Effect on different organic fertilizers on the Growth parameters, Biochemical and Phytochemical constituents of *Abelmoschus esculentus* (L.) Moench and *Amaranthus tricolor* (L.)

By

ANITHA, D.
(16PBO001)

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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I. INTRODUCTION

Vegetables are certain parts of plants that are consumed by humans as food as part of a savoury meal. The modern-day culinary usage of the term vegetable may exclude food derived from plants such as fruits, nuts and cereal grains, but include seeds such as pulses; the term vegetable is somewhat arbitrary and can be largely defined through culinary and cultural tradition.

At first, plants which grew locally would have been cultivated, but as time went on, trade brought exotic crops from elsewhere to add to domestic types. Nowadays, most vegetables are grown all over the world as climate permits and crops may be cultivated in protected environments in less suitable locations. China is the largest producer of vegetables and global trade in agricultural products allows consumers to purchase vegetables grown in faraway countries. The scale of production varies from subsistence farmers supplying the needs of their family for food, to agribusinesses with vast acreages of single-product crops. Depending on the type of vegetable concerned, harvesting the crop is followed by grading, storing, processing and marketing.

Vegetables can be eaten either raw or cooked and play an important role in human nutrition, being mostly low in fat and carbohydrates, but high in vitamins, minerals and dietary fiber. Many nutritionists encourage people to consume plenty of fruit and vegetables. Throughout recorded history, the rich have been able to afford a varied diet including meat, vegetables and fruit, but for poor people, meat was a luxury and the food they ate was very dull, typically comprising mainly some staple product made from rice, rye, barley, wheat, millet or maize. The addition of vegetable matter provided some variety to the diet.

The staple diet of the Aztecs in Central America was maize and they cultivated tomatoes, avocados, beans, peppers, pumpkins, squashes, peanuts
and amaranth seeds to supplement their tortillas and porridge. In Peru, the Incas subsisted on maize in the lowlands and potatoes at higher altitudes. They also used seeds from quinoa, supplementing their diet with peppers, tomatoes and avocados (Lambert Tim, 2015)

In Ancient China, rice was the staple crop in the south and wheat in the north, the latter made into dumplings, noodles and pancakes. Vegetables used to accompany these included yams, soybeans, broad beans, turnips, spring onions and garlic. The diet of the

ancient Egyptians was based on bread, often contaminated with sand, which wore away their teeth. Meat was a luxury but fish was more plentiful. These were accompanied by a range of vegetables including marrows, broad beans, lentils, onions, leeks, garlic, radishes and lettuces. The Romans grew broad beans, peas, onions and turnips and ate the leaves of beets rather than their roots (Forbes and James, 1965).

Nutrition and Health

Vegetables play an important role in human nutrition. Most are low in fat and calories but are bulky and filling (Fruits & Vegetables, 2015). 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 (Vegetables, 2015; Terry & Leon, 2011). Research has shown that, compared with individuals who eat less than three servings of fruits and vegetables each day, those that eat more than five servings have an approximately twenty percent lower risk of developing coronary heart disease or stroke (Vegetables & Fruits, 2015).The nutritional content of vegetables varies considerably; some contain useful amounts of protein though generally they contain little fat and varying proportions of vitamins such as vitamin A, vitamin K and vitamin B_6; provitamins; dietary mineralsand carbohydrates.

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 and may become contaminated during their preparation by an infected food handler. Hygiene is important when handling foods to be eaten raw and such products need to be properly cleaned, handled and stored to limit contamination (Centres for Disease Control & Prevention, 2013).

With the increase in population, we are in a state not only to stabilize the agricultural production, but also to increase it further in a sustainable manner. Excessive use of agro-chemicals like pesticides and fertilizers has affected the soil health and lead to decrease in crop yields as well as quality of products. Hence, a natural balance has to be maintained to make the life and property exist. Organic farming is the best way for sustainable production of vegetable crops.

Azospirillum is a micro-Europhilic nitrogen fixer. It fixes nitrogen in an environment of low oxygen tension. *Azospirillum* are free-living N_2 fixing bacteria that in the rhizospheric

zone have the ability to synthesize and secrete some biologically active substances that enhance root growth.

The bacteria induce the plant roots and secrete mucilage, which creates low oxygen environment that helps to fix atmospheric nitrogen. It fixes N_2 10-14 kg/ha/season in many vegetable crops. They also increase germination and vigour in young plants, leading to improved crop stands. *Azospirillum* can freely fix molecular nitrogen and be considered as biological fertilizer (Amiri *et al*., 2013).

The *Azospirillum* and VAM are the main bio fertilizers which are biologically active products containing bacteria or fungi as a single or composite cultures and thus they improve soil health and fertility. The VAM fungi and soil microorganisms develop special characteristic structures called as apostles and vesicles.

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 solubilising bacteria are capable of solubilising unavailable form of phosphorus into available form and make it available to plants (Veena *et al*., 2009; Shankarappa *et al*., 2012).

The organic sources are directly or indirectly helpful in increasing the availability and uptake of nutrients from the soil and ultimately to boost up the yield and quality of plant. Application of bio-fertilizers such as PSB and *Azospirillum* has reduced the use of chemical fertilizers, but, provided high quality organic products free of harmful agrochemicals for the safety of public health.

Bio-fertilizers are one of the best modern tools for agriculture. They contain microorganisms which promote the adequate supply of nutrients to the host plants to ensure their proper development (Uma Maheswari and Elakkiya, 2014).

The organic fertilizers are directly or indirectly helpful in increasing the availability and uptake of nutrients from the soil and ultimately to boost up the yield and quality of plant (Rajesh *et al*., 2015)

Okra, also termed as lady's finger, is a flowering plant in the mallow family. This plant is known for its edible green fruits, or long green pods. It's scientific name is "*Abelmoschusesculentus*" and also "*Hibiscus esculentus*". For centuries, this green vegetable has been widely grown across the entire African region. The species apparently originated in

the Ethiopian Highlands, though the manner of distribution from there is undocumented. African slaves brought it to USA.It is cultivated in the entire warm temperate and tropical regions of the world for its fibrous fruits or pods containing round, white seeds. The fruits are harvested when immature and eaten as a vegetable. The plant prefers warm climate and tolerates poor soils with heavy clay and intermittent moisture. It is in the same plant family as hibiscus and cotton.

Okra/lady's finger is one of the most common vegetables of the South-Asian countries. It is used in preparing many yummy and delicious dishes. When cut, it releases a sticky material with thickening properties, often used in soups and stews. Gumbos, Brunswick stew and pilaus are some well-known dishes which frequently use okra.

Amaranthus species are being cultivated since centuries as a leafy vegetable, as well as an important subsidiary food grain crop in many parts of the world. Vegetable amaranth serves as an alternative source of nutrition for people in developing countries since it is a rich and inexpensive source of carotenoid, protein, vitamins and dietary fiber. Unlike other leafy vegetables, vegetable amaranth is cultivated during hot summer months when no other green vegetables are available in the market. Besides immense nutritional importance, it can also be successfully grown under varied soil and agro climatic conditions. Recently, current interest in amaranth also resides in the fact that it has a great amount of genetic diversity and phenotypic plasticity. Amaranth is extremely adaptable to adverse growing conditions, resists heat and drought, has no major disease problem and is among the easiest of plants to grow. Vegetable amaranth (*Amaranthus tricolor* L.) remains a subsidiary under exploited crop for vegetable purose. Inspite of immense nutritional qualities, not much work has been done for its genetic improvement.

A. tricolor (Tambdi Bhaji/Lal Saag) is native to a large part of India and forms an integral part of the Goan staple diet. Its mild spinach like flavour, high nutritive value, ability to grow in hot weather and lower cost, have made it a very popular vegetable. The plant is well known for its purple betalain pigments, such as amaranthine and isoamaranthine.

Amaranthus make up a large well-known family of primarily tropical plants. *Amaranthus*, collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial plants. Some amaranth species are cultivated as leafy vegetables, pseudocereals and ornamental plants. Most of the *Amaranthus* species are summer annual weeds and are commonly referred to as pigweed. Catkin-like cymes of

densely packed flowers grow in summer or autumn. Members of this genus share many characteristics and uses with members of the closely related genus *Celosia*.

"Amaranth" derives from Greek word *amárantos*, meaning "unfading". *Amaranthus* shows a wide variety of morphological diversity among and even within certain species. Although the family (Amaranthaceae) is distinctive, the genus has few distinguishing characters among the 70 species included.

Amaranth species are cultivated and consumed as a leafy vegetable in many parts of the world. Four species of *Amaranthus* are documented as cultivated vegetables in eastern Asia: *Amaranthus cruentus*, *Amaranthus blitum, Amaranthus dubius* and *Amaranthus tricolor*. A traditional food plant in Africa, amaranth has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care.

A very popular, tender and tasty dark green leafy vegetable

- Can produce huge yields of 10 tons per hectare edible greens in 30-40 days.
- Comes up quickly, can often be harvested in 3-4 weeks
- Seeds readily available
- Regrows so that up to 4 leaf harvests can be made before replanting
- Protein quality is excellent for leaf crop
- Amaranth can tolerate high aluminium content in soil. High aluminium levels in the soil are often a factor limiting growth in tropical areas where malnutrition is prevalent.

The use of organic fertilizers could increase the yield of the vegetable crop as well as improve the fertility of the soil. The present study on the vegetable crop *Abelmoschus esculentus* (L.) Moench and green leafy vegetable (*Amaranthus tricolor* L.) is an initiative to grow the plant under different organic fertilizers and estimate the biochemical constituents and phytochemical constituents available in both the plants.

Objective

The objective of the present study is to evaluate the

(1) Effect of different organic fertilizers on different growth parameters of *Abelmoschus esculentus* (L.) Moench and *Amaranthus tricolor* L. Parameters such as shoot length, root length, number of leaves, fresh weight,dry weight and number of fruits were measured.

(2) Biochemical parameters such as chlorophyll, protein and carbohydrate of control and treated plants were calculated.

(3) Analyze the preliminary phytochemical constituents using different solvent extracts.

II. REVIEW OF LITERATURE

The available literature pertaining to the use of organic fertilizer on the growth, biochemical studies and phytochemical aspects relevant to the present investigation has been reviewed and presented in this chapter.

A field experiment conducted by Mounika *et al*. (2017) on the effect of individual and combined application of bio-fertilizers, micronutrients on the growth and yield of coriander have shown a significant increase in growth and yield parameters.

The evaluation of bio-fertilizers on growth and establishment of cashew grafts under green house condition was carried out by Shankarappa *et al*. (2017).

Singh *et al*. (2017) have carried out experiment on the role of bio-fertilizer and chemical fertilizer for sustainable onion production.

The study carried out by Jhankar *et al.* (2017) on the effect of INM practices on the yield of coriander have shown a significant increase in the number of compound leaves of coriander.

Pulipati *et al*. (2017) have carried out research on the total phenol, tannin and flavonoid content of *Amaranthus tricolor* (L.).

Earlier work by Sarkar *et al.* (2017) on the effect of organic resources of nutrients for corms and cormlet production of *Gladiolus* have shown that the use of different organic sources in combination with bio-fertilizers could increase the rate of production.

Singh *et al.* (2013) have studied the influence of chemical fertilizers and biofertilizers on dry matter yield and NPK uptake by cabbage and found that the interaction of inorganic fertilizer and biofertilizer to be highly significant.

Earlier work by Nalawde and Bhalerao (2015) on the comparative account of effect of bio-fertilizers on the growth and biochemical parameters of *Vigna* sp. have shown excellent results in both morphological as well as biochemical parameters as compared to control plants.

Bio-fertilizer when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Kowsar Jan and Boswal, 2015).

Singh *et al*. (2014) has studied the effect of organics on the growth, yield and biochemical parameters of chilli (*Capsicum annum* L.).

The application of bacterial bio-fertilizer could improve the growth and yield of *Phaseolus vulgaris* L. (Kalaiarasi and Sivakumar, 2014).

Sharma *et al.* (2013) have studied the effect of doses of bio-fertilizers on the growth and production of cabbage.

Experiments were carried out by Javed and Panwar (2013) on the effect of bio-fertilizer, Vermicompost and chemical fertilizer on different biochemical parameters of *Glycine max* and *Vigna mungo.*

The combined application of FYM, inorganic fertilizer and bio-fertilizer showed a significant deference of number of primary branches per plant in comparison to other treatments (Tripathi *et al*., 2013; Nagar *et al.,* 2009).

Ainika *et al*. (2012) studied the effect of organic and inorganic fertilizer on the growth and yield of *Amaranthus caudatus* L. Their result showed best edible yield of amaranth in 300 Kg/ha NPK and 5 t/ha FYM.

Earlier, work was carried by Dhanasekar and Dhandapani (2012) on the effect of bio-fertilizers on the growth of *Helianthus annuus.*

Studied on the phytochemical constituents of *Amaranthus tricolor* Linn. leaf by Tharun *et al*. (2012) have shown the presence of carbohydrates, tannins and flavonoids in ethyl acetate fraction and steroids in petroleum ether fraction.

The work done by Gendy *et al.* (2012) on the effect of different levels of cattle manure and bio-fertilizers showed an increase in all the parameters studied in *Hibiscus sabdariffa* L.

Ajayi *et al*. (2011) carried out qualitative phytochemical screening of seven different plant species and found that saponins, flavonoids and reducing sugars were present in all the aqueous extract of the plants, but none contain phlobatannins, carotenoids, steroids, cardiac glycosides and confirmed anthraquinones.

Maneemegalai and Nandakumar (2011) aimed to study the effect of biochemical activity on germinated seeds of *Vigna radiata*, *Vigna mungo* and *Pennisetum typhoides*. Their study confirmed a corresponding increase in the nutritive quality of the germinated seeds compared to dry seeds.

In recent years, *Azospirillum* have gained importance because of this N_2 fixing capacity in root of different crop like – cereals, vegetable and spices. *Azospirillum spp.* are also known to produce growth regulators like IAA, IBA, NAA, GA_1, GA_3, Vitamins, etc (Singh, 2010).

Padmapriya *et al.* (2010) have conducted an experiment on the effect of organic amendments and growth promoters on morphology and yield of *Gymnema sylvestre*. The result showed higher growth in plants treated with combination of fertilizers along with foliar spraying of panchagavya and Manchurian mushroom extract.

An experiment was carried out to determine the effects of different doses of organic and inorganic fertilizers on growth, yield and quality of broccoli. The results showed higher chlorophyll content when inorganic fertilizer was added along with organic manure (Ouda and Mahadeen, 2008).

A field experiment conducted to study the effect of organic manures on plant growth, seed quality and yield of soybean showed higher seed quality by the application of recommended dose of fertilizer along with FYM (Maheshbabu *et al*., 2008).

Studies carried out by Ray *et al*. (2007) on the growth of lady's finger have shown that the application of *Azospirillum* / *Azotobacter* supplemented with FYM could have a beneficial effect in sustaining the growth and yield of the crop.

Vermicompost and FYM might fulfill the nutritional requirements if used appropriately. Inoculation with bio-fertilizers like *Azotoacter*/ *Rhizobium* might increase the productivity by 10 – 20 % (Gill and Sarlach, 2006).

In a field study, Mirza *et al*. (2005) have studied the effects of agroforestry (*Sesbania*) and farm yard manure on rice (*Oryza sativa*). Results revealed that both grain and straw yield of paddy were significantly improved by the application of *Sesbania* and FYM.

Earlier work by Mahantesh Sajan *et al*. (2002) on the growth and yield of chilli have shown that the application of bio-fertilizers along with reduced level of chemical fertilizers has been beneficial compared to the application of chemical fertilizers or bio-fertilizers alone.

Kathiresan and Venkatesh (2002) have reported that combined application of *Azospirillim* and VAM along with recommended dose of NPK increase the number cormels per plant in *Gladiolus.*

Earlier studies have shown that medicinal plants respond best to organic source of nutrients which are also environment friendly (Menon and Potty, 1998 and Kurian *et al.,* 2000).

III. MATERIALS AND METHODS

The plant materials taken for the present study were *Abelmoschus esculentus* (L.) Moench and *Amaranthus tricolor* (L.) belonging to the family Malvaceae and Amaranthaceae respectively. A pot study was carried out on the growth parameters and biochemical parameters under different organic fertilizer treatments. Preliminary phytochemmical screening was also done for both the plants.

Collection of seeds

Seeds of both *Abelmoschus esculentus* (L.) Moench and *Amaranthus tricolor* (L.) were obtained from Tamil Nadu Agricultural University, Coimbatore.

Collection of Fertilizers

The bio-fertilizers such as *Azospirillum*, VAM and Phosphobacteria were collected from TNAU, Coimbatore. The dosage used were as per the TNAU Agriportal.

Morphology of the Plants

Abelmoschus esculentus (L.) Moench

Systematic Position

Kingdom - Plantae

Order - Malvales

Family - Malvaceae

Genus - *Abelmoschus*

Species - *A. esculentus* (L.) Moench

Description

- They are specially found in tropical region of the world.
- Okra, *Abelmoschus esculentus*, is an herbaceous annual plant in the family Malvaceae.
- It is grown for edible seed pods.
- Okra stem is erect, mucilage sacs occur abundantly in the tissues.
- The leaves are simple, alternate and hairless with heart – shaped leaves, mucilage sacs are also found.

- The plant produces flowers with five white to yellow petals which are 4-8 cm (1.6-3.1 in) in diameter.

Plate 1 Habit of *Abelmoschus esculentus* (L.) Moench

- The leaves are 10-20 cm (4-8 inch) long with 5-7 lobes.78
- The seed pod is a capsule upto 25cm (10 inch) long, containing numerous seeds.
- Okra can grow to a height of 1.2-1.8m (4-6 ft) tall and as an annual plant, survives only in growing season.
- Okra may also be refrered to as lady's finger and is believed to have originated in Ethiopia. (Pandey 2006).

Medicinal values

- Okra is known to aid in the prevention of diabetes`
- The folates present in okra reduce the neural defects in a newborn baby.
- Controls Obesity
- High fiber content
- Prevents kidney diseases
- Helps in digestion
- Good source of antioxidants

Description

Amaranthus tricolor (L.)

Systematic Position

Kingdom - Plantae

Order - Caryophyllales

Family - Amaranthaceae

Genus - *Amaranthus*

Species - *A. tricolor* (L.)

- *Amaranthus tricolor* belongs to the family Amaranthaceae.
- The family is well represented in the tropical regions of the world.
- The plants are ascending or erect annual herb.

Plate 2 - Habit of *Amaranthus tricolor* (L.)

- The plants grow up to 125cm tall, usually much branched.
- The leaves are generally spiral, simple, usually covered with hairs, without stipules.
- The petiole is upto 8 cm long.
- Usually the flowers are very minute.
- Inflorescence is axillary, globose cluster upto 2.5 cm in diameter.
- The fruits are generally dry.

Medicinal values

- The plant is rich in vitamins and minerals.
- The whole plant is an astringent.
- A decoction of the root is used with *Cucurbita* tocontrol haemorrhage following abortion.

- A decoction of very old plants is taken internally to improve vision and strengthen the liver.

ORGANIC FERTILIZERS

Azospirillum

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

Vesicular Arbuscular Mycorrhiza (VAM)

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

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

Phosphobacteria

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

Methods

Pot culture experiment

Pot culture experiment was conducted with the two test plants. The experiment was carried out in the period from December 2017 to February 2018. The size of the experimental pot was 30 cm × 24 cm × 30 cm. Triplicates were maintained for each treatment.

The soil was cleaned by removing stones and other unwanted materials. The red soil and sand soil were mixed in the ratio of 1:1 and filled in pots of 7 kg capacity. A study was

undertaken to assess the effect of different bio-fertilizers on the growth, biochemical and yield parameters of both the plants. A preliminary phytochemical study was also conducted.

The seeds were soaked in different bio-fertilizers for 12 hours. The bio-fertilizers used for the study were *Azospirillum,* Phosphobacteria and Vesicular Arbuscular Mycorrhizal (VAM) fungi. In the growing stages of the plants, the bio-fertilizers were sprayed on the plants and growth and biochemical assays were carried out on 30^{th}, 45^{th} and 60^{th} days of the plants.

The infection to the plants by various insects were controlled by spraying thulasi extract on the leaves of both the plants.

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Materials and methods

I. GROWTH PARAMETERS

Plant samples were uprooted carefully on 30^{th}, 45^{th} and 60^{th} day. 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 (g)
5. Dry weight (g)

Root Length

The plants were taken from control pot and other treatment pots and washed to get rid off 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 as cm/seedling.

Shoot Length

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

Number of leaves

The number of leaves present in the uprooted plants was also 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 55º C for 24 hours. Then, the dry weight of the plants was measured using an electronic digital balance and expressed in grams.

II. BIOCHEMICAL PARAMETERS

The biochemical parameters studied in the leaves of the two plants were chlorophyll, protein and carbohydrate. In *Abelmoschus esculentus* (L.) Moench the biochemical parameters were estimated on 30^{th}, 45^{th} and 60^{th} day and in *Amaranthus tricolor* (L.) it was estimated on 30^{th} and 45^{th} days.

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 100 ml volumetric flask. This procedure was repeated until the residue became colourless.
- The washing was collected and the volume was made up to 100 ml 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,

- mg chlorophyll '*a*'/gm tissue = 12.7 A_{663} - 2.69 A_{645} x V/(1000xW)
- mg chlorophyll '*b*'/gm tissue = 22.9A_{645}-4.68A_{663}x V/(1000xW)
- mg total chlorophyll /gm tissue = 20.2A_{645}+8.02A_{663}x V/(1000xW)

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 ($CuSO_4.5H_2O$) in 1 percent potassium sodium tartarate (Reagent B).
- Alkaline copper solution: mix 50 ml of reagent A and 1ml of reagent B prior to use (Reagent C).
- Folin- Ciocalteau reagent (Reagent D)
    - Protein solution (stock standard): Accurately 50 mg of bovine serum albumin (fraction V) was weighed and dissolved in distilled water and made up to 50ml in a standard flask.
    - Working standard: Ten ml of stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 mg protein.

Procedure

Extraction of protein from samples

Extraction was carried out with buffers. About 500 mg of the sample was weighed and ground well with a pestle and mortar in 5-10 ml 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 ml and 0.2 ml of the sample was pipetted 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 5 ml of reagent C was added to each tube including the blank. Mixed well and allowed to stand for 10 min.
- To this, 0.5 ml of reagent D was added, mixed well and incubated in dark for 30 minutes. Blue colour developed was read at 660 nm.
- 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 mg / gm = mg of protein/volume of test standard x concentration of the standard.

3. Estimation of Carbohydrate (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 colorimetrically at 630 nm.

Materials Required

- 2.5 N HCl
- Anthrone reagent was prepared by dissolving 200 mg anthrone in 100 ml of ice cold 95% H_2SO_4. Prepared freshly before use.
- Stock standard: 100 mg of glucose was dissolved in 100 ml of water.
- Working standard: 5 ml of stock standard solution was diluted to 100 ml using distilled water (50 mg/ ml).

Procedure

- About 100 mg of the sample was taken in a boiling tube and was hydrolysed by keeping it in boiling water bath for three hours with 5 ml 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 100 ml 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, 4 ml 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 630 nm.
- 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 x 100

III. YIELD PARAMETERS

1. Number of fruits

The number of fruits obtained on 45th day and 60th day was calculated for *Abelmoschus esculentus* (L.) Moench. For *Amaranthus tricolor* (L.), the study was carried out upto 45th day only because, the plant started losing its vigour after that period.

IV. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preparation of plant extracts

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, acetone, petroleum ether, ethanol and chloroform for overnight extraction and filtered through whatman No.1. filter paper. Qualitative tests were conduted on these extracts according to the method of Harborne (1984).

The following phytochemical parameters were observed in the leaves of the two plants taken for study.

Test for Alkaloids

Mayer's test

To 1 ml of extract, 2 ml of Mayer's reagent was added. Appearance of dull white precipitate indicates the presence of alkaloids.

Test for Tannins

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

Test for Flavonoids

To 1 ml of extract, 1 ml 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 of phlobatannins

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

Test for Phenol

To 1 ml of extract, lead acetate solution was added and the precipitate formation indicates the presence of phenolic compounds**.**

Test for Carbohydrates

Molisch's Test

Two drops of Molisch's reagent was added to an aqueous or hydrochloric acid solution of the extract and 2 ml of concentrated sulphuric acid was added by the side of the test tube.

The formation of reddish violet ring at the junction of the liquids indicated the presence of carbohydrates.

Test for Steroids

Liebermann – Burchard Test

The extract were dissolved in 2 ml of chloroform to which 10 drops of acetic acid and 5 drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

Test for Terpenoids

To 5 ml of filterate, 2 ml of chloroform was added and 3 ml of concentrated sulphuric acid was added carefully. An interface with a reddish brown colouration indicates the presence of terpenoids.

Test for fixed oil and fat

To 1 ml 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 biometric and biochemical observations were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978). The significance and critical differences of various treatments were analysed.

IV. RESULTS AND DISCUSSION

The experiments conducted in *Abelmoschus esculentus* (L.) Moench and *Amaranthus tricolor* (L.) using different organic fertilizers treatments showed the following results.

I. GROWTH PARAMETERS

1. ***Abelmoschus esculentus*** (L.) Moench

The growth parameters such as root length, shoot length, fresh weight, dry weight and number of leaves were analysed on 30th, 45th and 60thday and tabulated (Plate 3, 4 and 5)

The shoot length and root length was observed to be maximum in T_4 and the values were found to be 25.37 ± 1.19 cm and 15.20 ± 0.36 cm respectively (Table 1; Fig. 1).

The number of leaves was also higher in plants treated with *Azospirillum,* VAM and Phosphobacteria (12.67 ± 1.15).

Similarly, the fresh weight and dry weight of the plants were also found to be higher in T_4 is combination of *Azospirillum,* VAM and Phosphobacteria. The readings were found to be 8.80 ± 0.76 g and 2.59 ± 0.36 g respectively. The values were found to be significant at 5 % level (Table 1).

The shoot length and root length of lady's finger was found to be higher in T_4 on the 45th day (Table 2). The values were found to be 35.20 ± 3.67 cm and 20.00 ± 1.42 cm respectively on the 45th day, the number of leaves of leaves was found to be more in plants treated with phosphobacteria (21.00 ± 3.00).

Similar to the shoot length and root length of the plants, the fresh weight and dry weight of lady's finger on the 45th day was higher in T_4 and the values were found to be 18.50 ± 1.32 g and 4.16 ± 0.34 g respectively (Table 2; Fig. 2).

On the 60th day again the shoot length and root length of lady's finger was observed to be more in T_4 (i.e.,) 48.53 ± 1.20 cm and 25.30 ± 2.97 cm (Table 3; Fig. 3). The number of leaves was higher in T_3 on the 60th day (27.67 ± 1.53). But, the fresh weight and dry weight of the plants were higher in T_2 i.e., VAM treated. The values were 28.90 ± 0.928 and 6.81 ± 0.238 respectively. This might be due to the accumulation of nutrients in the plants that has resulted because of VAM treatment.

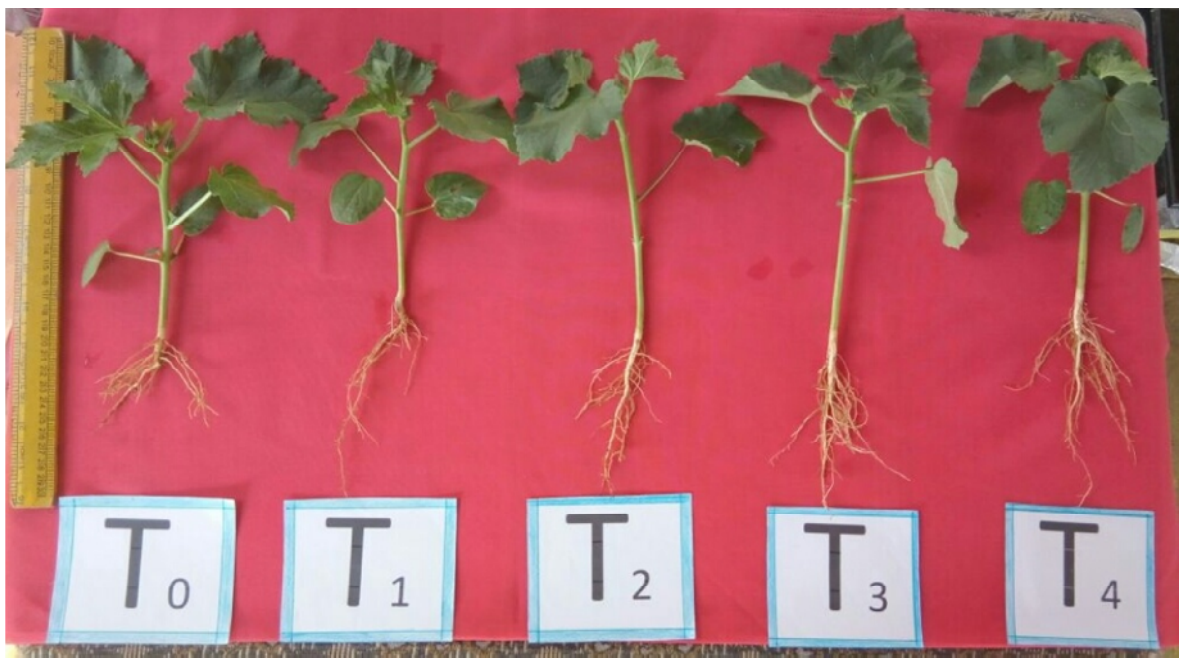


Plate 3 – Growth of *Abelmoschus esculentus* (L) Moench on 30th day

Table 1: Growth parameters of *Abelmoschus esculentus* (L.) Moench using different organic fertilizers on the 30th day

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T_0	22.57 ± 1.00	7.17 ± 1.11	7.00 ± 1.00	6.68 ± 0.65	0.75 ± 0.61
T_1	27.9 ± 0.82	14.47 ± 0.55	9.33 ± 0.58	7.41 ± 0.50	2.24 ± 0.44
T_2	25.30 ± 1.05	13.23 ± 1.85	10.67 ± 1.53	7.97 ± 0.86	1.73 ± 0.46
T_3	24.87 ± 0.38	12.23 ± 1.85	10.33 ± 2.52	8.54 ± 1.05	1.70 ± 0.30
T_4	**25.37** ± 1.19	**15.20 ± 0.36**	**12.67 ± 1.15**	**8.80 ± 0.76**	**2.59 ± 0.36**
SEd	0.7616	0.8345	1.2293	0.6426	0.3656
Cd ($p<0.05$)	1.6969	1.8595	2.7390	1.4319	0.8146

Values are mean ± SD of three samples in each group

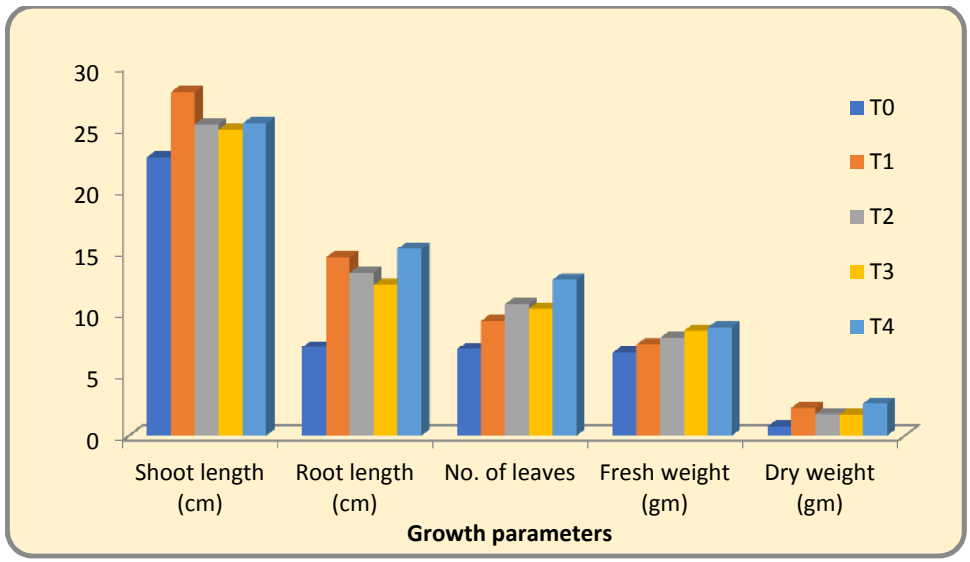


Fig. 1 Growth parameters of *Abelmoschus esculentus* (L.) Moench using different organic fertilizers on the 30th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

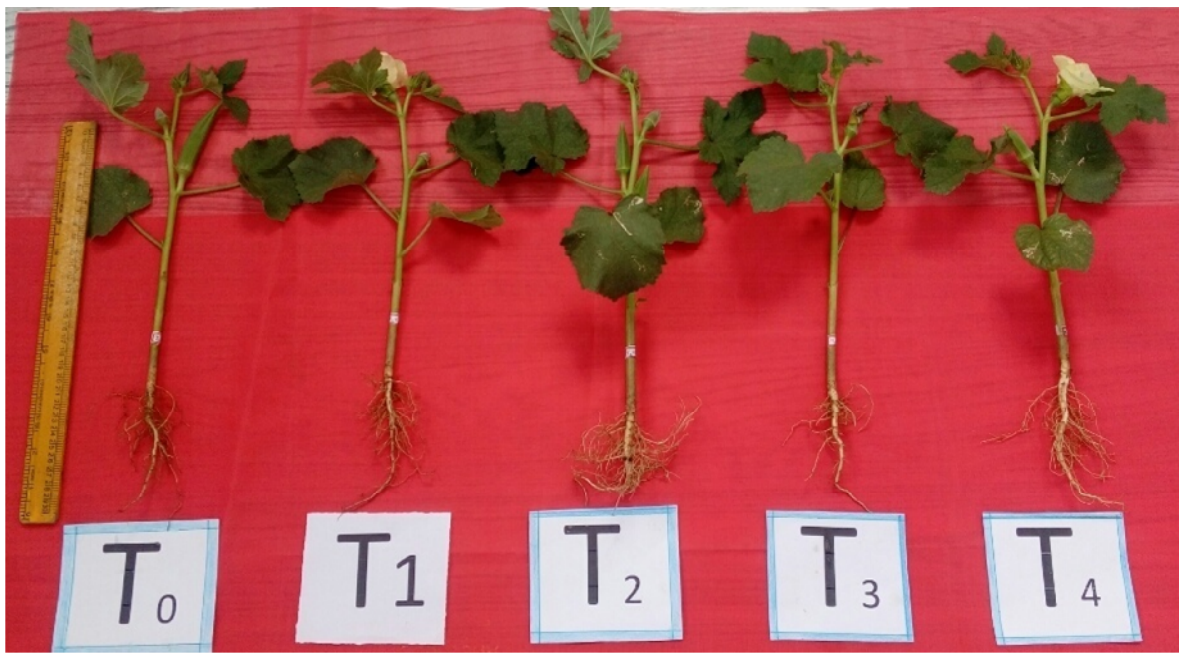


Plate 4 – Growth of *Abelmoschus esculentus* (L) Moench on 45th day

Table 2: Growth parameters of *Abelmoschus esculentus* (L.) Moench using different organic fertilizers on the 45th day

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T_0	28.83 ± 0.32	12.47 ± 1.12	14.00 ± 1.00	12.09 ± 0.65	2.52 ± 0.39
T_1	35.03 ± 4.02	15.90 ± 0.80	17.33 ± 1.15	13.31 ± 0.52	4.24 ± 0.68
T_2	33.17 ± 2.04	16.80 ± 0.70	18.00 ± 1.00	15.70 ± 1.15	4.16 ± 0.34
T_3	34.40 ± 4.35	16.00 ± 1.06	**21.00 ± 3.00**	17.30 ± 1.87	3.80 ± 0.99
T_4	**35.20 ± 3.67**	**20.00 ± 1.42**	20.67 ± 3.79	**18.50 ± 1.32**	**4.45 ± 0.25**
SEd	59.7399	0.8566	1.8856	0.9838	0.4878
Cd ($p<0.05$)	133.1094	1.9087	4.2014	2.1921	1.0869

Values are mean ± SD of three samples in each group

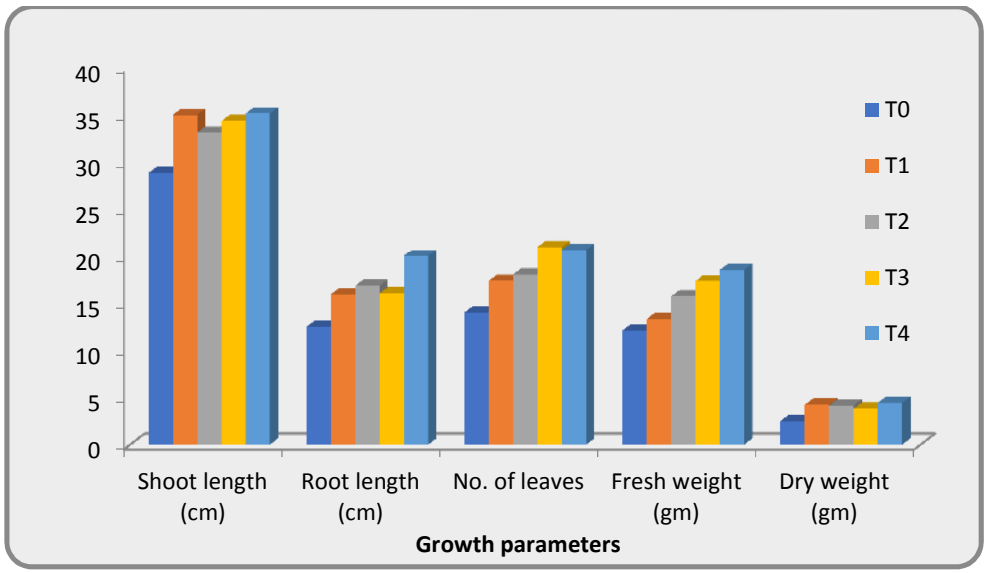


Fig. 2 Growth parameters of *Abelmoschus esculentus* (L.) Moench using different organic fertilizers on the 45th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Plate 5 – Growth of *Abelmoschus esculentus* (L) Moench on 60th day

***Table 3: Growth parameters of Abelmoschus esculentus (L.) Moench using different* organic fertilizers on the 60th day**

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T_0	37.17 ± 1.22	17.57 ± 0.78	20.33 ± 0.58	21.89 ± 0.50	4.72 ± 0.78
T_1	43.30 ± 4.81	19.13 ± 0.35	23.00 ± 1.00	27.93 ± 0.61	6.48 ± 0.40
T_2	42.17 ± 3.65	22.57 ± 1.66	25.67 ± 0.58	**28.90 ± 0.92**	**6.81 ± 0.23**
T_3	44.67 ± 2.15	24.23 ± 0.45	**27.67 ± 1.53**	28.70 ± 0.54	6.19 ± 0.60
T_4	**48.53 ± 1.20**	**25.30 ± 2.97**	27.67 ± 3.21	27.31 ± 0.70	6.16 ± 0.77
SEd	2.912	1.2912	1.3824	595.3327	0.4859
Cd ($p<0.05$)	6.4532	2.8769	3.0803	1326.4903	1.0826

Values are mean ± SD of three samples in each group

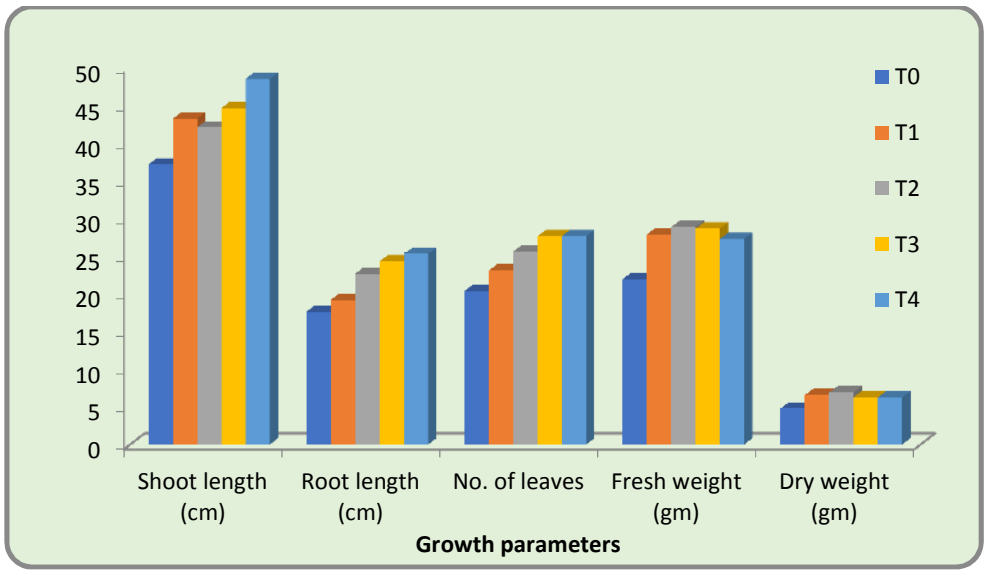


Fig. 3 Growth parameters of *Abelmoschus esculentus* (L.) Moench using different organic fertilizers on the 60th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

2. ***Amaranthus tricolor*** *(L.)*

This is a green leafy vegetable which grows for a maximum period of one and half month. So, the growth parameters were studied in this plant on the 30th day and 45th day. After that, the plant started dying (Plate 6 and 7)

On the 30th day, the shoot length and root length (Table 4; Fig. 4) under different organic fertilizer treatment was found to be higher in T_1 (38.00 + 2.34 cm) and T_4 (19.07 ± 0.93 cm) respectively. The number of leaves was more in T_4 (249.33 ± 10.02).

The fresh weight and dry weight of *Amaranthus tricolor* (L.) was also higher in T_4 (55.08 ± 3.36 g and 5.51 ± 0.43 g) respectively.

On the 45th day of growth, the green leafy vegetable showed a higher shoot length (88.90 ± 3.99 cm), root length (28.50 ± 2.88 cm), number pf leaves (562.33 ± 30.01), fresh weight (91.26 ± 4.33 g) and dry weight (18.89 + 0.69 g) in pants treated with the combination of organic fertilizers such as *Azospirillum*, VAM, phosphobacteria (Table 5; Fig. 5). This shows that when the organic fertilizers of vegetable crops could be increased there by an increase in the yield could be obtained.

The study on the influence of chemical fertilizers and bio-fertilizers on dry matter yield and NPK uptake by Cabbage (Singh *et al*., 2013) had shown a significant variation in dry matter yield/ plant due to the inoculation of *Azospirillum*. The maximum dry matter yield obtained might be due to the ability of *Azospirillum* to produce some growth promoting substances involved in increasing the accumulation of food in plant.

Plate 6 - Growth of *Amaranthus tricolor* (L.) on 30th day

Table 4

Table 4: Growth parameters *Amaranthus tricolor* (L.) using different organic fertilizers on the 30th day

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T_0	25.88 ± 0.54	12.67 ± 0.81	97.67 ± 4.51	26.74 ± 0.63	2.47 ± 0.27
T_1	**38.00 ± 2.34**	16.50 ± 0.56	116.00 ± 12.12	51.82 ± 5.69	5.31 ± 0.59
T_2	31.50 ± 1.32	16.87 ± 1.76	216.00 ± 26.06	48.22 ± 1.44	4.05 ± 0.07
T_3	35.57 ± 2.40	17.83 ± 0.93	234.33 ± 31.56	43.03 ± 1.65	4.75 ± 0.19
T_4	36.43 ± 1.99	**19.07 ± 0.93**	**249.33 ± 10.02**	**55.08 ± 3.36**	**5.51 ± 0.43**
SEd Cd ($p<0.05$)	1.5179 3.3821	0.8796 1.9600	16.0955 35.8633	2.5512 5.6844	0.2938 0.6546

Values are mean ± SD of three samples in each group

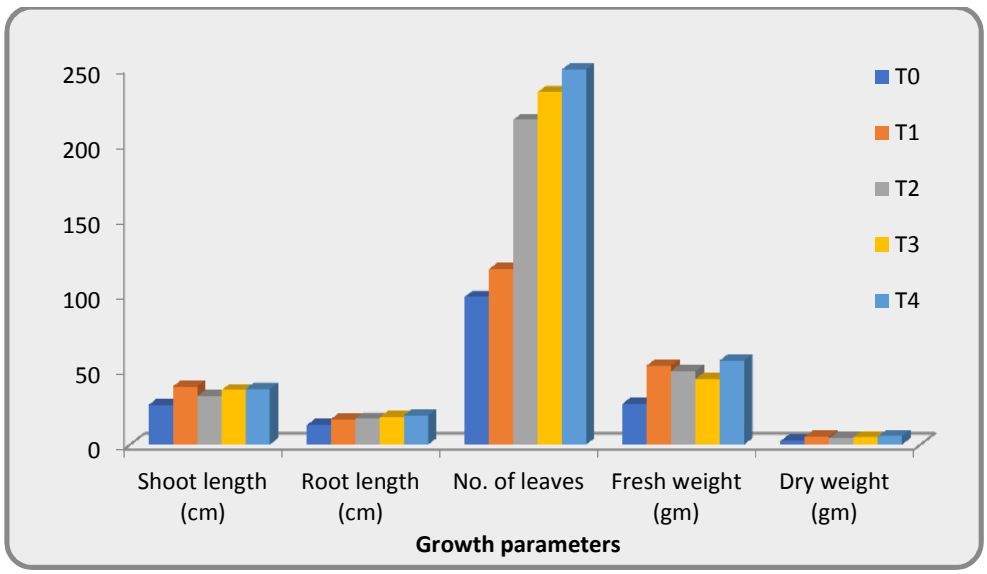


Fig. 4 Growth parameters *Amaranthus tricolor* (L.) using different organic fertilizers on the 30th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Plate 7- Growth of *Amaranthus tricolor* (L.) on 45th day

Table 5: Growth parameters *Amaranthus tricolor* (L.) using different organic fertilizers on the 45th day

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh weight (gm)	Dry weight (gm)
T_0	67.10 ± 6.27	21.43 ± 0.68	289.33 ± 9.87	289.33 ± 9.87	12.02 ± 0.53
T_1	73.17 ± 4.95	26.77 ± 2.73	293.67 ± 85.56	293.67 ± 85.56	18.34 ± 1.32
T_2	84.40 ± 2.79	26.67 ± 0.76	453.33 ± 20.13	453.33 ± 20.13	17.05 ± 0.63
T_3	80.67 ± 6.15	26.10 ± 2.88	460.67 ± 50.00	460.67 ± 50.00	17.68 ± 0.72
T_4	**88.90 ± 3.99**	**28.50 ± 2.88**	**562.33 ± 30.01**	**562.33 ± 30.01**	**18.89 ± 0.69**
SEd	4.0890	1.8305	38.6862	38.6862	0.6761
Cd ($p<0.05$)	9.1108	4.0786	86.1986	86.1986	1.5064

Values are mean ± SD of three samples in each group

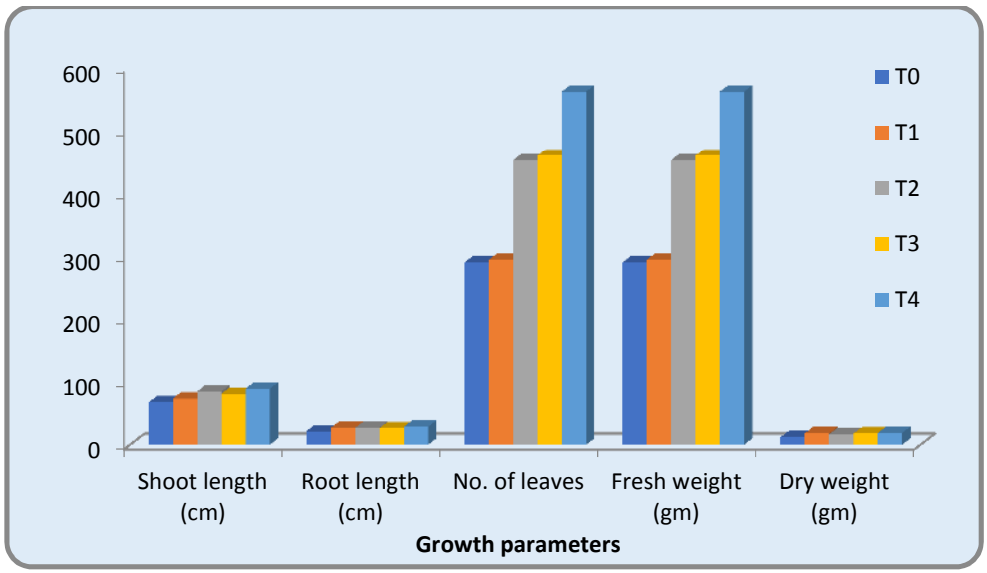


Fig. 5 Growth parameters *Amaranthus tricolor* (L.) using different organic fertilizers on the 45th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

II. **Biochemical parameters**

The biochemical parameters such as chlorophyll '*a'*, chlorophyll '*b'*, total chlorophyll, protein and carbohydrate were analysed on 30th, 45th and 60th day for *Abelmoschus esculentus* (L.) Moenchand on 30th and 45th day for *Amaranthus tricolor* (L.) and tabulated.

1. ***Abelmoschus esculentus*** (L.) Moench

Cholorophyll '*a*', chlorophyll '*b*' and total chlorophyll

Cholorophyll '*a',* chlorophyll '*b'* and total chlorophyll content was found to be higher on the 30th day in plants treated with combination of *Azospirillum*, VAM and Phosphobacteria and the values were 0.0760 ± 0.0135 mg/g and 0.1570 ± 0.0192mg/g (Table 6; Fig. 6) respectively. But, the chlorophyll *'b'* content was observed to more in T_1 i.e., plants treated with *Azospirilllum* (0.1733 ± 0.2166 mg/g).

Similarly, on the 45th day of growth, the chlorophyll *'a'* and total chlorophyll content was observed to be more in T_4 (0.2313 ± 0.0798 mg/g and 0.3587 ± 0.0984 mg/g) respectively. But, the chlorophyll 'b' content was higher in T_2 (0.1230 ± 0.0460) mg/g (Table 7; Fig. 7).

On the 60th day, all the three chlorophyll parameters i.e., chlorophyll '*a',* chlorophyll '*b'* and total chlorophyll was found to be higher in plants treated with combination of organic fertilizers (Table 8; Fig. 8). The values were 0.2087 ± 0.0117 mg/g (chlorophyll '*a*'), 0.1850 ± 0.0078 mg/g (chlorophyll '*b*') and 0.4210 ± 0.0201 mg/g (total chlorophyll).

The studies carried out by Uma Maheswari and Elakkiya (2014) have shown that combined inoculation of liquid bio-fertilizers such as *Rhizobium*, *Azospirillum* and *Azotobacter* could enhance the growth parameters as well as the biochemical constituents.

Seed treatment with the organic fertilizers such as *Azospirillum,* VAM fungi and Phosphobacteria had shown an increase in growth and yield of the vegetable crops taken for study. This result is in accordance with the work carried out by Mounika *et al.* (2017) and Rahimi *et al*. (2009) in Coriander ; Mehta *et al*.,(2012) in fenugreek.

Protein

The protein content was estimated in 0.1 ml and 0.2 ml of the leaf sample on 30th day, 45th day and 60th day. On all the days, the protein content was higher in plants treated with

Phosphobacteria (Table 9; Fig. 9). It shows that the presence of phosphate solubilizing bacteria has an effect on the protein content of the vegetable crop.

Carbohydrarte

The carbohydrate content was estimated on the 30th day, 45thday and 60th day also, the carbohydrate content was more in T_1 (Table 10; Fig. 10).

The beneficial effect of different organic fertilizers on total carbohydrate content has been reported by Hussein *et al.* (2012). They have reported that the benefit may be due to the role of macro and micro nutrients provided by the organic fertilizers which stimulate the metabolic processes and phytosynthetic apparatus resulting in more photosynthesis and carbohydrate synthesis.

Table 6

Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Abelmoschus esculentus* (L.) Moench on the 30th day (mg/g.f.wt)

Treatments	Chlorophyll '*a*'	Chlorophyll '*b*'	Total chlorophyll
T_0	0.0577 ± 0.0075	0.0373 ± 0.0185	0.0910 ± 0.0406
T_1	0.0607 ± 0.0172	**0.1733 ± 0.2166**	0.1067 ± 0.0350
T_2	0.0630 ±0.0087	0.0333 ± 0.0139	0.1017 ± 0.0067
T_3	0.0627 ± 0.0390	0.0473 ± 0.0093	0.1430 ± 0.0066
T_4	**0.0760 ± 0.0135**	0.0710 ± 0.0231	**0.1570 ± 0.0192**
SEd	0.0168	0.0801	0.0211
CD (P<0.05)	0.0375	0.1784	0.0469

Values are mean ± SD of three samples in each group

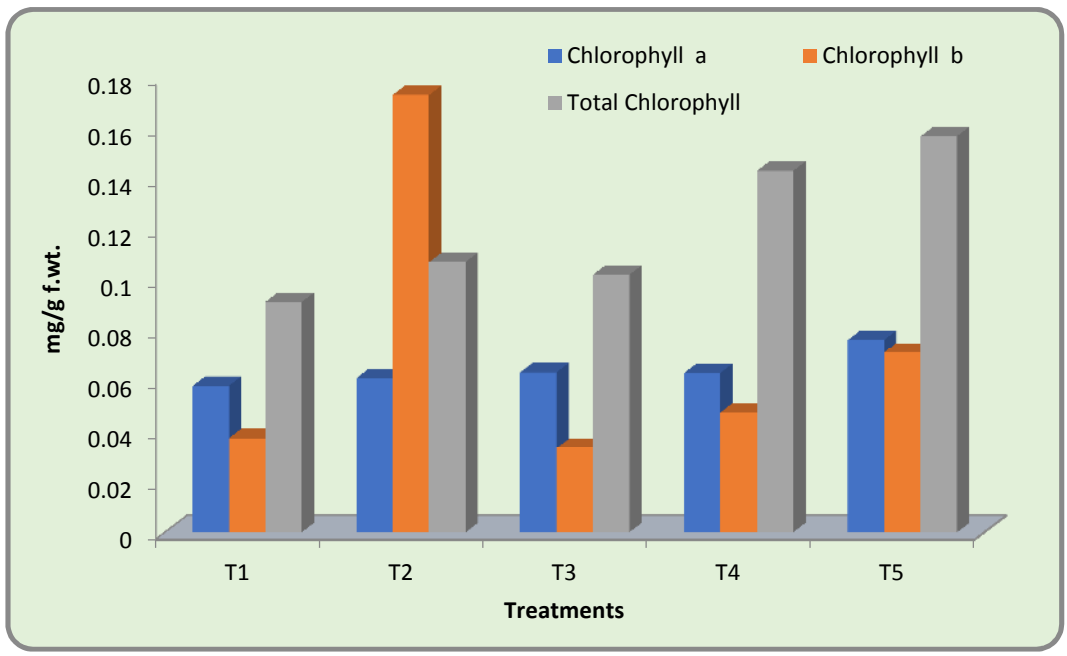


Fig 6: Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Abelmoschus esculentus* (L.) Moench on the 30th day

Table 7: Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Abelmoschus esculentus* (L.) Moench on the 45th day (mg/g.f.wt)

Treatments	Chlorophyll '*a*'	Chlorophyll '*b*'	Total chlorophyll
T_0	0.1007 ± 0.0774	0.0643 ± 0.0140	0.2303 ± 0.0150
T_1	0.1077 ± 0.0517	0.0853 ± 0.0249	0.2627 ± 0.0304
T_2	0.1760 ± 0.0452	**0.1230 ± 0.0460**	0.3227 ± 0.0969
T_3	0.1583 ± 0.0303	0.1047 ± 0.0391	0.2840 ± 0.0722
T_4	**0.2313 ± 0.0798**	0.0963 ± 0.0129	**0.3587 ± 0.0984**
SEd	0.0490	0.0248	0.0582
CD (P<0.05)	0.1091	0.0554	0.1297

Values are mean ± SD of three samples in each group

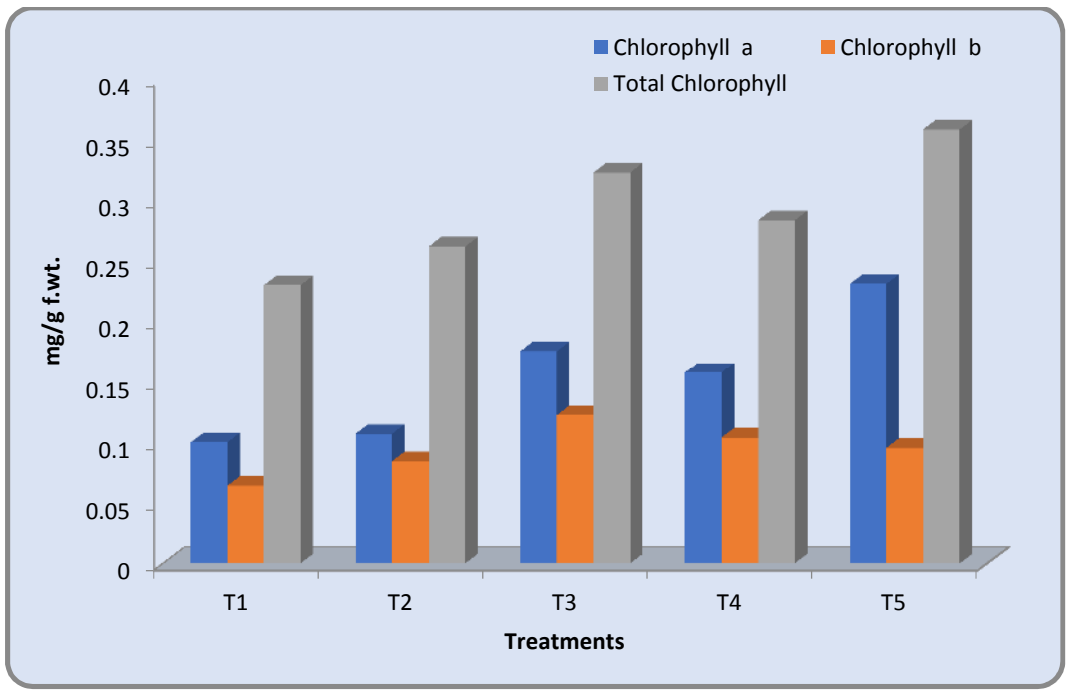


Fig 7: Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Abelmoschus esculentus* (L.) Moench on the 45th day

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Table 8: Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Abelmoschus esculentus* (L.) Moench on the 60th day (mg/g.f.wt)

Treatments	Chlorophyll '*a*'	Chlorophyll '*b*'	Total chlorophyll
T_0	0.1590 ± 0.0030	0.1677 ± 0.0258	0.3410 ± 0.0406
T_1	0.1747 ± 0.0376	0.1793 ± 0.0312	0.3770 ± 0.0662
T_2	0.1937 ± 0.0263	0.1310 ± 0.0330	0.3503 ± 0.0572
T_3	0.1827 ± 0.0437	0.1687 ± 0.0195	0.3753 ± 0.0693
T_4	**0.2087 ± 0.0117**	**0.1850 ± 0.0078**	**0.4210 ± 0.0201**
SEd	0.0236	0.0206	0.0440
CD (P<0.05)	0.0525	0.0458	0.0980

Values are mean ± SD of three samples in each group

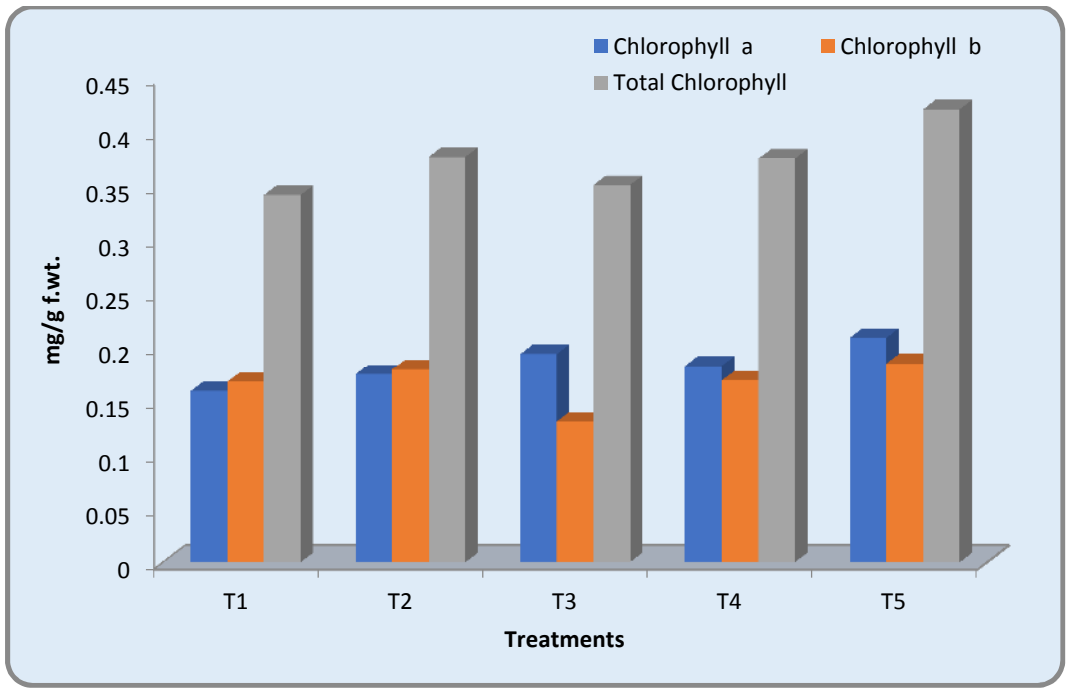


Fig. 8: Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of the *Abelmoschus esculentus* (L.) Moench on the 60th day (mg/g.f.wt)

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Table 9

Protein Content of *Abelmoschus esculentus* (L.) Moench on the 30th day, 45th day and 60th day (mg/g f.wt.)

TREATMENTS	30TH DAY		45TH DAY		60TH DAY	
	0.1	0.2	0.1	0.2	0.1	0.2
T_0	3.10 ± 0.26	2.20 ± 0.17	5.07 ± 0.38	2.73 ± 0.25	5.77 ± 0.06	3.47 ± 0.42
T_1	3.67 ± 0.87	2.40 ± 0.53	5.03 ± 0.81	3.40 ± 0.30	6.53 ± 0.40	4.37 ± 0.25
T_2	3.77 ± 0.76	2.63 ± 0.55	5.53 ± 0.40	3.47 ± 0.45	6.20 ± 0.10	3.73 ± 0.40
T_3	**4.97 ± 0.47**	**3.13 ± 0.06**	**6.13 ± 1.21**	**4.07 ± 0.23**	**8.40 ± 0.17**	**4.73 ± 0.15**
T_4	4.63 ± 0.31	2.83 ± 0.49	6.03 ± 0.55	3.77 ± 0.45	7.40 ± 0.92	4.43 ± 0.38
SEd	0.33830					
CD(P<0.05)	0.67670					

Values are mean ± SD of three samples in each group

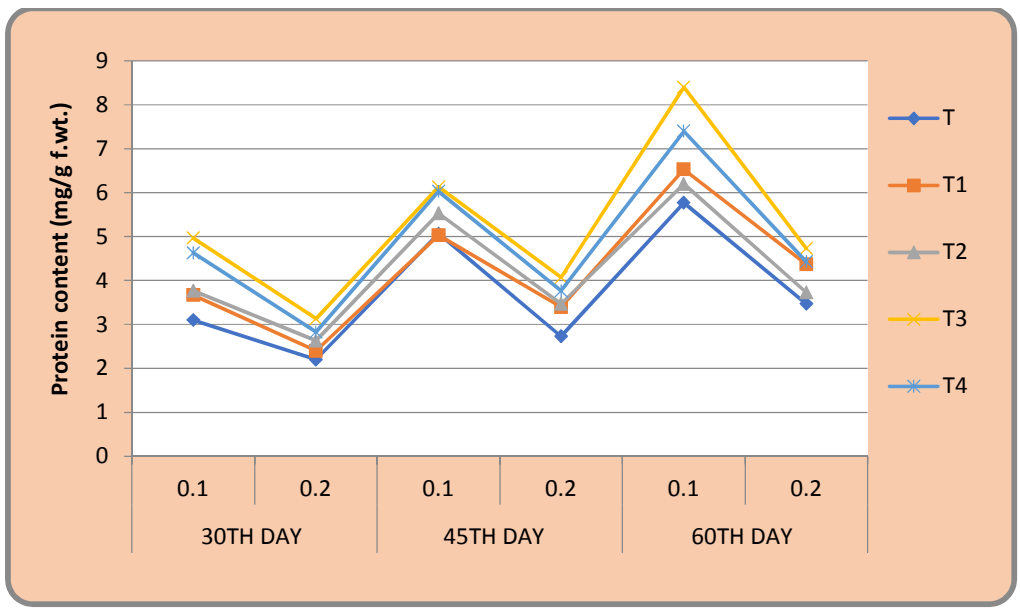


Fig.9 Protein Content of *Abelmoschus esculentus* (L.) Moench on the 30th day, 45th day and 60th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Table 10

Carbohydrates content of *Abelmoschus esculentus* (L.) Moench on the 30th day, 45th day and 60th day (mg/g f. wt)

TREATMENTS	30th day		45thday		60th day	
	0.1	0.2	0.1	0.2	0.1	0.2
T_0	2.37 ± 0.38	1.47 ±0.25	3.60 ±0.40	2.13 ± 0.23	4.23 ± 0.67	2.67 ± 0.55
T_1	**3.20 ± 0.17**	**2.07 ±0.06**	**4.00 ±0.20**	**2.53 ± 0.47**	**5.63 ± 0.78**	**3.87 ± 0.91**
T_2	2.90 ± 0.20	1.90 ±0.26	3.40 ±0.35	2.50 ± 0.20	5.43 ± 1.29	3.67 ± 0.57
T_3	2.53 ± 0.32	1.73 ±0.06	3.50 ±0.20	2.37 ± 0.38	4.57 ± 0.31	3.10 ± 0.46
T_4	2.63 ± 0.35	1.73 ±0.06	3.63 ±0.38	2.13 ± 0.25	4.77 ± 0.42	3.47 ± 0.61
SEd	0.38413					
CD(P<0.05)	0.76838					

Values are mean ± SD of three samples in each group

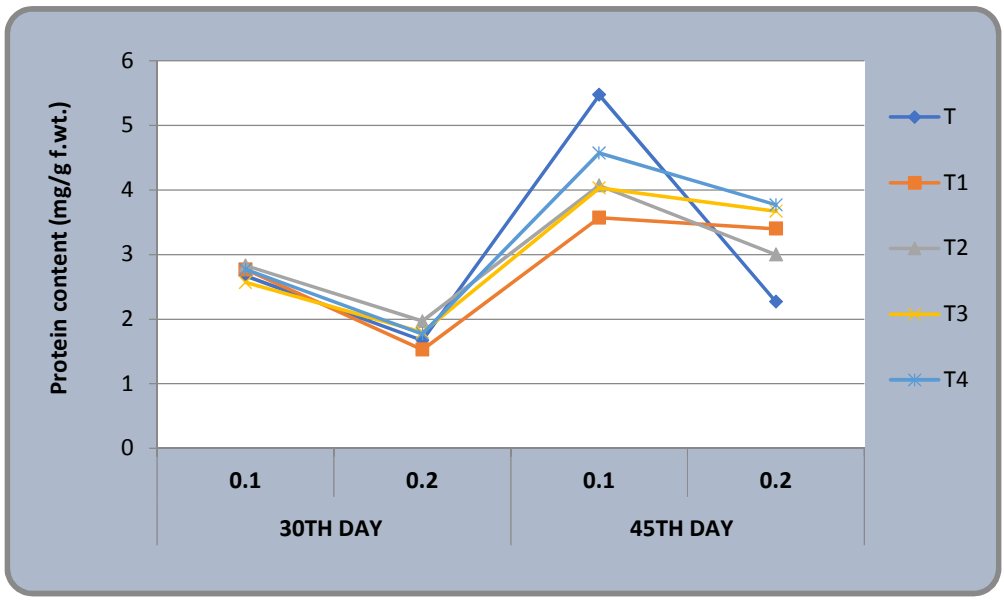


Fig. 10 Carbohydrates content of *Abelmoschus esculentus* (L.) Moench on the 30th day, 45th day and 60th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

2. ***Amaranthus tricolor*** (L.)

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

In *Amaranthus tricolor* (L.), the chlorophyll '*a'* and chlorophyll '*b'* content was found to be higher in T_1 (*Azospirillum* treated plants) on the 30th day (Table 11; Fig. 11) and the values were 0.3723 ± 0.4050 mg/g and 0.3507 ± 0.4844 mg/g respectively. The total chlorophyll content was observed to be more (0.3680 ± 0.0960 mg/g) in T_2 (VAM treated plants).

On the 45th day, all the chlorophyll parameters namely chlorophyll '*a*', chlorophyll '*b'* and total chlorophyll contents were higher in T_4 i.e., plants treated with the combination of organic fertilizers (Table 12; Fig. 12).

Chlorophyll is one of the important pigment content which is used as an index of plant production capacity. The pigment content is an indication of photosynthetic and metabolic activity. The chlorophyll is an integral part of plant pigments and play an important role in the process of photosynthesis. The highest chlorophyll content recorded in *Arachis hypogea* L. in vermicompost and AM fungi applied plants (Lenin *et al.,* 2012) correlate with the result obtained in the present study on lady's finger and Amaranth.

The results of the study carried out by Nalawde and Bhalerao (2015) on the growth of *Vigna* sp showed a significant improvement in the growth parameters. The total chlorophyll content were also found to be significantly higher in treated plants than the control plants.

Protein

The protein content was estimated at two different concentrations on 30th day and 45th day and presented in (Table 13; Fig. 13). The protein content was higher in T_2 on the 30th day and T_4 on the 45th day. The values were significant at 5 % level.

Carbohydrate

The carbohydrate content of *Amaranthus tricolor* (L.) was estimated on the 30thday and 45th day at two different concentration levels, it was found to be higher in T_4 treatment i.e., plants treated with the combination of organic fertilizer. The values were found to be 2.83 ± 0.06 mg/g and 4.63 ± 0.75 mg/g at 0.1 ml concentration on the 30th day and 45th day. Similarly, the higher value of 1.73 ± 0.21 mg/g and 3.77 ± 0.78 mg/g was found in 0.2 ml concentration on the 30th day and 45th day (Table 14; Fig. 14).

Table 11: Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Amaranthus tricolor* (L.) on the 30th day (mg/g.f.wt)

Treatments	Chlorophyll '*a*'	Chlorophyll '*b*'	Total chlorophyll
T0	0.1000 ± 0.0151	0.1017 ± 0.0023	0.1910 ± 0.0217
T1	**0.3723 ± 0.4050**	**0.3507 ± 0.4844**	0.2387 ± 0.0346
T2	0.1640 ± 0.0480	0.1480 ± 0.0957	**0.3680 ± 0.0960**
T3	0.1013 ± 0.0354	0.0417 ± 0.0271	0.1563 ± 0.0386
T4	0.1490 ± 0.0530	0.1520 ± 0.0148	0.3247 ± 0.0561
SEd	0.1508	0.1807	0.0455
CD (P<0.05)	0.3361	0.4025	0.1014

Values are mean ± SD of three samples in each group

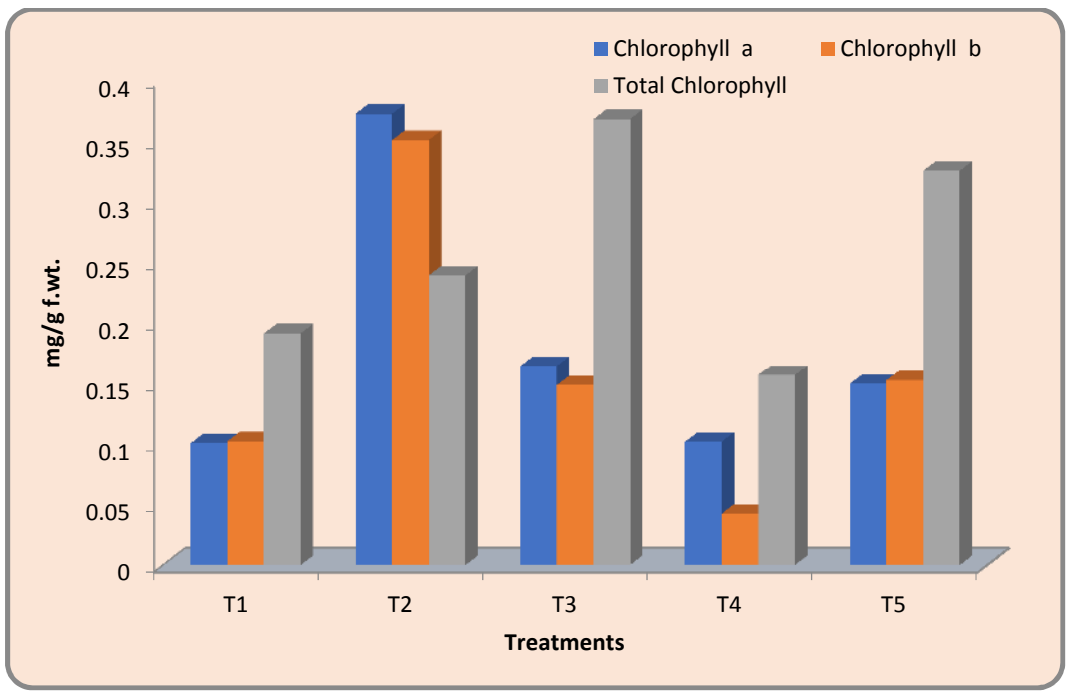


Fig 11: Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Amaranthus tricolor* (L.) on 30th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Table 12: **Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of *Amaranthus tricolor* (L.) on the 45th day (mg/g.f.wt)**

Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll
T0	0.1533 ± 0.0155	0.1133 ± 0.0253	0.2867 ± 0.0206
T1	0.2430 ± 0.0471	0.0787 ± 0.0200	0.3543 ± 0.0541
T2	0.2023 ± 0.0310	0.1287 ± 0.0049	0.3543 ± 0.0345
T3	0.1627 ± 0.0177	0.1240 ± 0.0508	0.3337 ± 0.0354
T4	**0.3063 ± 0.0127**	**0.1420 ± 0.0090**	**0.4883 ± 0.0095**
SEd	0.0228	0.0223	0.0280
CD (P<0.05)	0.0508	0.0496	0.0624

Values are mean ± SD of three samples in each group

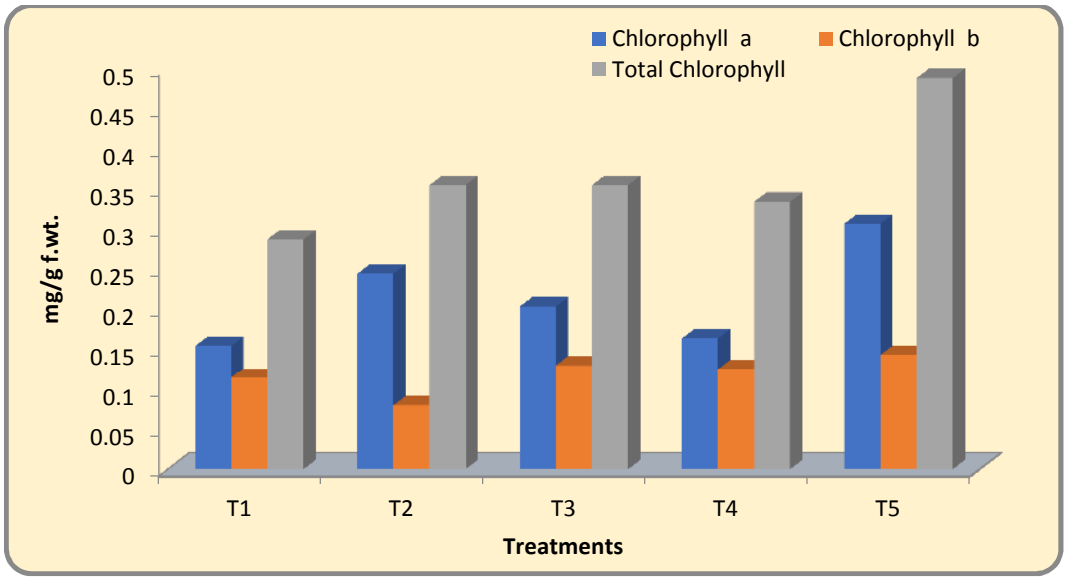


Fig 12: **Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll content of the *Amaranthus tricolor* (L.) on 45th day**

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

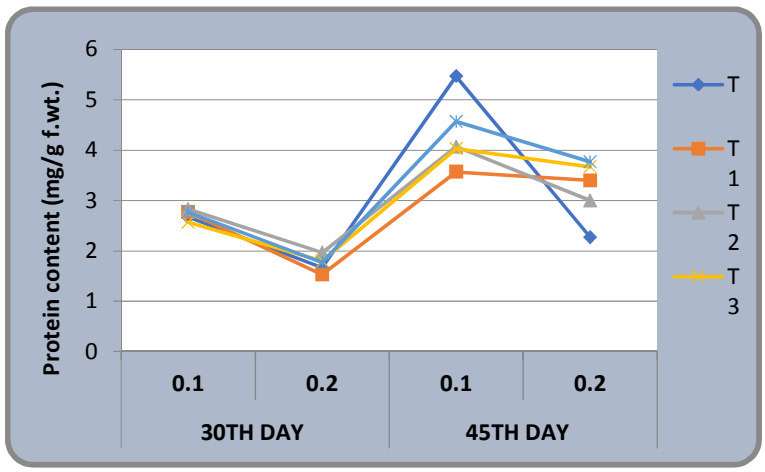
T_4 – *Azospirillum* + VAM + Phosphobacteria

Table 13

Protein Content of *Amaranthus tricolor* (L.) on the 30th day and 45th day (mg/g f.wt.)

TREATMENTS	30TH DAY		45TH DAY	
	0.1	0.2	0.1	0.2
T0	2.67 ± 0.21	1.67 ± 0.15	5.47 ± 0.31	2.27 ± 0.12
T1	2.77 ± 0.15	1.53 ± 0.31	3.57 ± 0.40	3.40 ± 0.10
T2	**2.83 ± 0.68**	**1.97 ± 0.21**	4.07 ± 0.31	3.00 ± 0.30
T3	2.57 ± 0.21	1.80 ± 0.10	4.03 ± 0.68	3.67 ± 0.78
T4	2.77 ± 0.21	1.77 ± 0.15	**4.57 ± 0.85**	**3.77 ± 0.78**
SEd	0.34960			
CD(P<0.05)	0.70659			

Values are mean ± SD of three samples in each group



Protein Content of *Amaranthus tricolor* (L.) on the 30th day and 45th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Table 14: Carbohydrate content of *Amaranthus tricolor* (L.) on the 30th day and 45th day (mg/g f. wt)

TREATMENTS	30th day		45th day	
	0.1	0.2	0.1	0.2
T0	2.13 ± 0.47	1.47 ± 0.15	2.80 ± 0.10	1.73 ± 0.15
T1	2.40 ± 0.10	1.57 ± 0.21	2.87 ± 0.06	1.83 ± 0.06
T2	2.53 ± 0.21	1.70 ± 0.10	3.93 ± 0.42	2.63 ± 0.40
T3	2.37 ± 0.15	1.57 ± 0.12	4.03 ± 0.68	3.67 ± 0.78
T4	**2.83 ± 0.06**	**1.73 ± 0.21**	**4.63 ± 0.75**	**3.77 ± 0.78**
SEd	0.31868			
CD(P<0.05)	0.64409			

Values are mean ± SD of three samples in each group

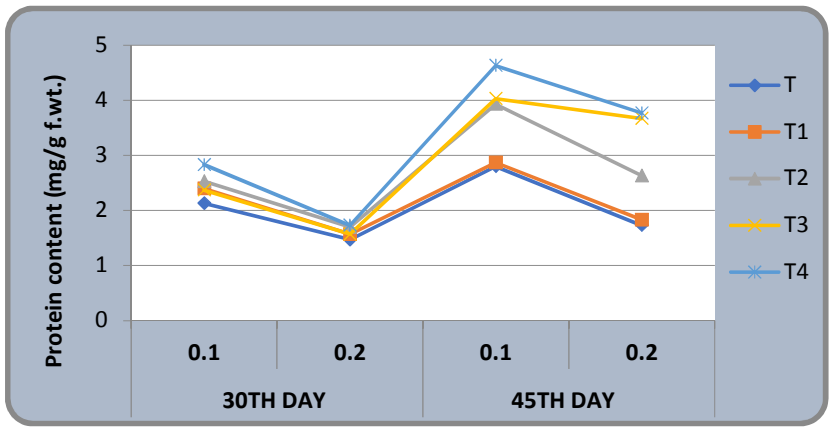


Fig 14: Carbohydrate content of *Amaranthus tricolor* (L.) on the 30th day and 45th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

Bio-fertilizers are used to hasten the biological activity of the plants to improve the availability of plant nutrient (Kumari *et al*., 2015). 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.

The results on the application of microbial inoculants to onion produced maximum plant height, number of leaves per plant and fresh weight of plant. This result is on par with the current study, where the use of organic fertilizers such as *Azospirillum,* VAM fungi and Phosphobacteria has resulted in height growth parameters of lady's finger and amaranth. The current result also correlate with the findings of Rather *et al*. (2003) Yadav *et al*. (2005) and Jha *et al*., (2006).

Earlier studies by Srivastava (2017) have shown that integration of bio-fertilizers significantly improved the yield of Kalmegh.

The higher content of protein and carbohydrate on the 45th day in plants treated with *Azospirillum,* VAM and phosphobacteria in combination indicates that the plants are able to mobilize the phosphorus content from the soil through Phosphobacteria and also grow well with the help of the other organic fertilizers.

III. Yield parameters

The flowers started coming after 35 days of growth. On the 45thday, fruits were formed and it was more in plants treated with the combination of organic fertilizers (T_4). The value was found to be 4.00 ± 1.00. Similarly, on the 60th day, the number of fruits was found to be higher in T_4 (10.33 ± 1.53). The values were found to be significant at 5 % level (Table 15; Fig. 15).

The effect of bacterial bio-fertilizer on the yield of sunflower had shown a positive effect on the growth and yield (Dhanasekar and Dhandepani, 2012). These results are in correlation with the current result on *Abelmoschus* and *Amaranthus.*

Amaranthus is one of the plants that accumulate nitrates especially when soil fertility is very high (Alegbejo, 2013). Green leafy vegetables represent an excellent component of the habitual dot in the tropical and temperate countries (Ashok kumar *et al.*, 2013).

Table 15

Number of Fruits of *Abelmoschus esculentus* (L.) Moench on the 45th day and 60th day

TREATMENTS	NUMBER OF FRUITS 45th DAY	NUMBER OF FRUITS 60th DAY
T0	1.33 ± 0.58	3.33 ± 0.58
T1	1.67 ± 0.58	6.00 ± 1.73
T2	2.33 ± 0.58	7.67 ± 1.53
T3	3.00 ± 1.00	8.67 ± 1.53
T4	**4.00 ± 1.00**	**10.33 ± 1.53**
SEd	0.6992	1.1738
Cd ($p<0.05$)	1.5579	2.6154

Values are mean ± SD of three samples in each group

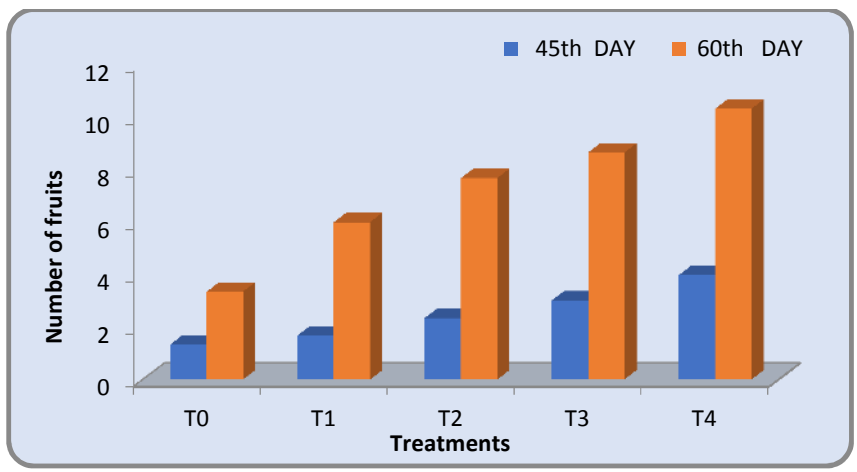


Fig 15: Number of Fruits of *Abelmoschus esculentus* (L.) Moench on 45th day and 60th day

TREATMENTS

T_0 – Control

T_1 – *Azospirillum*

T_2 – VAM

T_3 - Phosphobacteria

T_4 – *Azospirillum* + VAM + Phosphobacteria

IV. Preliminary Phytochemical Screening

Preliminary phytochemical analysis was carried out on the 60th day in *Abelmoschus esculentus* (L.) Moench and on the 45th day in *Amaranthus tricolor* (L.). The solvents used for extraction were water, petroleum ether, chloroform, acetone and ethanol. 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. Abelmoschus esculentus (L.) Moench

The phytochemical tests were carried out for all the treatments on the 60th day in lady's finger. In the extract using water, it was observed that the quinones were completely absent in the plant. Phlobatannins was present only in the control plant. All the other secondary metabolites were observed in the plant (Table 16).

In the leaf extract using petroleum ether, phenol and phlobatannins were absent in the organic fertilizer treated plants. Other secondary metabolites such as alkaloids, tannins, flavonoids, carbohydrates, fats & oil, steroids and terpenoids were present in all the treated plant on the 60th day (Table 17).

In the leaves extracted with chloroform, the alkaloids, tannins, carbohydrates, flavonoids, steroid, terpenoids and fats & oil were present in all the organic fertilizer treated plants. Quinones, phenol and phlobatannins were completely absent in the fertilizer treated plants (Table 18).

When the dried leaves were extracted with acetone, the following secondary metabolites were observed – alkaloids, flavonoids, tannins, phenol, carbohydrates, steroids, terpenoids and fats &oil. Quinones and phlobatannins were completely absent in the plants on the 60th day (Table 19).

The dried leaf powder extract of lady's finger in ethanol showed the presence of alkaloids, tannins, flavonoids, phenol, steroids, terpenoids and fats & oil. Carbohydrates and phlobatannins were seen only in the control plants in acetone extract (Table 20).

Table 16: Preliminary phytochemical Analysis of *Abelmoschusesculentus* (L.) Moench in water extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	+	+	+	+
2	Tannins	-	+	-	+	+
3	Flavonoids	+	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	-	-	-	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

Table 17: Preliminary phytochemical Analysis of *Abelmoschusesculentus* (L.) Moench in petroleum ether extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	+	+	+	+	+
3	Flavonoids	+	+	+	+	+
4	Quinones	+	-	-	-	-
5	Phlobatannins	-	-	-	-	-
6	Phenol	+	-	-	-	-
7	Carbohydrates	+	+	+	+	+
8	Steroids	-	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

Table 18: Preliminary phytochemical Analysis of *Abelmoschus esculentus* (L.) Moench in chloroform extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	+	+	-	+	+
3	Flavonoids	-	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	-	-	-	-	-
6	Phenol	+	-	-	-	-
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

Table 19: Preliminary phytochemical Analysis of *Abelmoschus esculentus* (L.) Moench in acetone extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	-	+	+	+	+
3	Flavonoids	-	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	-	-	-	-	-
6	Phenol	-	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

Table 20: Preliminary phytochemical Analysis of *Abelmoschusesculentus* (L.) Moench in ethanol extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	+	+	+	+	+
3	Flavonoids	-	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	-	-	-	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	-	-	-	-
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

***2. Amaranthus tricolor* (L.)**

The phytochemical tests carried out in *Amaranthus tricolor* (L.) using different solvent extracts showed the following results.

In the leaf powder extracted with water, alkaloid and flavonoids were completely absent. The other secondary metabolites such as tannins, quinones, phlobatannins, phenol, carbohydrates, steroids, terpenoids and fats & oil were present in the leaves of amaranth on the 45th day (Table 21).

In the petroleum ether extract of the leafy vegetable, alkaloid, phlobatannins and terpenoids were completely absent. All the other secondary metabolites were present in the leaves of amaranth (Table 22).

In the chloroform extract of the dried leaf powder, the quinones were completely absent in control plants as well as the organic fertilizer treated plants. The alkaloids, flavonoids, phlobatannins, phenol, carbohydrates, steroids, terpenoids and fats & oil were present in the green leafy vegetable on the 45th day (Table 23).

The acetone extract of the leafy vegetable showed the presence of phenol, carbohydrates, steroids and terpenoids in control as well as in the treated plants. Flavonoids and quinones were completely absent in the acetone extract. Alkaloids, tannins and fats & oil were present in few of the treated plants (Table 24).

The ethanol extract of the leafy vegetable on the 45th day showed the presence of almost all the secondary metabolites except, alkaloid and phlobatannin (Table 25).

Studies on the phytochemical constituents of *Amaranthus tricolor* (L.) have shown the presence of carbohydrates, proteins, aminoacids, steroids, cardiac glycosides, alkaloids, tannins and flavonoids (Pulipati *et al*., 2017).

Earlier studies have shown that medicinal plants respond best to organic source of nutrients which are also environment friendly (Menon and Potty, 1998 and Kurian *et al*., 2000). Vermicompost and FYM might fulfill the nutritional requirements if used appropriately. Inoculation with bio-fertilizers like *Azotobacter / Rhizobium* might increase the productivity by 10-20 % (Gill and Sarlach, 2006).

Sharma *et al.* (2013) have studied the effect of doses of bio-fertilizers on the growth and production of cabbage. Pulipati *et al*. (2017) have carried out research on the total phenol, tannin and flavonoid content of *Amaranthus tricolor* (L.).

Studied on the phytochemical cconstituents of *Amaranthus tricolor* Linn. leaf by Tharun *et al.* (2012) have shown the presence of carbohydrates, tannins and flavonoids in ethyl acetate fraction and steroids in petroleum fraction.

The study on the phytochemical constituents of lady's finger on the 60^{th} day and amaranth on the 45^{th} day showed the presence of a number of secondary metabolites that could be utilized in pharmacology. Further studies are to be carried out to isolate the compound from the plants, so that it could be used in pharmacy.

Table 21: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) in water extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	-	-	-	-
2	Tannins	+	+	+	+	+
3	Flavonoids	-	-	-	-	-
4	Quinones	+	+	+	+	+
5	Phlobatannins	-	+	+	+	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	+	+	+	+
10	Fats and oil	+	+	+	+	+

Table 22: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) in petroleum ether extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	-	-	-	-
2	Tannins	+	+	+	+	+
3	Flavonoids	+	+	+	+	+
4	Quinones	+	+	+	+	+
5	Phlobatannins	-	-	-	-	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	-	-	-	-
10	Fats and oil	+	+	+	+	+

Table 23: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) in chloroform extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	-	+	+	-	+
3	Flavonoids	+	+	+	-	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	+	+	+	+
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	-	+	+	+
10	Fats and oil	+	+	+	+	+

Table 24: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) in acetone extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	-	-	-	-
2	Tannins	-	+	+	-	+
3	Flavonoids	-	-	-	-	-
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	+	+	+	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	+	+	+	+
10	Fats and oil	-	-	-	+	-

Table 25: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) ethanol extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	-	-	-	-
2	Tannins	+	+	+	+	+
3	Flavonoids	-	-	-	-	-
4	Quinones	+	+	+	+	+
5	Phlobatannins	-	+	+	+	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	+	+	+	+
10	Fats and oil	+	+	+	+	+

V. SUMMARY AND CONCLUSION

Fertilizers are commonly used for growing all crops, with application rates depending on the soil fertility. Fertilizers are applied to crops both as solids as well as liquid. In general, organic fertilizers release nutrients over an extended period of time. They act much like the slow-release fertilizers. Organic fertilizers can describe those fertilizers with an organic - biologic -origin i.e., fertilizers derived from living or formerly living materials. Organic fertilizers can also describe commercially available and frequently packaged products that strive to follow the expectations and restrictions adopted by "organic agriculture". The "organic fertilizer" products typically contain both organic materials as well as acceptable additives such as nutritive rock powders, ground sea shells (crab, oyster, etc.), other prepared products such as seed meal or kelp and cultivated microorganisms and derivatives.

Growth Parameters

The growth parameters of tomato plants were measured on the 30^{th}, 45^{th} and 60^{th} day. It was found that the use of combination of organic fertilizers such as *Azospirillum*, VAM fungi and Phosphobacteria showed a higher growth in terms of root length and shoot length on all the days tested.

The number of leaves was higher in plants treated with Phosphobacteria on the 45^{th} day and 60^{th} day. The fresh weight and dry weight was higher in T_4 on 30^{th} and 45^{th} day. On the 60^{th} day, it was maximum in VAM treated plants.

The growth parameters of amaranth were tested on the 30^{th} and 45^{th} day. On the 30^{th} day, shoot length and root length was higher in T_1 and T_4 respectively. The other growth parameters such as number of leaves, fresh weight and dry weight were higher in T_4 only. On the 45^{th} day, all the growth parameters tested were maximum in plants treated with combination of fertilizers.

Biochemical Parameters

The biochemical parameters such as chlorophyll *a*, chlorophyll *b* and total chlorophyll were estimated on the 30^{th}, 45^{th} and 60^{th} day in lady's finger. On the 30^{th} and 60^{th} day, the chlorophyll contents were found to be maximum in T_4. On the 45^{th} day, chlorophyll b was higher in T_2.

In amaranth, the biochemical parameters were tested on the 30th and 45th day. On the 30th day, chlorophyll a and chlorophyll b was more in T_1 and total chlorophyll was higher in T_2. But, on the 45th day, combination of fertilizers showed better chlorophyll contents.

In lady's finger, the protein content was maximum in plants treated with Phosphobacteria on all the days tested. Carbohydrate content was observed to be maximum in plants treated with *Azospirillum*.

In amaranth, the protein content was higher in T_2 on 30th day and T_4 on 45th day. The carbohydrate content was observed to be maximum on both the days in plants treated with *Azospirillum*, VAM and Phosphobacteria.

The vegetable yield of lady's finger was also maximum in T_4.

Phytochemical Analysis

Preliminary phytochemical screening was done using water, petroleum ether, chloroform, acetone and ethanol for lady's finger on the 60th day and amaranth on the 45th day of growth.

Quinone was completely absent in water extract of lady's finger. Phenol and phlobatannin were absent in petroleum ether extract. Most of the secondary metabolites tested were present in all the solvent extracts used.

In amaranth, alkaloids and flavonoids were absent in water extract, quinone in chloroform and acetone extract. Ethanol extract showed the presence of most of the secondary metabolites tested.

CONCLUSION

Use of bio-fertilizer is needed as an alternative source to bring forth the eco-friendly methods of farming. The extent of benefit from the microorganisms depends on their number and their efficiency, which however, is governed by soil and environmental factors.

Azosprillum might have fixed higher amount of nitrogen in soil and made available to the plants resulting in better uptake of N by plants. VAM or PSB would have caused more mobilization and solubilisation of insoluble P in the soil and improve the availability of phosphorus to plants.

The use of organic sources enhances the absorption and release of macro as well as well as micronutrients and thus ensure their availability to the plant throughout its growing season. Through bio-fertilizers, fertilizer application can be reduced by 50%.

Amaranthus, green leafy vegetable is used due to its antioxidant property. The presence of various phytochemical is responsible for its high antioxidant activity. The plant has to be further investigated to isolate its active constituents.

Traditionally, the boiled leaves of *Amaranthus* are used as laxative, diuretic, anti-gonorrheal, expectorant, to relieve breathing in acute bronchitis. The leaves, shoots, tender stems and grains are eaten as pot herb in sauces or soups, cooked with other vegetables with a main dish or by itself. They are highly nutritious and contain vitamins and minerals.

Amaranth is a multipurpose crop supplying high nutritional quality grains and leafy vegetables for food and animal feed.

Green leafy vegetables are the cheapest of all the vegetables within the reach of poor men, being richest in nutritional value. Green leafy vegetables represent an important proportion of foods with medicinal value. Organic agriculture encourages vigorous management of soil and atmosphere avoiding chemical pollution by chemical fertilizers. It decreases the entry of toxic residues in to the soil by promoting production of fresh, quality foods.

To improve and maintain the productivity of agricultural lands, the integrated approach to determine the most favourable plant. Microorganism interaction is essential. The uses of AM fungi in field for many crops could contribute to the sustainable agriculture systems. AM fungi are known to colonize a number of tropical plants including vegetables.

The advantages and benefits of adopting mycorrhizae in agriculture, allows us to better visualize the scope of this phenomenon at the crop level and inturn, the impact of its long term adoption on the quality of life in the improvement of nutrition, tolerance to stress and resistance against pathogens.

In the present study, the application of *Azosprillum*, VAM fungi and Phosphobacteria separately and in combination gave highest values with regard to growth and biochemical aspects when compared to the control. The increase in vegetative growth due to bio-fertilizer application might be due to the vital role of bacteria present in the applied bio-fertilizer.

Therefore, application of VAM and other bio-fertilizer is suggested to be used in order to improve the effective growth and yield of crop plants. The present conclusion is based on the investigation done using pot culture experiments. Further studies are required under field trial to support the current study.

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