1.0502	12.5892	8.4891	2.2038
Cd (p<0.05)	4.7527	2.6321	28.0507	18.9150	4.9103

Values are mean ± SD of three samples in each group



**Fig.2 Growth Parameters of *Solanum lycopersicum* L. using different biofertilizers on 45<sup>th</sup> day**

### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

Bio-fertilizers such as *Azospirillum*, Phosphorus solubilizing bacteria and mycorrhiza are capable of improving the mineral nutrients of plants and enhance the soil fertility. Phosphorus solubilizing bacteria are capable of solubilizing unavailable form of phosphorous into available form and make it available to plants (Veena *et al.*; 2009; Shakarappa *et al.*; 2012).

Earlier studies by Ghanti and Sharangi (2009) have revealed better growth, yield and quality of onion when *Azotobacter* was used in combination with *Azospirillum*.

The fresh weight and dry weight of tomato on the 60<sup>th</sup> day of growth showed a high value in plants treated with Phosphobacteria (T<sub>3</sub>). The values obtained were 148.11±42.87g and 49.37±14.29g respectively (Table 3).

*Pongamia pinnata* treated with VAM increased the plant height, root length and dry material (Venkatesh *et al.* 1998). Singh (2014) studied the yield parameters of coriander and found significant increase in plants treated with bio-fertilizers when compared to control. The result obtained in the present study on the uses of bio-fertilizers is in accordance with the studies carried out by Singh (2014).

## **2. *Amaranthus viridis* L.**

The green leafy vegetables selected for the present study showed growth only up to 45 days. Therefore, the growth parameters were calculated for 30<sup>th</sup> day and 45<sup>th</sup> day (Plate 6 and 7).

The growth parameters such as shoot length and root length were observed to be more in T<sub>1</sub> (55.00±5.30cm) and T<sub>4</sub> (15.60±2.48cm) respectively. The number of leaves was found to be higher on 30<sup>th</sup> day in T<sub>4</sub> and the value was 40.33±4.93.

The fresh weight and dry weight of the plant on the 30<sup>th</sup> day was found to be higher in plants treated with *Azospirillum* (T<sub>1</sub>) and the values were 39.57±1.77g and 4.74±0.42g respectively (Table 4).

Earlier, Ahmed and Jha (1997) and Mohammed *et al.* (1989) have reported increased phosphorous 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).

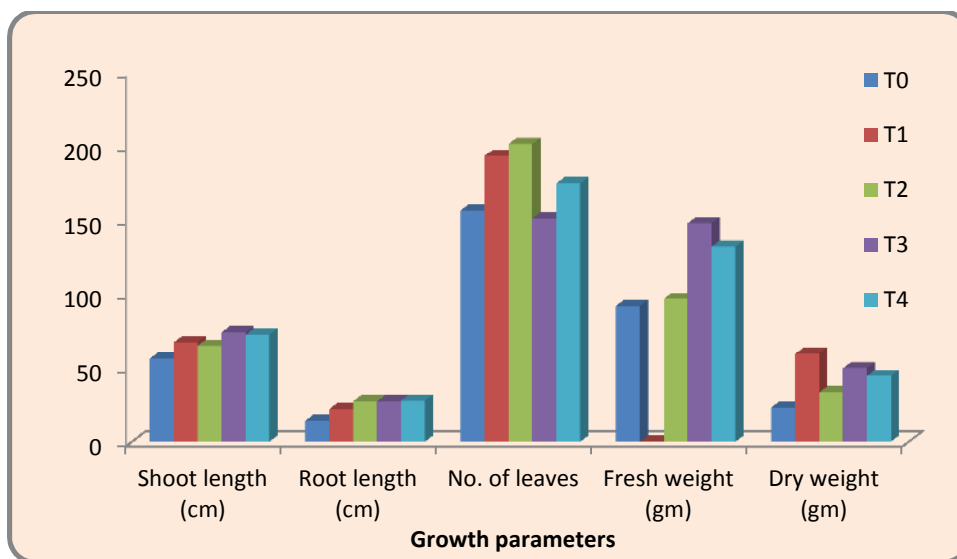


**Plate 5- Growth of *Solanum lycopersicum* L. on 60<sup>th</sup> day**

**Table 3**  
**Growth Parameters of *Solanum lycopersicum* L. using different bio-fertilizers**  
**on 60<sup>th</sup> day**

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T <sub>0</sub>	55.97 ± 3.38	14.10 ± 0.87	156.67 ± 7.09	91.89 ± 6.61	23.07 ± 1.36
T <sub>1</sub>	67.47 ± 3.31	21.83 ± 3.17	193.33 ± 15.50	178.40 ± 41.73	59.45 ± 13.88
T <sub>2</sub>	65.07 ± 3.81	27.07 ± 5.15	<b>201.67 ± 3.21</b>	96.70 ± 2.96	33.11 ± 1.83
T <sub>3</sub>	<b>74.20 ± 5.55</b>	27.03 ± 3.32	151.33 ± 15.04	<b>148.11 ± 42.87</b>	<b>49.37 ± 14.29</b>
T <sub>4</sub>	72.53 ± 0.97	<b>27.47 ± 1.27</b>	174.67 ± 26.31	132.46 ± 21.01	44.15 ± 7.00
SEd Cd (p<0.05)	4.3214 7.0068	1.3881 2.2188	12.7523 28.4141	23.3049 51.9269	7.7544 17.2780

Values are mean ± SD of three samples in each group



**Fig.3 Growth Parameters of *Solanum lycopersicum* L. using different biofertilizers on 60<sup>th</sup> day**

### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

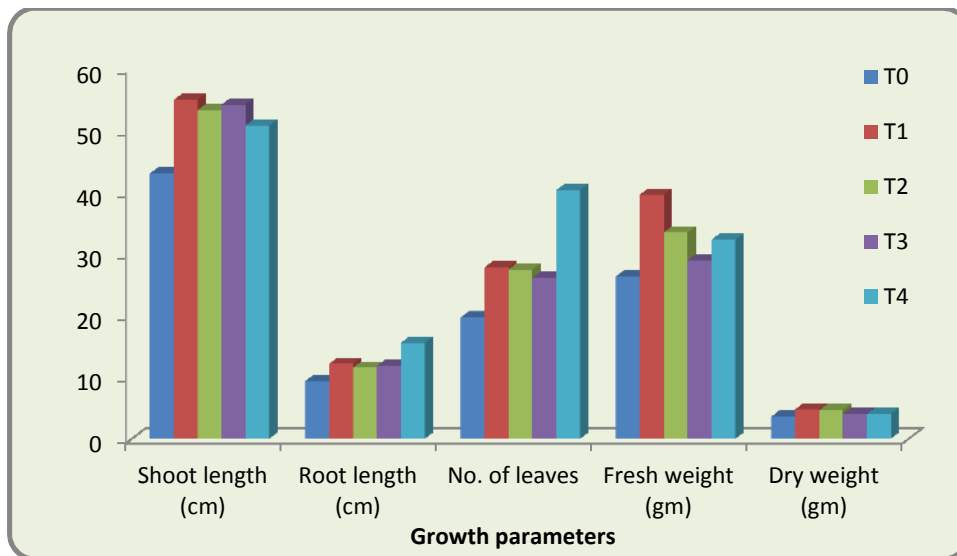


**Plate 6 - Growth of *Amaranthus viridis* L. on 30<sup>th</sup> day**

**Table 4****Growth Parameters of *Amaranthus viridis* L. using different bio-fertilizers  
on 30<sup>th</sup> day**

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T <sub>1</sub>	43.00 ± 1.48	9.33 ± 0.21	19.67 ± 2.08	26.24 ± 2.77	3.56 ± 0.59
T <sub>1</sub>	<b>55.00 ± 5.30</b>	12.07 ± 1.99	27.67 ± 2.89	<b>39.57 ± 1.77</b>	<b>4.74 ± 0.42</b>
T <sub>2</sub>	53.33 ± 2.73	11.43 ± 0.31	27.33 ± 6.66	33.45 ± 1.64	4.61 ± 0.65
T <sub>3</sub>	54.23 ± 3.37	11.77 ± 0.90	26.00 ± 1.00	28.83 ± 1.12	4.04 ± 0.18
T <sub>4</sub>	50.87 ± 4.13	<b>15.60 ± 2.48</b>	<b>40.33 ± 4.93</b>	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



**Fig.4 Growth Parameters of *Amaranthus viridis* L. using different biofertilizers on 45<sup>th</sup> day**

#### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria



**Plate 7 - Growth of *Amaranthus viridis*L. on 45<sup>th</sup> day**

The growth of *Amaranthus viridis* L. was measured on the 45<sup>th</sup> day (Table 5). The shoot length and root length was found to be higher in T<sub>1</sub> (99.20 ± 4.35 cm) and T<sub>2</sub> (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 45<sup>th</sup> day was 66.33 ± 17.01 which was higher and observed in T<sub>2</sub> (Table 5).

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

The fresh weight and the dry weight of the plant was measured on the 45<sup>th</sup> day and found to be higher in T<sub>4</sub> ie., combination of different bio-fertilizers (Table 5).

## **II. Biochemical Parameters**

### **1. *Solanum lycopersicum* L.**

#### **Chlorophyll *a*, *b* and Total chlorophyll**

In *Solanum lycopersicum* L. Grown under different bio-fertilizer treatments, the chlorophyll contents were measured on the 30<sup>th</sup> day, 45<sup>th</sup> day and 60<sup>th</sup> day and tabulated (Table 6, 7 and 8).

Chlorophyll *a* was found to be higher in T<sub>1</sub> (0.166 ± 0.034 mg/g) on 30<sup>th</sup> day, T<sub>1</sub> on 45<sup>th</sup> day (0.242 ± 0.076 mg/g) and T<sub>2</sub> (0.386 ± 0.049) on the 60<sup>th</sup> day. This shows that *Azospirillum* increases the chlorophyll pigments initially, but at later stage of growth, presence of VAM increases the chlorophyll *a* pigment.

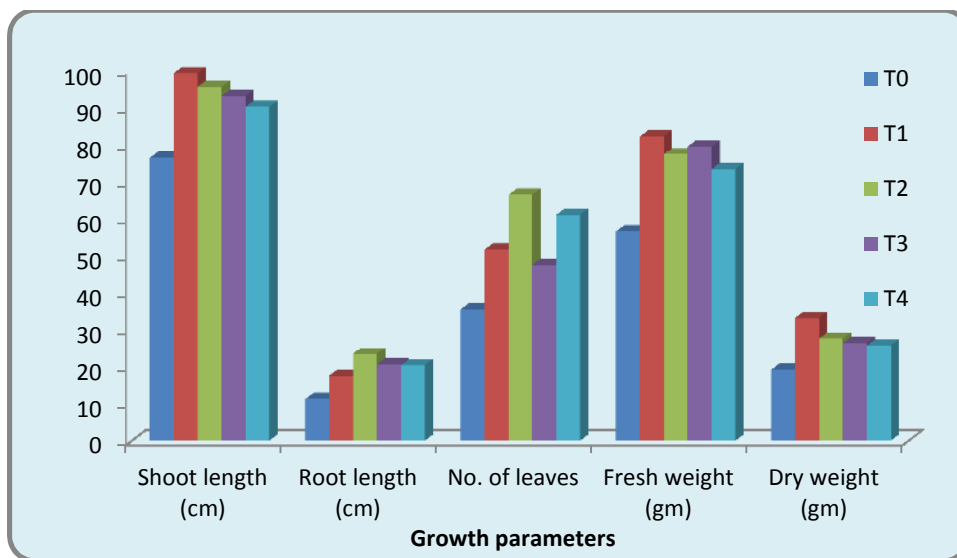
The chlorophyll *b* content was found to be higher in T<sub>2</sub> (0.131 ± 0.020 mg/g) on 30<sup>th</sup> day, T<sub>1</sub> (0.498 ± 0.051 mg/g) on 45<sup>th</sup> day and T<sub>4</sub> (0.498 ± 0.204 mg/g) on the 60<sup>th</sup> day. This result shows that the chlorophyll *b* pigment gradually increases when there is combination of bio-fertilizers and on the 60<sup>th</sup> day higher chlorophyll *b* content is observed in T<sub>4</sub> (Table 8).

Similarly the total chlorophyll content was found to be higher in T<sub>1</sub> (Table 6) on 30<sup>th</sup> day and 45<sup>th</sup> day (Table 7). The values were 0.280 ± 0.023 mg/g and 0.682 ± 0.098 mg/g respectively. On the 60<sup>th</sup> day, total chlorophyll content was higher in T<sub>4</sub> and the value was 0.724 ± 0.204 mg/g. (Table 8).

**Table 5****Growth Parameters of *Amaranthus Viridis* L. using different bio-fertilizers on 45<sup>th</sup> day**

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T <sub>0</sub>	76.17 ± 4.29	11.10 ± 2.62	35.33 ± 1.15	56.55 ± 11.44	18.89 ± 3.78
T <sub>1</sub>	<b>99.20 ± 4.35</b>	17.30 ± 0.56	51.67 ± 5.51	<b>82.16 ± 21.40</b>	<b>33.08 ± 0.80</b>
T <sub>2</sub>	95.53 ± 5.84	<b>23.27 ± 1.11</b>	<b>66.33 ± 17.01</b>	77.21 ± 17.07	27.49 ± 7.02
T <sub>3</sub>	93.07 ± 1.70	20.37 ± 6.56	47.33 ± 8.50	79.40 ± 18.48	26.24 ± 6.53
T <sub>4</sub>	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	13.198	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



**Fig.5 Growth Parameters of *Amaranthus viridis* L. using different biofertilizers on 45<sup>th</sup> day**

#### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

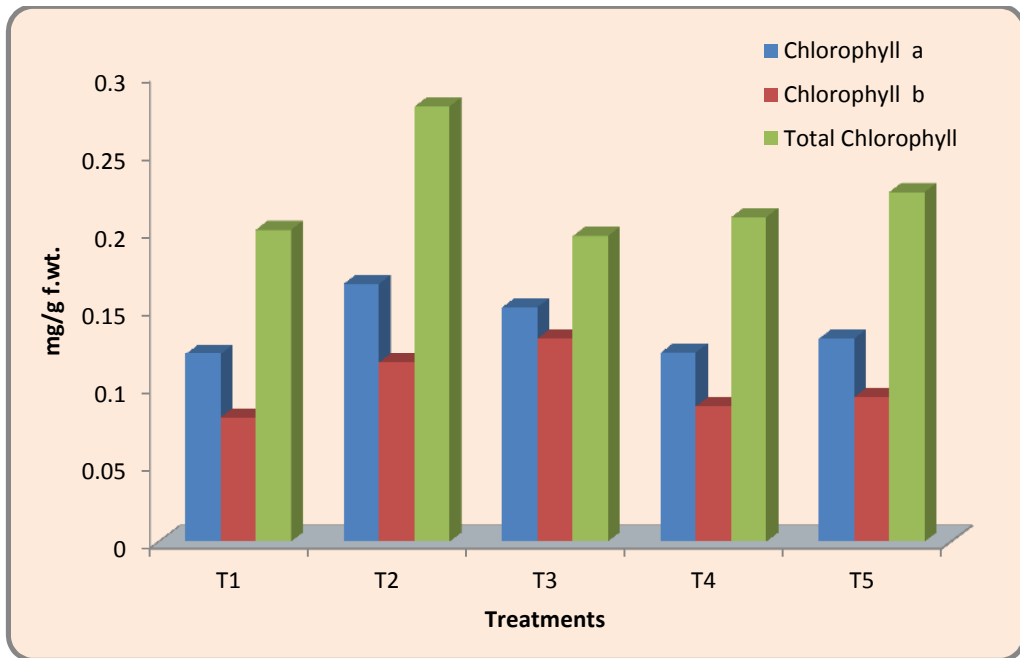
T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

**Table 6**

**Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the  
*Solanum lycopersicum* L. on the 30<sup>th</sup> day (mg/g f.wt.)**

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T <sub>0</sub>	0.121 ± 0.010	0.080 ± 0.005	0.201 ± 0.012
T <sub>1</sub>	<b>0.166 ± 0.034</b>	0.115 ± 0.014	<b>0.280 ± 0.023</b>
T <sub>2</sub>	0.151 ± 0.092	<b>0.131 ± 0.020</b>	0.197 ± 0.146
T <sub>3</sub>	0.122 ± 0.013	0.087 ± 0.005	0.209 ± 0.016
T <sub>4</sub>	0.131 ± 0.004	0.093 ± 0.009	0.225 ± 0.012
SEd	0.0364	0.0098	0.0547
CD(P<0.05)	0.0810	0.0219	0.1219

Values are mean ± SD of three samples in each group



**Fig. 6 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Solanum lycopersicum* L. on the 30<sup>th</sup> day**

**TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

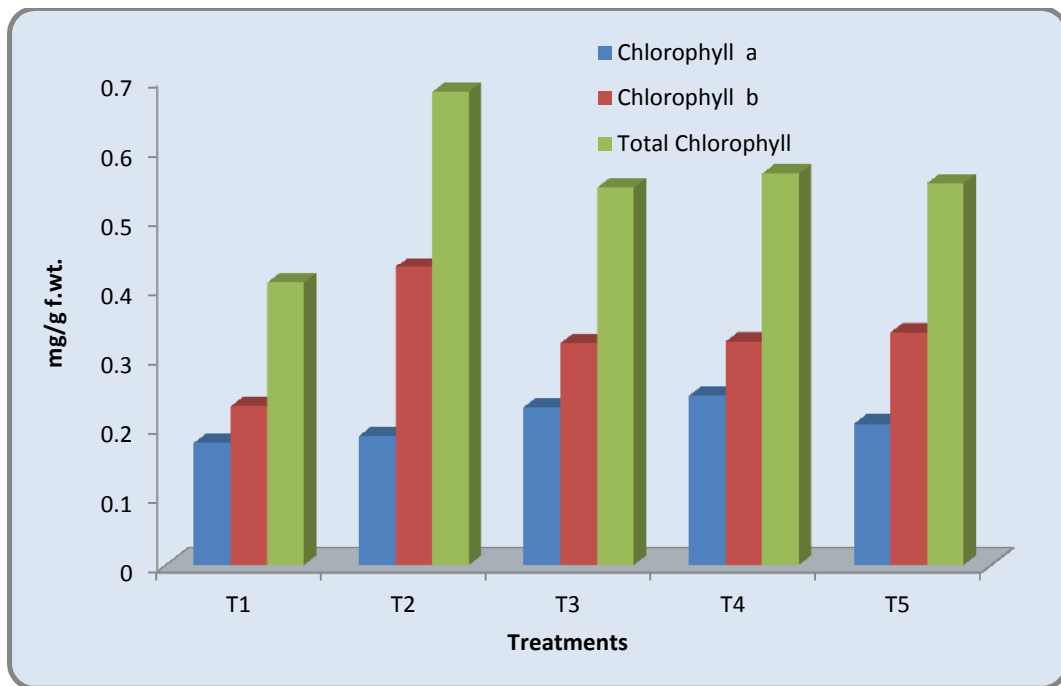
T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

**Table 7**

**Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the  
*Solanum lycopersicum* L. on the 45<sup>th</sup> day (mg/g f.wt.)**

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T <sub>0</sub>	0.175 ± 0.041	0.227 ± 0.054	0.406 ± 0.071
T <sub>1</sub>	0.185 ± 0.047	<b>0.429 ± 0.051</b>	<b>0.682 ± 0.098</b>
T <sub>2</sub>	0.226 ± 0.036	0.319 ± 0.098	0.544 ± 0.126
T <sub>3</sub>	<b>0.242 ± 0.076</b>	0.321 ± 0.072	0.564 ± 0.069
T <sub>4</sub>	0.202 ± 0.064	0.334 ± 0.051	0.550 ± 0.016
SEd	0.0448	0.0553	0.0689
CD(P<0.05)	0.0999	0.1231	0.1535

Values are mean ± SD of three samples in each group



**Fig. 7 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Solanum lycopersicum* L. on the 45<sup>th</sup> day**

#### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

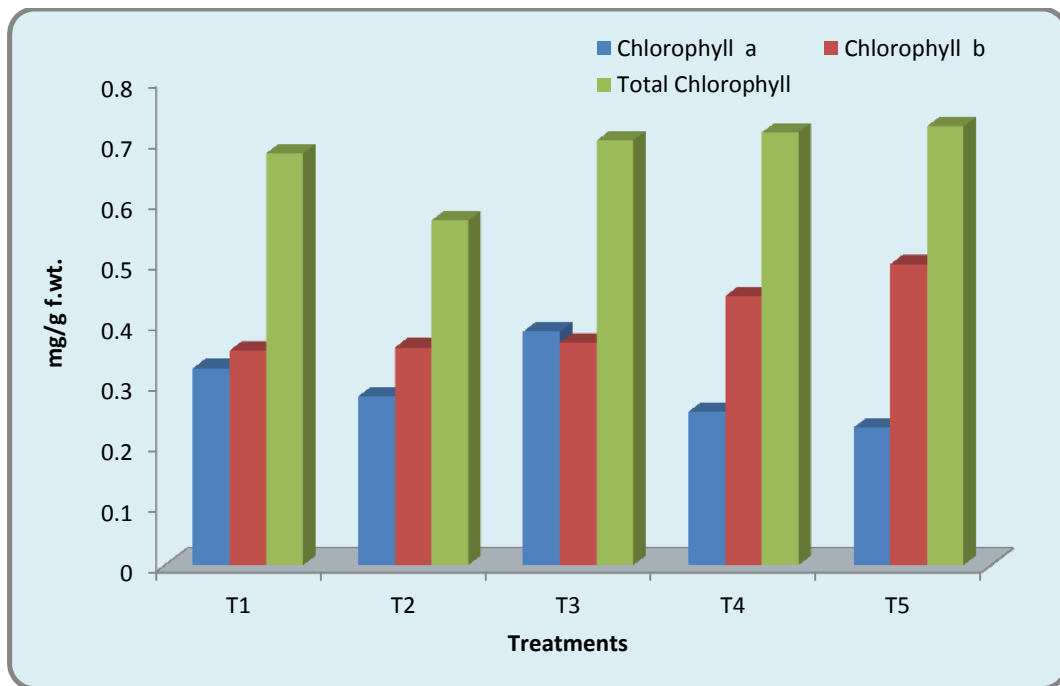
T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

**Table 8**

**Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the  
*Solanumlycopersicum* L. on the 60<sup>th</sup> day (mg/g f.wt.)**

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T <sub>0</sub>	0.326 ± 0.024	0.355 ± 0.079	0.680 ± 0.095
T <sub>1</sub>	0.278 ± 0.031	0.360 ± 0.077	0.569 ± 0.182
T <sub>2</sub>	<b>0.386 ± 0.049</b>	0.368 ± 0.022	0.701 ± 0.099
T <sub>3</sub>	0.253 ± 0.049	0.444 ± 0.071	0.714 ± 0.123
T <sub>4</sub>	0.227 ± 0.013	<b>0.498 ± 0.204</b>	<b>0.724 ± 0.204</b>
SEd	0.0295	0.0887	0.1205
CD(P<0.05)	0.0656	0.1976	0.2685

Values are mean ± SD of three samples in each group



**Fig. 8 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the**

***Solanum lycopersicum* L. on the 60<sup>th</sup> day**

**TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

Earlier, similar results were reported by Selvakumar and Thamizhiniyan (2011) in chilli and Selvakumar *et al.* (2012) in black gram. Higher chlorophyll and protein content have been reported due to the application of *Azotobacter* and *Azospirillum*.

## Protein

The protein content of *Solanum lycopersicum* L. was estimated on the 30<sup>th</sup> day, 45<sup>th</sup> day and 60<sup>th</sup> day and tabulated (Table 9). Two different concentrations 0.1 ml and 0.2 ml of the sample were used for estimation.

On the 30<sup>th</sup> day, the protein content was found to be  $3.30 \pm 0.63$  mg/g in T<sub>2</sub> in 0.1ml concentration. On the 45<sup>th</sup> day, the protein was estimated to be higher in T<sub>4</sub> ( $4.77 \pm 0.06$  mg/g) in 0.1ml concentration. On the 60<sup>th</sup> day, the protein content was higher in T<sub>3</sub> ( $6.13 \pm 0.45$  mg/g).

Similarly in 0.2ml concentration of the sample, the protein was higher in T<sub>2</sub> on the 30<sup>th</sup> day ( $2.07 \pm 0.40$  mg/g), T<sub>4</sub> on the 45<sup>th</sup> day ( $2.93 \pm 0.15$  mg/g) and T<sub>3</sub> on the 60<sup>th</sup> day ( $4.37 \pm 0.35$  mg/g). As the concentration varies, there is drastic variation in the protein content also under different bio fertilizer treatments (Table 9).

Earlier studies by Javed and Panwar (2013) on the effect of biofertilizers, vermicompost and chemical fertilizer on different biochemical parameters of *Glycine max* and *Vigna mungo*

## Carbohydrate

The carbohydrate content was estimated for *Solanum lycopersicum* L. under different bio fertilizer treatment on 30<sup>th</sup> day, 45<sup>th</sup> day and 60<sup>th</sup> day and tabulated (Table 10).

The carbohydrate content was found to be higher in T<sub>2</sub> on 30<sup>th</sup> day in both the concentrations and the values were  $3.47 \pm 0.35$  mg/g and  $2.03 \pm 0.15$  mg/g (Table 10).

On the 45<sup>th</sup> day of the plant growth, the carbohydrate content was estimated to be higher in T<sub>1</sub> in both the concentrations and the values were  $4.97 \pm 0.32$  mg/g and  $3.63 \pm 0.50$  mg/g (Table 10).

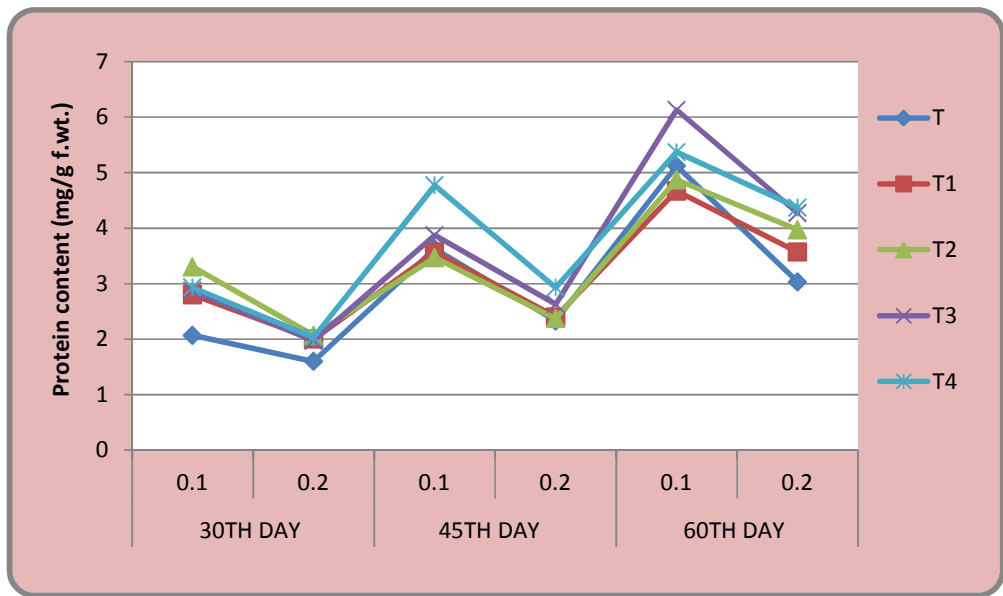
On the 60<sup>th</sup> day, the carbohydrate content was found to be more in T<sub>4</sub> in both the concentrations and the readings obtained were  $6.47 \pm 0.38$  mg/g and  $5.50 \pm 0.82$  mg/g (Table 10).

Table 9

Protein Content Of *Solanum lycopersicum* L. On The 30<sup>th</sup> Day, 45<sup>th</sup> Day and 60<sup>th</sup> Day (mg/g f.wt.)

TREATMENTS	30 <sup>TH</sup> DAY (mg/g)		45 <sup>TH</sup> DAY(mg/g)		60 <sup>TH</sup> DAY(mg/g)	
	0.1	0.2	0.1	0.2	0.1	0.2
T <sub>0</sub>	2.07 ± 0.25	1.60 ± 0.52	3.63 ± 0.21	2.33 ± 0.12	5.12 ± 0.40	3.03 ± 0.12
T <sub>1</sub>	2.80 ± 0.30	2.00 ± 0.36	3.57 ± 0.25	2.40 ± 0.10	4.67 ± 0.15	3.57 ± 0.15
T <sub>2</sub>	<b>3.30 ± 0.62</b>	<b>2.07 ± 0.40</b>	3.47 ± 0.38	2.37 ± 0.21	4.87 ± 0.47	3.97 ± 0.31
T <sub>3</sub>	2.87 ± 0.25	1.97 ± 0.12	3.87 ± 0.40	2.63 ± 0.12	6.13 ± 0.45	4.27 ± 0.49
T <sub>4</sub>	2.93 ± 0.72	2.03 ± 0.21	<b>4.77 ± 0.06</b>	<b>2.93 ± 0.15</b>	<b>5.37 ± 0.38</b>	<b>4.37 ± 0.35</b>
SEd	0.40925					
CD(P<0.05)	0.81961					

Values are mean ± SD of three samples in each group



**Fig. 9 Protein Content Of *Solanum lycopersicum* L. On The 30<sup>th</sup> Day, 45<sup>th</sup> Day and 60<sup>th</sup> Day**

**TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

Table 10

Carbohydrates content of *Solanum lycopersicum* L. on the 30<sup>th</sup> day, 45<sup>th</sup> day and 60<sup>th</sup> day (mg/g f.wt.)

TREATMENTS	30 <sup>TH</sup> DAY		45 <sup>TH</sup> DAY		60 <sup>TH</sup> DAY	
	0.1	0.2	0.1	0.2	0.1	0.2
T <sub>0</sub>	3.23 ± 0.40	1.83 ± 0.06	4.00 ± 0.10	2.27 ± 0.23	4.63 ± 0.15	2.33 ± 0.32
T <sub>1</sub>	2.90 ± 0.26	1.80 ± 0.44	<b>4.97 ± 0.32</b>	<b>3.63 ± 0.50</b>	5.90 ± 0.66	3.80 ± 0.10
T <sub>2</sub>	<b>3.47 ± 0.35</b>	<b>2.03 ± 0.15</b>	3.70 ± 0.36	2.20 ± 0.35	6.10 ± 0.36	3.70 ± 0.46
T <sub>3</sub>	3.10 ± 0.62	1.83 ± 0.40	4.70 ± 0.10	3.07 ± 0.15	5.83 ± 0.67	3.63 ± 0.50
T <sub>4</sub>	3.00 ± 0.17	1.87 ± 0.32	4.70 ± 0.85	3.00 ± 0.20	<b>6.47 ± 0.38</b>	<b>5.50 ± 0.82</b>
SEd	0.28674					
CD(P<0.05)	0.57358					

Values are mean ± SD of three samples in each group

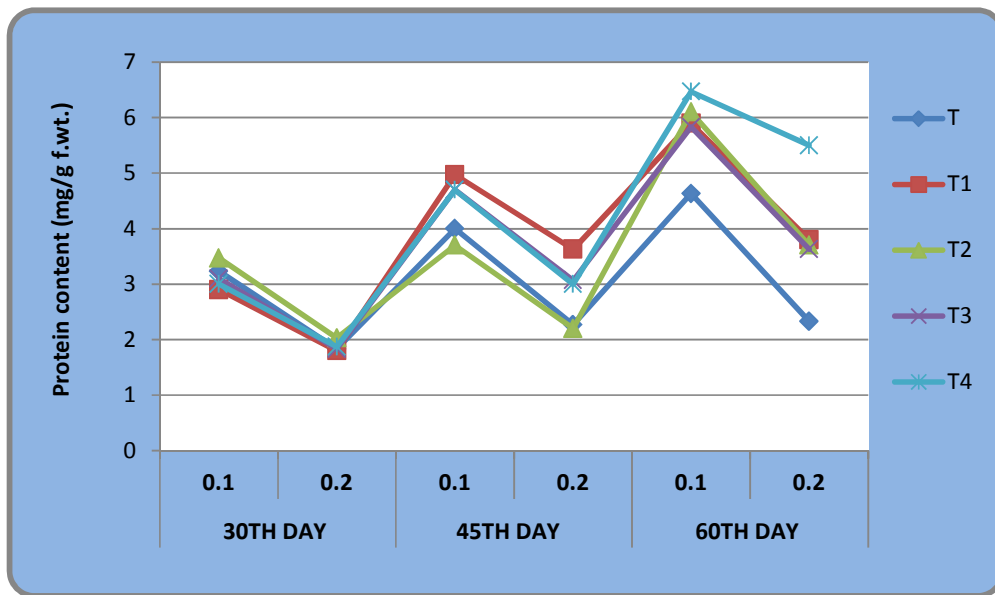


Fig. 10 Carbohydrates content Of *Solanum lycopersicum* L. on the 30<sup>th</sup> day, 45<sup>th</sup> day and 60<sup>th</sup> day

#### TREATMENTS

T<sub>0</sub>-Control

T<sub>1</sub>-Azospirillum

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-Azospirillum + VAM + Phosphobacteria

## 2. *Amaranthus viridis* L.

### Chlorophyll *a*, *b* and total chlorophyll

The chlorophyll pigments present in the green leafy vegetables were calculated on the 30<sup>th</sup> day and 45<sup>th</sup> day and tabulated (Table 11& 12).

The chlorophyll *a* content was higher in T<sub>1</sub> (0.133±0.012mg/g) on the 30<sup>th</sup> day. Chlorophyll *b* was observed to be more in T<sub>2</sub> (0.225±0.129mg/g) on the 30<sup>th</sup> day and the total chlorophyll content on the 30<sup>th</sup> day was more in T<sub>1</sub> (0.173±0.015mg/g).

On the 45<sup>th</sup> day, there was variation in chlorophyll pigments. The chlorophyll *a* was higher in T<sub>4</sub> (0.300±0.045mg/g). The chlorophyll *b* and total chlorophyll was found to be higher in T<sub>3</sub> and the values were estimated to be 0.450±0.141mg/g and 0.678±0.147mg/g respectively (Table 12).

### Protein

The protein content of *Amaranthus viridis* L. was estimated on the 30<sup>th</sup> day and 45<sup>th</sup> day Table 13. On the 30<sup>th</sup> day, the protein content was found to be higher in T<sub>2</sub> (2.80±1.10mg/g) at 0.1ml concentration. On the 45<sup>th</sup> day, the protein content was found to be higher in T<sub>4</sub> (5.97±0.81mg/g).

### Carbohydrate

The carbohydrate content of *Amaranthus viridis* L. was estimated on the 30<sup>th</sup> day and 45<sup>th</sup> day and tabulated (Table 14). Higher carbohydrate content was observed in T<sub>2</sub> on the 30<sup>th</sup> day and the value was 2.83±0.83mg/g. On the 45<sup>th</sup> day, the carbohydrate content was found to be higher in T<sub>3</sub> (Phosphobacteria treated plants) and the value was 5.97±0.12mg/g (Table 14).

In *Amaranthus dubius*, Manoharan *et al.*, (2011) showed an increase in the amount of protein and carbohydrate in plant treated with cyanospray compared to other treatments.

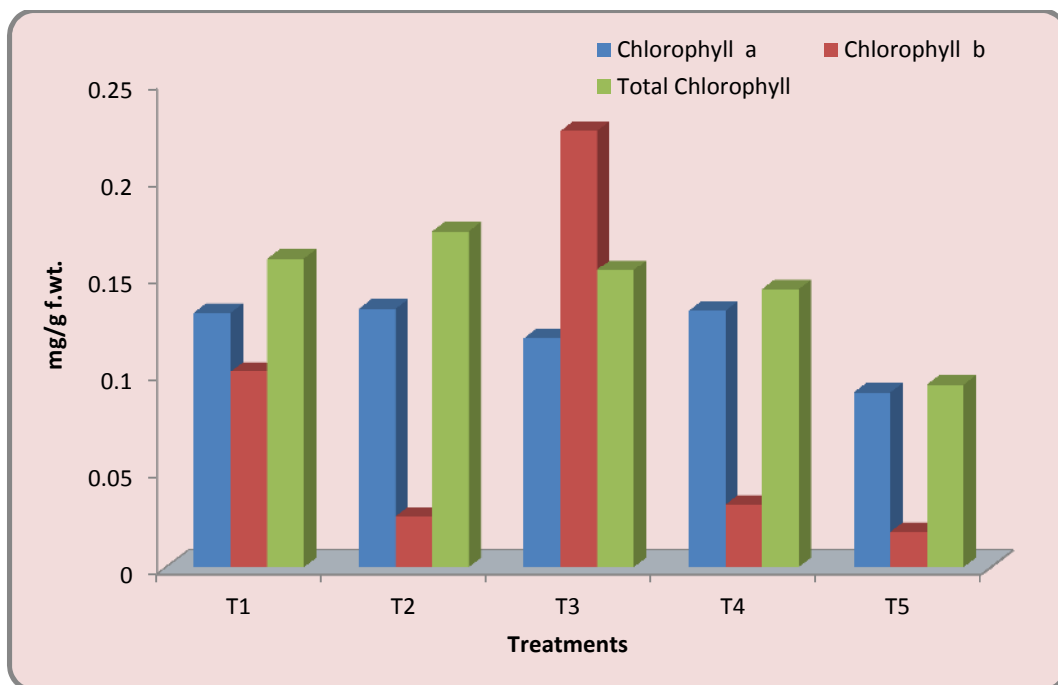
Bio-fertilizers are used to hasten the biological activity of the plants to improve the availability of plant nutrient. The work on the growth and establishment of cashew grafts under green house condition by Shankarappa *et al.* (2017) have shown that the bio fertilizers used increased the growth and nutrient uptake of the cultivar.

**Table -11**

**Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the  
*Amaranthusviridis* L. on the 30<sup>th</sup> day (mg/g f.wt.)**

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T <sub>0</sub>	0.131 ± 0.013	0.101 ± 0.129	0.159 ± 0.010
T <sub>1</sub>	<b>0.133 ± 0.012</b>	0.026 ± 0.012	<b>0.173 ± 0.015</b>
T <sub>2</sub>	0.118 ± 0.016	<b>0.225 ± 0.189</b>	0.153 ± 0.031
T <sub>3</sub>	0.132 ± 0.022	0.032 ± 0.021	0.143 ± 0.010
T <sub>4</sub>	0.090 ± 0.011	0.018 ± 0.002	0.094 ± 0.036
SEd	0.0124	0.0842	0.0190
CD(P<0.05)	0.0276	0.1875	0.0424

Values are mean ± SD of three samples in each group



**Fig. 11 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Amaranthusviridis* L. on the 30<sup>th</sup> day**

#### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

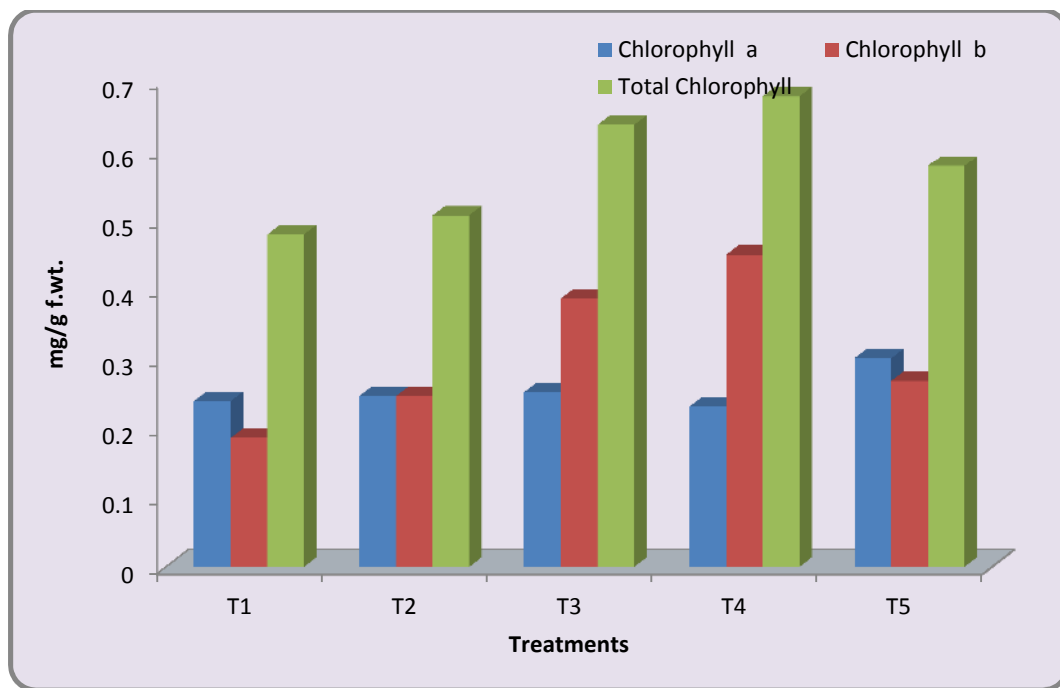
T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

**Table -12**

**Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the  
*Amaranthusviridis* L. on the 45<sup>th</sup> day (mg/g f.wt.)**

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T <sub>0</sub>	0.237 ± 0.042	0.184 ± 0.097	0.479 ± 0.070
T <sub>1</sub>	0.244 ± 0.080	0.244 ± 0.167	0.506 ± 0.141
T <sub>2</sub>	0.250 ± 0.053	0.386 ± 0.091	0.637 ± 0.133
T <sub>3</sub>	0.229 ± 0.027	<b>0.450 ± 0.141</b>	<b>0.678 ± 0.147</b>
T <sub>4</sub>	<b>0.300 ± 0.045</b>	0.266 ± 0.307	0.577 ± 0.291
SEd	0.0429	0.1460	0.1407
CD(P<0.05)	0.0955	0.3253	0.3136

Values are mean ± SD of three samples in each group



**Fig. 12 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Amaranthusviridis* L. on the 45<sup>th</sup> day**

#### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

**Table 13**  
**Protein Content of *Amaranthus viridis* L. on the 30<sup>th</sup> Day, and**  
**45<sup>th</sup> day (mg/g f.wt.)**

TREATMENTS	30 <sup>TH</sup> DAY		45 <sup>TH</sup> DAY	
	0.1	0.2	0.1	0.2
T <sub>0</sub>	2.20 ± 0.10	1.23 ± 0.15	3.87 ± 0.57	3.77 ± 2.20
T <sub>1</sub>	2.30 ± 0.44	1.57 ± 0.32	5.07 ± 1.38	2.90 ± 0.70
T <sub>2</sub>	<b>2.80 ± 0.10</b>	1.33 ± 0.32	3.63 ± 0.46	2.67 ± 0.70
T <sub>3</sub>	2.60 ± 0.46	<b>1.67 ± 0.49</b>	4.87 ± 1.00	3.30 ± 0.95
T <sub>4</sub>	2.43 ± 0.58	1.37 ± 0.21	<b>5.97 ± 0.81</b>	<b>3.77 ± 1.27</b>
SEd	0.67594			
CD(P<0.05)	1.36615			

Values are mean ± SD of three samples in each group

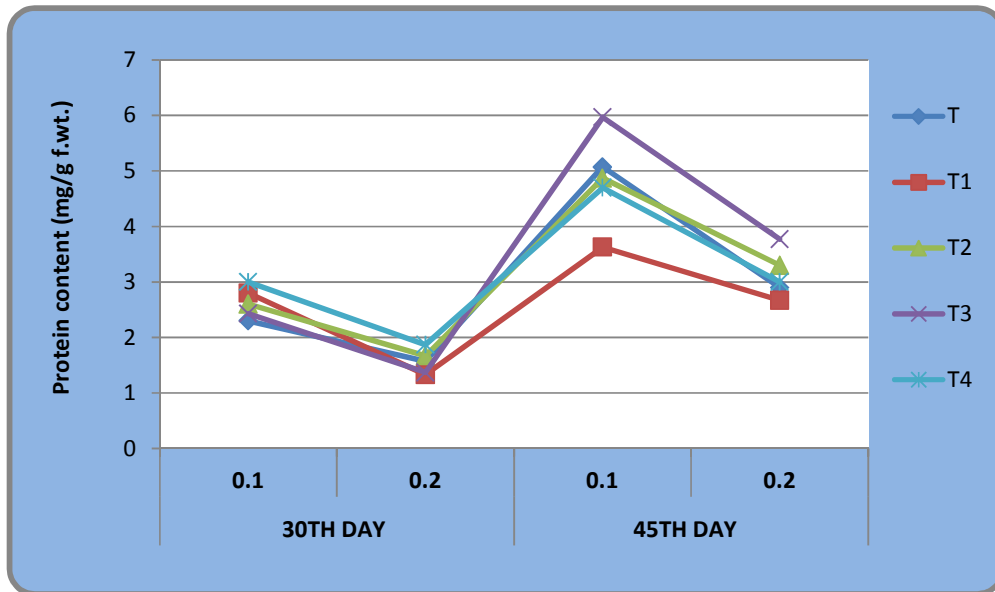


Fig. 13 Protein Content of *Amaranthus viridis* L. on the 30<sup>th</sup> Day, and 45<sup>th</sup> day

#### TREATMENTS

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

**Table 14**

**Carbohydrates Content of *Amaranthus viridis* L. on the 30<sup>th</sup> day and 45<sup>th</sup> day (mg/g f.wt.)**

TREATMENTS	30 <sup>TH</sup> DAY		45 <sup>TH</sup> DAY	
	0.1	0.2	0.1	0.2
T <sub>0</sub>	1.37 ± 0.32	1.07 ± 0.31	3.33 ± 0.15	2.13 ± 0.23
T <sub>1</sub>	2.63 ± 0.25	1.47 ± 0.06	3.63 ± 0.12	2.03 ± 0.15
T <sub>2</sub>	<b>2.83 ± 0.83</b>	1.73 ± 0.23	3.77 ± 0.15	2.20 ± 0.10
T <sub>3</sub>	2.47 ± 0.40	<b>1.83 ± 0.15</b>	<b>5.97 ± 0.12</b>	<b>3.43 ± 0.42</b>
T <sub>4</sub>	2.47 ± 0.06	1.80 ± 0.10	3.50 ± 0.26	2.97 ± 0.38
SEd	0.20303			
CD(P<0.05)	0.41035			

Values are mean ± SD of three samples in each group

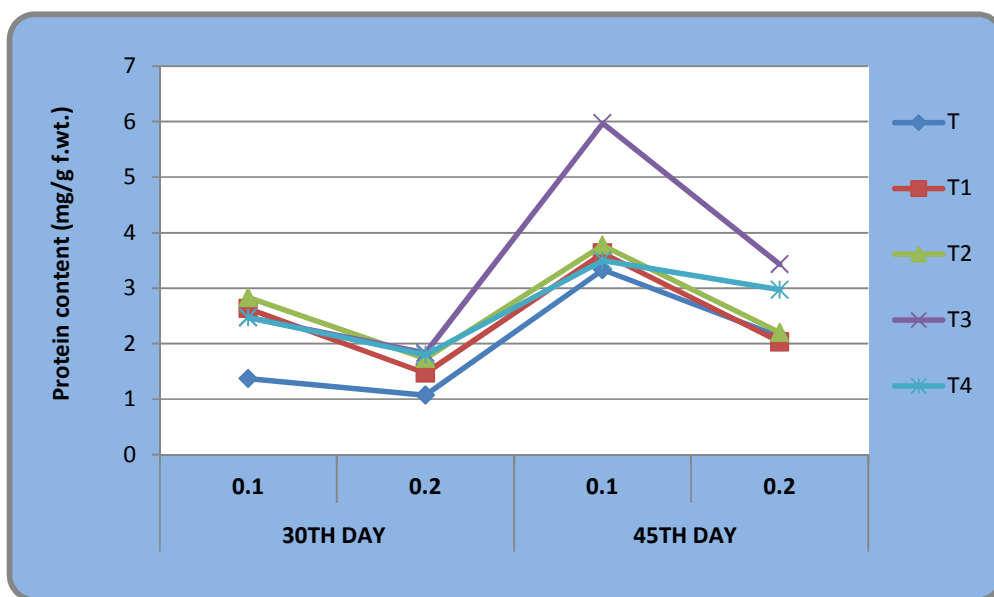


Fig. 14 Carbohydrates Content of *Amaranthus viridis* L. on the 30<sup>th</sup> day and 45<sup>th</sup> day

### TREATMENTS

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

### III. Yield parameters

## Number of fruits

The number of fruits of tomato was calculated on 45<sup>th</sup> day and 60<sup>th</sup> day and tabulated (Table 15). On the 45<sup>th</sup> day, the number of fruits was higher in T<sub>4</sub>, but on the 60<sup>th</sup> day the number of fruits was found to be higher in T<sub>3</sub> (Phosphobacteria treated plants). This indicates that the phosphate solubilizing bacteria increase the yield of tomato at the later stage of its growth.

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 and biochemical parameters showed significance at 5% level.

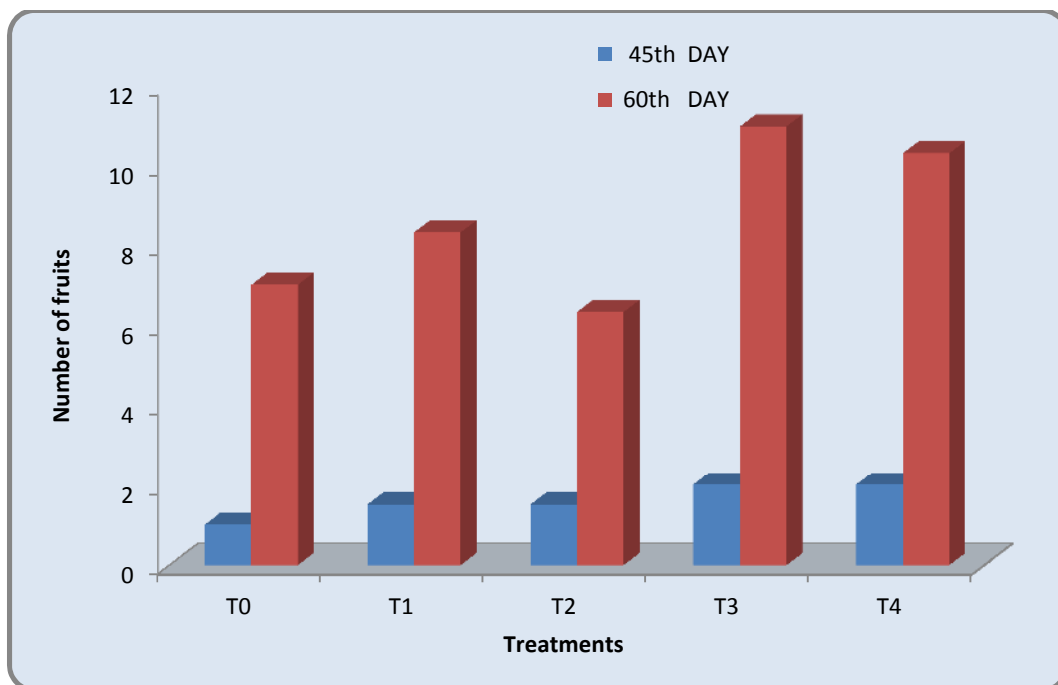
Studies on the effect of PSB, *Azospirillum* and *Azotobacter* by Choudhary *et al.* (2017) have indicated that the application of bio-fertilizers not only improves the quality of Knol-Khol, but also gives a maximum monetary benefit. They have concluded that the use of PSB, *Azospirillum* and *Azotobacter* 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, solubilizing 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.

**Table 15**

**Number of Fruits of *Solanum lycopersicum* L. on the 45<sup>th</sup> day and 60<sup>th</sup> day**

<b>TREATMENTS</b>	<b>NUMBER OF FRUITS 45th DAY</b>	<b>NUMBER OF FRUITS 60<sup>th</sup> DAY</b>
T <sub>0</sub>	1.00 ±#DIV/0!	7.00 ± 2.00
T <sub>1</sub>	1.50 ± 0.17	8.33 ± 0.58
T <sub>2</sub>	1.50 ± 0.17	6.33 ± 1.15
T <sub>3</sub>	<b>2.00 ± 1.41</b>	<b>11.00 ± 1.73</b>
T <sub>4</sub>	2.00 ± 0.00	10.33 ± 3.21

Values are mean ± SD of three samples in each group



**Fig. 15** Number of Fruits of *Solanum lycopersicum* L. on the 45<sup>th</sup> day and 60<sup>th</sup> day

#### **TREATMENTS**

T<sub>0</sub>-Control

T<sub>1</sub>-*Azospirillum*

T<sub>2</sub>-Vesicular Arbuscular mycorrhiza

T<sub>3</sub>-Phosphobacteria

T<sub>4</sub>-*Azospirillum* + VAM + Phosphobacteria

#### **IV. Preliminary Phytochemical Screening**

Preliminary phytochemical screening was done on the 60<sup>th</sup> day in *Solanum lycopersicum* L. and on the 45<sup>th</sup> day in *Amaranthus viridis* L. The solvents used for extraction were water, acetone, chloroform, ethanol and petroleum ether. The phytochemical screening was carried out to analyse the presence of various secondary metabolites such as alkaloids, tannins, flavonoids, quinones, phlobatannins, phenol, carbohydrates, steroids, terpenoids and fats & oil.

##### **1. *Solanum lycopersicum* L.**

In tomato, the phytochemical screening on the dry leaf powder extract using water showed the presence of alkaloids, tannins, flavonoids, phlobatannins, phenol, carbohydrate, terpenoids and fats & oil. Quinones and steroids were completely absent in water extract (Table 16).

The dry leaf powder extracts of tomato with acetone showed the presence of alkaloids, carbohydrates, terpenoids and fats & oil. The other secondary metabolites such as quinones, phlobatannins and phenol were altogether absent in the leaves of tomato. Tannins and flavonoids were present in T<sub>3</sub> and T<sub>4</sub> (Table 17).

The chloroform extract of the dry leaf powder of tomato showed the presence of alkaloids, tannins, phenol, carbohydrates, terpenoids and fats & oil. Quinones and phlobatannins were absent completely. Flavonoids were present in plants treated with VAM, Phosphobacteria and the combination of *Azospirillum*, VAM and Phosphobacteria (Table 18).

The ethanol extract of dry leaf powder of tomato showed the presence of alkaloids, tannins, phlobatannins, phenol, carbohydrates, terpenoids and fats & oil. Quinone was absent in all the treated plants as well as the control plant. Similarly, terpenoids was absent in control and *Azospirillum* treated plants of tomato (Table 19).

The dried leaf powder of tomato was overnight extracted with petroleum ether and later analysed for the presence of secondary metabolites. This showed the presence of alkaloids, tannins, flavonoids, phenol, carbohydrates, terpenoids and fats & oil in all the plants both control as well as treated plants. Quinones and phlobatannins were completely absent in tomato (Table 20).

**Table 16**

**Preliminary Phytochemical Screening of *Solanum lycopersicum* L.  
in water extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Tannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Flavonoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Quinones</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phlobatannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>-</b>	<b>-</b>
<b>Phenol</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Steroids</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Terpenoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

**Table 17**  
**Preliminary Phytochemical Screening of *Solanum lycopersicum* L. in acetone extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Tannins</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>+</b>	<b>+</b>
<b>Flavonoids</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>+</b>	<b>+</b>
<b>Quinones</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phlobatannins</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phenol</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Terpenoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

**Table 18**

**Preliminary Phytochemical Screening of *Solanum lycopersicum* L. in chloroform extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Tannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Flavonoids</b>	<b>-</b>	<b>-</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Quinones</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phlobatannins</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phenol</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Terpenoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

**Table 19**  
**Preliminary Phytochemical Screening of *Solanum lycopersicum* L. in ethanol extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Tannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Flavonoids</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>+</b>	<b>+</b>
<b>Quinones</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phlobatannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>-</b>
<b>Phenol</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Terpenoids</b>	<b>-</b>	<b>-</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

**Table 20**

**Preliminary Phytochemical Screening of *Solanum lycopersicum* L. in  
Petroleum ether extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Tannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Flavonoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Quinones</b>	<b>+</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phlobatannins</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phenol</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Terpenoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

**Table 21**

**Preliminary Phytochemical Screening of *Amaranthus viridis* L.  
in water extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>+</b>
<b>Tannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Flavonoids</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Quinones</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Phlobatannins</b>	<b>+</b>	<b>+</b>	<b>-</b>	<b>+</b>	<b>-</b>
<b>Phenol</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Steroids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Terpenoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

**Table 22**

**Preliminary Phytochemical Screening of *Amaranthus viridis* L. in petrolrum ether extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	+	-	-	-	+
<b>Tannins</b>	+	+	+	+	+
<b>Flavonoids</b>	+	+	+	+	+
<b>Quinines</b>	+	+	+	+	+
<b>Phlobatannins</b>	-	-	-	-	-
<b>Phenol</b>	+	+	+	+	+
<b>Carbohydrates</b>	+	+	+	+	+
<b>Steroids</b>	+	+	+	+	+
<b>Terpenoids</b>	-	-	-	-	-
<b>Fats and oil</b>	+	+	+	+	+

**Table 23**

**Preliminary Phytochemical Screening of *Amaranthus viridis* L.  
in acetone extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Tannins</b>	<b>-</b>	<b>+</b>	<b>+</b>	<b>-</b>	<b>+</b>
<b>Flavonoids</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Quinines</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phlobatannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Phenol</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Steroids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Terpenoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>-</b>	<b>+</b>	<b>-</b>	<b>-</b>

**Table 24**

**Preliminary Phytochemical Screening of *Amaranthus viridis* L. in chloroform extract**

<b>TEST</b>	<b>T<sub>0</sub></b>	<b>T<sub>1</sub></b>	<b>T<sub>2</sub></b>	<b>T<sub>3</sub></b>	<b>T<sub>4</sub></b>
<b>Alkaloids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Tannins</b>	<b>-</b>	<b>+</b>	<b>-</b>	<b>+</b>	<b>+</b>
<b>Flavonoids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Quinines</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>Phlobatannins</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Phenol</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Carbohydrates</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Steroids</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Terpenoids</b>	<b>+</b>	<b>-</b>	<b>+</b>	<b>+</b>	<b>+</b>
<b>Fats and oil</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

Tharun *et al.*, (2012) have carried out studies on the pharmacognostic, phytochemical, antimicrobial and antioxidant activity of *Amaranthus tricolor* Linn. Leaf and concluded that the plant is endowed with significant antimicrobial and antioxidant activity due to the presence of active phytochemical constituents.

## **2. *Amaranthus viridis* L.**

The green leafy vegetable was also tested for the presence of phytochemical constituent on the 45<sup>th</sup> day of its growth. The leaf was dried and extracted with water, petroleum ether, acetone and chloroform.

The dry leaf powder extracted with water showed the presence of quinones, phenol, carbohydrates, steroids, terpenoids and fats & oil. Flavonoids were absent in the plant.

Alkaloid was present only in the control plant. Phlobatannins was present in T<sub>0</sub>, T<sub>1</sub> and T<sub>3</sub> (Table 21).

The petroleum ether extract of the green leafy vegetable showed the presence of tannins, flavonoids, quinones, phenol, carbohydrates, steroid and fats & oil. Alkaloids, phlobatannins and terpenoids were absent in the vegetable crop (Table 22).

The dry leaf powder extracted with acetone showed the following results. The alkaloids, phlobatannins, phenol, carbohydrates, steroids and terpenoids were found to be present in the leaf in all the plants control as well as the organic fertilizer treated plants. Tannins was present only in T<sub>1</sub> and T<sub>2</sub> (Table 23).

The dry powder extracts of *Amaranthus* in chloroform showed the presence of all the secondary metabolites tested except quinones. Quinones was absent in control plants as well as organic fertilizer treated plants (Table 24).

In 54 Indian medicinal plants, the preliminary phytochemical analysis showed positive indication for alkaloids, flavonoids, steroids and terpenoids by Xavier and Kavitha (2012).

## V. SUMMARY AND CONCLUSION

Bio-fertilizers contain live cells of specific isolated strains of bacteria and fungi which is formulated in suitable carriers. These microbes when applied to soil under suitable conditions secrete metabolites and enzymes that make the deficient element available to the plant. Nitrogen fixing bacteria fixes the atmospheric nitrogen in the soil, while Phosphobacteria solubilizes the insoluble phosphorus in the soil into an available form. VAM infected root penetrate the soil effectively and make the unavailable forms of macro/microelements into an available form to the plant.

Bio-fertilizers are a suitable supplement to chemical fertilizers to meet the integrated nutrient demand of the crops. Application of bio-fertilizers results in increased mineral and water uptake, root development, vegetative growth and yield of the crop. They are eco-friendly, non-toxic, easy to use and cost effective that improves the soil fertility and crop productivity.

In the present study, two vegetable crops namely *Solanum lycopersicum* L. and *Amaranthus viridis* L. were taken and growth studies, biochemical parameters and phytochemical analysis were carried out. In the case of tomato, the study was carried out on 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> days and for amaranth, the study was carried out on the 30<sup>th</sup> and 45<sup>th</sup> days alone.

### **Growth Parameters**

The growth parameters carried out in the present study on *Solanum lycopersicum* L. showed a higher growth on 30<sup>th</sup> day in terms of root length, shoot length, number of leaves, fresh weight and dry weight when the combination of bio-fertilizers were used. On the 45<sup>th</sup> day, the root length, shoot length, fresh weight and dry weight was higher in plants treated with the combination of fertilizers. The number of leaves was higher in VAM treated plants. On the 60<sup>th</sup> day, the fresh weight and dry weight was found to be higher in Phosphobacteria treated plants.

In *Amaranthus viridis* 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 30<sup>th</sup> day. On the 45<sup>th</sup> 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.

## Biochemical Parameters

The chlorophyll *a* was found to be higher in tomato plants treated with *Azospirillum* on 30<sup>th</sup> day and 45<sup>th</sup> day, but on the 60<sup>th</sup> day, the chlorophyll *a* content was found to be higher in plants treated with VAM fungi. In the case of chlorophyll *b*, it was found that on the 30<sup>th</sup> day, the content was higher in VAM treated plants and on the 45<sup>th</sup> day and 60<sup>th</sup> day, it was observed to be more in *Azospirillum* treated and combination of fertilizers respectively.

Similarly, the total chlorophyll content was found to be higher in *Azospirillum* treated plants on the 30<sup>th</sup> and 45<sup>th</sup> day and combination of fertilizers on the 60<sup>th</sup> day.

In *Amaranthus viridis* L., the chlorophyll *a* was found to be higher in plants treated with *Azospirillum* on the 30<sup>th</sup> day. Chlorophyll *b* was found to be higher in plants treated with VAM fungi and total chlorophyll in plants treated with combination of fertilizers. On the 45<sup>th</sup> day, the chlorophyll *a* was found to be higher in T<sub>4</sub> and chlorophyll *b* and total chlorophyll was higher in plants treated with Phosphobacteria.

The protein content in tomato plant was higher in T<sub>2</sub> on the 30<sup>th</sup> day, T<sub>4</sub> on the 45<sup>th</sup> day and T<sub>3</sub> on the 60<sup>th</sup> day. The carbohydrate content was found to be more in T<sub>2</sub> on the 30<sup>th</sup> day, T<sub>1</sub> on the 45<sup>th</sup> day and T<sub>4</sub> on the 60<sup>th</sup> day.

In *Amaranthus viridis* L., the protein content was observed to be higher in T<sub>2</sub> on the 30<sup>th</sup> day and T<sub>4</sub> on the 45<sup>th</sup> day. Similarly, the carbohydrate content was also found to be more in T<sub>2</sub> on the 30<sup>th</sup> day and T<sub>3</sub> on the 45<sup>th</sup> day.

The yield of tomato plant was observed to be higher in the plants treated with combination of fertilizers.

## Phytochemical Analysis

The phytochemical screening using water, acetone, chloroform, ethanol and petroleum ether extract of tomato leaves on the 60<sup>th</sup> day showed the presence of most of the secondary metabolites screened. Quinones and steroids were absent in water extract. Quinones and phlobatannins were absent in acetone and petroleum ether extract of control as well as treated plants. Flavonoids were present in chloroform extract.

In *Amaranthus viridis* L., the phytochemical screening was done on the 45<sup>th</sup> day. The dry leaf powder extract was taken using water, petroleum ether, acetone and chloroform. Flavonoids was absent in water extract. Petroleum ether extract showed the presence of most

of the secondary metabolites except alkaloids, phlobatannins and terpenoids. In acetone extract, tannin was present only in T<sub>1</sub> and T<sub>2</sub>. Chloroform extract showed the presence of all the phytochemicals screened except quinones.

## CONCLUSION

Microbial flora of soil plays an important role in soil health. The microbes present in the environment around the roots influence the plant growth and crop yield. The microorganisms in Bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. The increased amount of chlorophyll content in leaves treated with organic fertilizers indicates the photosynthetic efficiency of the plants.

Mycorrhiza is a symbiotic relationship between Arbuscular mycorrhizal fungi (AMF) and roots of majority of vascular plants. The use of *Azospirillum*, Phosphobacteria and VAM fungi improves the growth potential of the crop plants. It also increases the yield and maintenance of soil health for sustainable agriculture. However, quantitative understanding of the ecological factors that control the performance of biological N<sub>2</sub> fixation systems of the bacterium in crop fields is essential for promotion and successful adoption of the bio-fertilizer production technology.

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 important as 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 or bio-fertilizer is the most effective way for higher growth and yield of the crop plants studied. 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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