

**Efficacy of Organic, Biofertilizers on the Growth and Yield of *Lycopersicon
esculentum* L. var. PKM 1 and its Effect on Green Synthesis of Silver
Nanoparticles**

**Thesis submitted in
Partial Fulfillment of the
Degree of Master of Philosophy (M.Phil)**

**By
MANJULA DEVI S
(19MPBOF001)
Department of Botany**

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore


February 2021

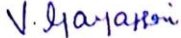
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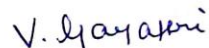

Signature of the Supervisor

CERTIFICATE

This is to certify that the dissertation entitled "**Efficacy of Organic, Biofertilizers on the Growth and Yield of *Lycopersicon esculentum* L. var. PKM 1 and its Effect on Green Synthesis of Silver Nanoparticles**" submitted for the degree of Master of Philosophy (M.Phil) by **S MANJULA DEVI** is the record of research work carried out by her during the period from July 2019 to June 2020 under my guidance and supervision, and that this work has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this University or any other similar Institution of Higher Learning.



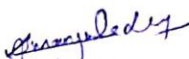
Signature of the Head of the Department



Signature of the supervisor

DECLARATION

I declare that the dissertation entitled "**Efficacy of Organic, Biofertilizers on the Growth and Yield of *Lycopersicon esculentum* L. var. PKM 1 and its Effect on Green Synthesis of Silver Nanoparticles**" submitted by me for the degree of Master of Philosophy (M.Phil) is the record of work carried out by me during the period from July 2019 to June 2020 under the guidance of **Dr. V.Gayathri**, Assistant Professor, Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this University or any University or other similar Institution of Higher Learning.


Signature of the Candidate

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CHAPTER I

INTRODUCTION

Agriculture is the main backbone of Indian economy. It not only provides food and raw materials but, also provides employment opportunities to a very large population of our country. The country has affluent flora and fauna. Both tropical and sub tropical climates provide, ample scope and potentiality for diversification. Human population increasing day by day has resulted in rising demand for food production. Globally, the number of malnourished was unacceptably high in 2010. In India, more than one third of the world's malnourished children are live. According to 2019 report, Global Hunger Index (GHI) ranked India 102nd out of 117 countries with a serious issue of children suffering from malnutrition. According to united Nations Food and Agriculture Organization (FAO) estimations, the average demand for the agricultural commodities will be 60% higher in 2030, than present time and more than 85% of this additional demand will be from developing countries, for over half a century, the world has relied on the concept of increasing crop yields to supply an ever increasing demand of food.

Agriculture production can be increased by increasing the land area which seems to be highly restricted due to industrialization and urbanization and hence can be achieved only through adapting judicial way of applying fertilizers without harming the environment. In agriculture the main reason to use fertilizer is to give full-fledged nutrients supplement of plant growth and environmental quality which usually soil lacks. 35-40 per cent of the crop productivity depends upon fertilizers. The chemical fertilizer contains high soluble nutrient content, which can be immediately absorbed by plant.

In today's era, high amount of chemical fertilizers and pesticides are being used by the farmers to get a better yield of various field crops. But the excess use of chemical fertilizers and pesticides is harmful to humans and plants and leads to some health problem. The uses of such fertilizers increases pathogen, reduces soil microflora, weaken the roots of plants, increase disease incidence, soil acidification and eutrophication of ground water (Chun-Li *et al.* 2014) that has caused tremendous effect to the environment. Chemical nutrients like nitrates leach to ground water and cause Blue Baby Syndrome also called 'acquired methemoglobinemia' (Knobeloch *et al.*, 2000). Such chemicals will have a great impact on the future generations. To overcome the situation, new mechanism must be developed to meet the increased food demands with sustainable food production that has the potential to provide adequate food nutrition without

hampering the fields. The best alternative of the present day environment degradation is to make proper use of the available unutilized organic biodegradable wastes to convert them to compost within a short period through “Biofertilizer”. Biofertilizers application can solve the problem of increased salinity of soil and chemical run off from the agricultural field.

Eco-friendly approaches are gaining popularity with a view on bio-safety of which Biofertilizers play a major role in sustainable agriculture (Kumar *et al.*, 2018). Biofertilizer is a natural product containing living microorganisms that play an important role in maintaining soil fertility and sustainability by fixing atmospheric N that convert nutrients from non soluble form to soluble form which have specific functions to enhance plant growth and reproduction. It is a component of organic farming i.e., Integrated Nutrient Management (INM) system to form sustained agricultural productivity and a healthy environment. Organic farming is a kind of agriculture that create the best possible relationship between the human and earth. The single gram of biofertilizer contains at least 10 million viable cells of a specific strain which is enough to produce enhancing results in plants (Anandaraj and Delapierre, 2010). Crop yield can be increased by 20-30% and chemical fertilizer usage can be reduced due to continuous use of biofertilizers.

Advantages of Biofertilizers

- Biofertilizers are eco-friendly in nature and low cost input, so affordable to farmers
- It leads to soil enrichment and the quality of the soil improves with time
- They increase the phosphorus content of the soil by solubilising and releasing unavailable phosphorus
- Biofertilizers improve root proliferation due to the release of growth promoting hormones (Sahu *et al.*, 2012).

Extensive researches have been carried out on the use of bacteria (*Azospirillum*, *Cyanobacteria* and phosphobacteria), vegetable compost, vermicompost and panchagavya as biofertilizers to supplement nitrogen and phosphorus fertilizers.

Azospirillum belongs to the family Spirillaceae that was found in 1970. It is an inoculant that has widespread distribution in soil and easy to culture and relatively efficient in the utilization of carbon. Apart from N₂ fixation, it stimulates plant growth and productivity. They are associated with roots of mainly C₄ crops having dicarboxylic pathway (Hatch-Slack pathway) of photosynthesis, because they grow and fix nitrogen on the organic salts of malic and aspartic

acid (Mishra and Dash, 2014). Nowadays, the crop production will be increased by the use of seeds treated with fertilizers. *Azospirillum* is used as seed treatment just before sowing without the application in slurry with other products (i.e. micronutrients, fungicides, insecticides, polymers *etc.*). Apart from BNF, it has also been proved as phytohormone and/or siderophore production and phosphate solubilization (Kumar *et al.*, 2018). Generally, the nitrogen requirement of non-leguminous crops such as horticultural crops is met partly from the activities of associative symbiotic bacteria—*Azospirillum* increases the yield and nitrogen economy of vegetables (Mukesh Kumar and Kuldeep Kumar, 2019). Application of liquid *Azotobacter* and *Azospirillum* biofertilizers with reduced recommended dose of nitrogen fertilizers is capable of improving soil health and fertility as well as to achieve more productivity (Reddy *et al.*, 2018). The inoculants of *Azospirillum* are considered significant and commercially viable under the present agricultural situation.

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. Several soil bacteria and fungi eg. *Pseudomonas*, *Bacillus*, *Penicillium* and *Aspergillus* *etc.* secrete organic acids that bring about solubilization of bound phosphates in soil. Increase in the yield of various crops were demonstrated due to inoculation of peat based cultures of phosphobacteria and saving up to 50% of recommended level of P_2O_5 was observed in many experiments. The chilli cultivars (Arka lohit, Arka jwala and Arka anamika) and Brinjal inoculated with Phosphobacteria found to increase crop growth and yield (Rao *et al.*, 2014). Tomato growth, yield and quality parameters such as TSS, ascorbic acid and lycopene contents were higher in plants grown with *Phosphobacteria* and *Azospirillum* (Mukesh Kumar and Kuldeep Kumar, 2019).

Cyanobacteria today are not only explored in agriculture as biofertilizer, but, also in pharmacology, in cancer treatment, bioremediation, as biofuel and also as a food supplement. In other words, we can say that cyanobacteria have ignited a revolution in wide area of health and agriculture (Ananya *et al.*, 2014). They are reported to be the first oxygen evolving organisms on earth. *Cyanobacteria* are free living as well as symbionts with lichens, ferns and cycads and their contribution in total biological nitrogen fixation comes next to Legume-*Rhizobium* symbiosis. *Cyanobacteria* are used as an alternative to chemical fertilizers for attaining the ultimate Goal of

increased productivity, especially in rice cultivation (Sao *et al.*, 2018). It contains 6 to 9% nitrogen; 1-3% phosphorus and 6-8 % ash. Cyanobacterial fertilizers are cost effective for farmers and increases spikelet count in rice field. Cyanobacteria has also attracted attention of environmentalist in bioremediation due to its ability to degrade pollutants including pesticides, crude oil, naphthalene, phenanthrene, phenol and catechol and xenobiotics. They also serve as effective bioabsorbant for removing heavy metals from contaminated water bodies. Not only this, cyanobacteria are effective in solving food crisis and malnutrition. Several strains of cyanobacteria eg *Spirulina*, *Anabaena* and *Nostoc* are consumed as human food in many countries including Chile, Mexico, Peru and Philippines. *Spirulina* as a food supplement serves to provide nutrition requirements and seems to be a ‘wonder food’. It has been stated by NASA that the nutritional value of 1000 kg of fruits and vegetables equals one kg of *Spirulina* (Ananya *et al.*, 2014).

In addition to organic farming, vermicompost provide enough requirements for proper growth of the plant and may enhance the uptake of nutrients. Vermicompost increases the surface area, provides strong absorption and retention of nutrients for a longer period of time. Vermicomposts are products of a non-thermophilic biodegradable organic materials found through interactions between earthworms and microorganisms.

Advantages of vermicompost in the field of vegetables crop (Ahirwar and Hussain, 2015).

- Vermicompost aids in protection of crop plants against pests and diseases.
- The use of vermicompost improves soil structure, texture, aeration and water holding capacity and prevents soil erosion. It will increase the physical and chemical properties of soil.
- It helps in reducing the toxicity of heavy metals.
- Application of vermicompost increases the bioavailability of phosphorus in the soil.
- Vermicompost is free from pathogens, toxic elements, weeds etc.
- It induces hormone like activities in the production of metabolites.

In India, organic farming was a well developed and systematic agricultural practice before the use of vermicompost. The ancient wisdom obtained through Indian knowledge systems such as ‘Vedas’ specify the use of ‘panchagavya’ in agriculture for the health of the plant and soil, as well as human. In South India, the farmers used modified formulation of panchagavya for sustainable agriculture and found them to enhance the biological efficiency of

the crop plants and the quality of fruits and vegetables. Panchagavya is prepared as a foliar nutrition, used widely for various agricultural and horticultural crops. It has been used in traditional Indian rituals. It is also called Cowpathy. The five products obtained from are called 'Gavya' and together termed as 'Panchagavya (Shakuntala *et al.* 2012). It can be applied as a foliar spray, soil application along with irrigation water, seed or seedling treatment etc. Cow dung acts as a medium for the growth of beneficial microbes. Milk gives protein, fat, carbohydrate, amino acid and calcium. Curd contains a *Lactobacillus* bacterium which acts as a catalyst in the digestion of organic wastes. Milk product of Ghee gives vitamin A and B, calcium and fat (Saritha and Vijayakumari, 2013). Cow urine has many properties particularly in the areas of agriculture and therapeutics. Cow urine contains 95% water, 2.5% urea, minerals, salt, hormones and 2.5% enzymes. It also contains iron, calcium, phosphorus, salts, carbonic acid, potash, nitrogen, ammonia, manganese, sulphur, phosphates, potassium, urea, uric acid, amino acids, enzymes, cytokinin, lactose, etc (Bhadauria, 2002).

The increasing anxiety for environmental safety and global demand for pesticide residue free food has suggested a keen interest in crop production using eco-friendly products that do not leave any harmful toxic residues besides conserving nature. Panchagavya, plays an important role in organic product development and provides immunity to the plant system. It contains N, P, K, S, Fe and Zn. Therefore balanced nutrition may have resulted in better development and stronger growth. Panchagavya is known to contain beneficial micro-organisms such as *Azospirillum*, *Azotobacter*, *Phosphobacteria* and *Pseudomonas* besides *Lactobacillus* (Yadav and Lourduraj, 2006) which promotes the plant growth parameters.

Among the families of angiosperms, Solanaceae family is well recognized for its economical, medicinal and agricultural importance. There are about 2000 species of *Solanum* in the world that are mainly situated in the tropical and sub-tropical areas. About 21 species of plants are used as herbal medicines. This species contain a rich source of Lycopene, β -carotene, carotenoids, lutein, total phenols, polyphenols, α -tocopherol, potassium and folate, flavonoids, ascorbic acid, dehydro-ascorbic acid, total vitamin C and vitamin E contents, fatty acids and carbohydrates as well as hydrophilic and lipophilic antioxidant activities which have shown pharmacological relevance (Gautam *et al.*, 2012).

Tomato (*Lycopersicon esculentum* Mill.) belongs to the Solanaceae family. It is a self pollinated crop and Peru Equador region is considered to be the centre of origin. Tomato was

introduced in India by the Portuguese. The plant grows to a height of 1–3 meters (3–10 ft) and it has a weak stem that often sprawls over the ground and vines over other plants and it is cultivated in tropics and subtropics of the world. It is being cultivated in kitchen gardens, commercial fields under green house or poly house conditions and soil less culture or hydroponic systems. In addition, tomato consumption leads to decreased serum lipid levels and low density lipoprotein oxidation (Agarwal *et al.*, 2001).

Tomato is one of the popular vegetables of great commercial value and is used in various forms like salad, soup, ketchup, sauce, chutney, pickles, powder, paste, juice, puree, whole canned fruits and also forms an important ingredient in the cocktails known as “bloody mary”. It is believed that consumption of one tomato per day enhances the health status of individuals and considered to be important in diet as it is quite high in nutritive value. It is one of the most important vegetable and ranks second in the world next to potato. It is often called poor man’s orange because of its high nutritive value and available to masses (Meena *et al.*, 2017). Tomatoes, beside being tasty and nutritious, is a good source of vitamin A, C and lycopene content (Ramakrishnan and Selvakumar, 2012). It covers 7.9% of total area grown under vegetable crops and 9% of total vegetable production.

The tomato plants are discarded after harvesting tomatoes because they are considered worthless for industrial processes. The remaining plant materials like leaves and stem contains some bioactive compounds. However, only few researches have focused on tomato leaves. The bioactive substance has antimicrobial, antiviral, and antioxidant compounds, giving them economic value in the food industry (Beltran *et al.*, 2015). The presence of phytochemical compounds such as secondary metabolites and essential oils proved to have ecofriendly biological activity used for various purposes such as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides and food additives.

Phytochemicals, such as phenolic compounds have great sparkle as antithrombotic, anticarcinogenic and anti-inflammatory agents and the presence of phytoalexins are specifically involved in defense mechanism. Flavonoids are structurally diverse nonvolatile plant specialized metabolites and their antimicrobial effect are useful as a food preservative to extend the shelf life and safety to foods. Polyphenols and carotenoids are important because of their contribution to human health with multiple biological effects such as anti-mutagenic and cytoprotective

activities (Ajila and Rao, 2008). The secondary metabolites and essential oils are proved to have ecofriendly biological activity (Ghormade *et al.*, 2011).

Fertilizer plays a major role in agriculture, which could give high yield. Though the research has achieved high productivity, still the nutrient use efficiency is surprisingly low. According to 2014-15 census, India's population is 1.27 billion with the concern of providing food to such a big population there has been a new technology giving more yield in short period. Nanotechnology can enhance crop yield and nutritional values thereby increasing environmental sustainability. Nano fertilizers increase the nutrient use efficiency (NUE) by 3 times and it also provides stress tolerance potential.

Nanotechnology is the study and application of small object which can be rapid growing in the field of science that combines engineering with physics, chemistry and biology and removes the traditional boundaries between them (Ray *et al.*, 2009). The term "Nanotechnology" was given by Norio Taniguchi of the Tokyo Science University in 1974. A nanometer is one billionth (10^{-9}) of a meter and nano refers to a size scale between 1 nanometer (nm) and 100 nm. It has been very useful in improving the growth, yield and health of fruit crops and its most important advantage has been in the form of Nano Fertilizers (NFs), accounting for the majority of research in this field.

Nanomaterial have properties that have made them extremely useful in biosensing, biological labeling, catalysis, antibacterial activity, antiviral activity, drug delivery, antioxidant applications, DNA sequencing and gene therapy in the recent year (Bharathi *et al.*, 2018). Mostly metallic and non-metallic nanoparticles are widely recognized. The non-metallic nanoparticles are carbon, silicon, nitric oxide, chitosan, fullerenes and grapheme oxide, some of the metallic nanoparticles include cobalt, titanium, aluminium oxide, copper, silver, gold, palladium, magnesium, manganese oxide, platinum and zinc oxide. Among these metallic nanoparticles, silver is one of the most commercialized nano-material with five hundred tons of silver nanoparticles production per year (Larue *et al.*, 2014). There are growing concerns over the potential adverse impact of nanoparticles to the environment.

Nano-fertilizers have novel properties that enhance the growth and yield of the plant by supplying one or more nutrients, whereas, nanomaterial-enhanced fertilizers improve the performance of conventional fertilizers. The effect of nanoparticles varies from plant to plant and depends on their mode of application and size. Nanosilver is applied in textiles, home water

purification system, medical devices, cosmetics, electronics and household appliances. It has strong bactericidal and broad spectrum antimicrobial activities and also reduces various plant diseases caused by spore producing fungal pathogens (Nair *et al.*, 2010).

There are many techniques of synthesizing nanoparticles, and they can be largely described as ‘wet or dry’ processes. Wet method of synthesis are frequently referred as “bottom up” synthetic method, since nanoparticles are assembled atom by atom through a process of nucleation while dry method of synthesis known as “top down” method, since they embroil breaking down bulk compound to nanoparticles (Rai *et al.*, 2016). The synthesis of nanoparticle using naturally occurring reagents such as sugars, biodegradable polymers, plant extracts and microorganisms as reductants and capping agents could be considered attractive for nanotechnology (Ahmed and Ikram, 2015). Numerous methods have been exploited for the synthesis of nanoparticles, however many research works aim to apply a biological green nano technique for the synthesis of silver nanoparticles using plant extract, because it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer product. These nanoparticles are characteristically categorized by their size, surface area, shape and dispersity nature. The common procedures implemented in their characterization are UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM) and X-ray diffraction (XRD).

Topical application of chitosan capped AgNPs has been recently shown to accelerate the healing of a burn wound where it decreased the inflammatory reaction and consequently, reduces the length of repair phase (Oryan *et al.*, 2018). AgNPs have been extensively used in the preparation of ointment for burns, dressing for pressure ulcer wound and devices for effective avoidance of microbial infections (Zhou *et al.*, 2016). It is also an essential class of nanomaterial for a range of medical applications that may have probable risks to human health, which have been documented through studies on the genotoxicity and cytotoxicity of AgNPs. In *Allium cepa* assay, the genotoxic potentials of green synthesized AgNPs with the occurrences of chromosomal aberrations have been reported (Yekeen *et al.*, 2017).

Objectives of the study

1. To study the productivity of tomato under various organic and biofertilizer treatments
2. To determine the presence of phytoconstituents in leaf sample of tomato

3. To synthesize silver nanoparticles and characterize them in the test plant using Scanning Electron Microscope
4. To analyze the fluorescent nature of test plant

CHAPTER II

REVIEW OF LITERATURE

Vermicompost

Application of vermicompost significantly enhanced the growth and flowering in *Crossandra udulaefolia* (Gajalakshmi and Abbasi, 2002).

Yadav and Vijayakumari (2003) have proved that application of vermicompost along with other organic manures improve the shoot length, root volume, number of leaves and fruits in chilli.

In strawberry, plant growth and yield significantly increased by the application of vermicompost which includes increase of up to 37% in leaf area and shoot biomass, 40% in flower numbers, 36% in plant runner numbers and 35% in marketable fruit weights by the application of vermicompost (Arancon *et al.*, 2004).

Senthilkumar *et al.* (2004) found that vermicompost \pm NPK fertilizers significantly enhanced rose growth, yield and quality over the untreated control, especially when used in combination.

According to Ismail (2005), vermicompost promotes growth of plants and it has been found to have a favorable influence on all yield parameters of crops like wheat, paddy and sugarcane. The application of vermicompost showed improvement in shoot length, number of leaves and fruits in chilli (Kurumkar *et al.*, 2005).

Natesh *et al.*, (2005) observed maximum plant height of 73.7 cm in chilli by using FYM, Mycorrhiza and Vermicompost.

Hidlago *et al.* (2006) have reported that incorporation of earthworm castings increases plant (including root) growth, stem diameter and flower numbers of marigold.

An improvement of the physical structure of the potting medium, increases the population of beneficial microorganisms which in turn enhances the availability of plant growth influencing substances produced by microorganisms in vermicomposts that have contributed to increased fruit yield in pepper (Arancon *et al.*, 2006).

According to Ansari (2008), the yield of potato was significantly higher in plots amended with vermicompost @ 6 tonnes / ha, whereas, the yield of spinach was considerably higher in plots treated with vermicompost @ 4 tonnes / ha.

Vermicompost has a significant positive effects on flower number, leaf growth and fresh and dry weights in *Petunia hybrida* (Chamani *et al.*, 2008).

Uma *et al.* (2009) have reported highest runners per plant in strawberry with PM + *Azotobacter* + wood ash + vermicompost + oil cake application.

The increase in height of the plant, plant spread, leaf area and number of leaves per plant with the application of bio-fertilizers and vermicompost has been observed by Tripathi *et al.* (2010) in strawberry.

Application of vermicompost at 15% and 20% concentration to chilli pepper showed higher growth parameters. Maximum moisture trapped by plants was 14.05% and 14.52% at 15% and 20% of vermicompost concentration respectively. Thus, 15 to 20% vermicompost treatment was found to be beneficial for plant growth (Narkhede *et al.*, 2011).

Bahrampour (2013) showed that the application of vermicompost @ 15 t/ha significantly (at $p < 0.05$) increased growth, yield and fruit quality of tomato (*Lycopersicum esculentum* var. Super Beta).

Vermicompost can be described as a complex mixture of earthworm faeces, humified organic matter and microorganisms, which when added to the soil or plant growing media increases germination, growth, flowering, fruit production and accelerates the development of a wide range of plant species (Priya and Santhi, 2014).

Jadhav *et al.*, (2014) have reported increase in leaf length (37.59cm), single radish leaf weight (13.20 gm), total no of leaves per plant (10.60), root length (23.11 cm) and weight (89.03 gm) by 1.2 kg vermicompost treatment in radish.

The highest fresh weight (85.4g), dry weight (79.6g) and yield (19924kg/ha) were obtained by the application of 9 ton/ha vermicompost in *Coriandrun sativum* L. (Shirkhodaie *et al.*, 2014).

Vermicompost plays a major role in improving growth and yield of different field crops, vegetables, flowers and fruits (Lekshmanaswamy, 2014).

According to Singh *et al.* (2015), the maximum plant height (20.26 cm), plant spread (25.64 cm), leaf area (97.87 cm²) and number of leaves (54.30) plant⁻¹ in strawberry were obtained in vermicompost + *Azotobacter* + PSB + AM treated plants.

Bindiya *et al.* (2016) have reported maximum growth, yield and yield attributes with good quality cucumber due to incorporation of 2 tons of vermicompost + 50% recommended

dose of fertilizers + biofertilizers (5 kg Azotobacter and 5 kg phosphate solubilizing bacteria) per hectare.

The greatest impact on fruit weight in cucumber due to application of INM [half recommended NPK + 10 tons of FYM + 2 tons of vermicompost + Biofertilizers (4.0 kg Azotobacter + 4.0 kg PSB)] per hectare has been observed by Dash *et al.* (2018).

The application of PSB at the rate of 4 kg/ha + vermicompost 5 t/ha resulted in significant increase in plant height, number of leaves, root length, root weight and root diameter and root yield in Radish (Mali *et al.*, 2018). Kurumkar *et al.*, (2005) have shown maximum carbohydrate content by the application of vermicompost.

Vegetable compost

According to Kibria *et al.*, (2013), the combined application of poultry manure (15t/ha), kitchen waste compost (30t/ha) and NPK (60:30:40 kg/ha) significantly influenced the growth and yield of lady's finger (*Abelmoschus esculentus* L.Moench).

Sharifian *et al.* (2014) observed that 25% and 50% compost significantly increased the plant height of Marigold. Han *et al.* (2016) found that tomato and cucumber residue compost promoted plant growth and improved vegetable yield and quality. The more the amount of compost returned, the better was the effect on plant growth and yield.

Food waste is comprised of organic matter which can be used for composting to convert it to a fertilizer. It is an effective and eco-friendly way of disposing food waste. Leftovers and other food waste from the kitchen can be converted into a highly organic products rich in nutrients that can be used to grow plants. Vegetables, fruit peelings, over-ripened fruits, nuts and egg shells or the remnants can be used for making vegetable compost (Courtney, 2017).

According to Weerasinghe and De silva (2017), plant height of *Zea mays* was increased with 1: 1.5 soil:compost ratio. Municipal solid waste (MSW) is largely made up of kitchen and yard waste and its composting has been adopted by many municipalities.

Compost from decomposed organic material can be produced when bacteria in soil break down garbage and biodegradable trash, resulting in a product rich in minerals that is an ideal garden amendment. It can be added to the soil any time of the year without the fear of pollution (Vinje, 2019).

Azospirillum

Azospirillum was found to fix more nitrogen and produces more grain yield in pearl millet (Tilak, 1995). Similar growth increase was recorded in black pepper earlier with combined inoculation of bio-fertilizers such as *Azospirillum*, *Azotobacter* and Phosphobacteria (Bopaiah and Khadeer, 1989).

Rokhzadi (2008) indicated that inoculation with bio-fertilizers containing *Azotobacter* and *Azospirillum* increased plant height, leaf number per plant, fruit mean weight and yield in Black gram.

Application of *Azotobacter* + *Azospirillum* resulted in maximum number of leaves (8.67) per bulb as recorded in onion (Ghanti and Sharangi, 2009). Kandil *et al.* (2011) have studied the effects of inoculation with *Azotobacter* sp. and *Azospirillum* sp. on wheat and observed higher plant height in inoculated wheat plants.

Naserirad *et al.*, (2011) investigated the effect of integrated application of biofertilizers on grain yield of maize. They found that inoculation with biofertilizers containing *Azotobacter* and *Azospirillum* increased plant height, leaf number per plant, fruit weight and yield.

According to Ramakrishnan and Selvakumar (2012), the growth and yield were significantly higher when the bio-fertilizers were inoculated with combined treatment (*Azotobacter* and *Azospirillum*) compared to individual inoculation and control plant in tomato (*Lycopersicon esculentum* Mill.).

Earlier, Khan and Pariari (2012) have shown maximum fruit yield (10.25 t/ha), number of fruits/plant (80.20), fruit length (6.72 cm) and oleoresin content (19.80%) in Chilli by the inoculation of plant with *Azospirillum* and 75% N along with P and K.

The maximum number of branches per plant (6.93), clusters per plant (9.83), flower per plant (40.80), fruit weight (70.24 g) and highest yield (663.60 q/ha) was recorded under the treatment of *Azospirillum* 2 kg/ha in tomato (Meena *et al.*, 2014).

According to Singh *et al.* (2014), application of increasing dose of *Azospirillum* + PSB showed better root growth, cell multiplication, elongation and cell expansion in the plant body which in turn increases the plant height.

The combined application of FYM @ 12.5t/ha, Vermicompost @ 2.5t/ha, *Azospirillum* and PSB @ 2.5t/ha showed better growth and yield in *Capsicum annuum* cv: Suryamukhi (Singh *et al.*, 2014). According to Choudhary *et al.* (2015), significant increase in yield per plant and

marketable yield was recorded by the application of *Azospirillum*+NPK in *Abelmoschus esculentus* L.

Application of 100% RDF in combination with vermicompost and bio-fertilizers (*Azotobacter*, *Azospirillum* and phosphorus solubilizing bacteria) increased the length of vine, number of primary branches per vine, whereas, the number of days of female and male flower appearance and node number to which male and female appear was earliest (Thriveni *et al.*, 2015).

According to Kanaujia and Daniel (2016), the earliness in flowering, larger fruits, high fruit set, high fruit weight, better vine length, branching and yield in Cucumber was observed due to application of bio-fertilizers.

The plant height (125.52cm), number of fruit cluster per plant (18.28) and yield per hectare (465.83q) was recorded maximum by the application of Bio-fertilizers (P solubilizing bacteria @ 2.5 kg/ha and *Azospirillum* @ 2.5 kg/ha) + RDF 100% in tomato (Singh *et al.*, 2017).

Esquivel-Cote *et al.* (2017) have reported the higher fruit yield (1,800 g per plant) of the Juanita tomato cultivar with the inoculation of *Azospirillum* sp. associated with application of 170 kg N ha⁻¹.

According to Vieira (2017), bacteria of the genus *Azospirillum* contribute positively for N₂ fixation, increased nutrients and water absorption, plant hormone production and increased phosphate solubilization, which potentiates plant growth.

Application of FYM 50% + *Azospirillum* 50% and *Azotobacter* 100% at optimum level is quite effective to promote growth, yield and quality of cabbage (Kumar *et al.*, 2017).

Singh *et al.* (2018) have reported that the application of vermicompost (6 t/ha) and PSB (2 kg/ha) and *Azospirillum* (5 kg /ha) increases the height of the plant and number of branches per plant in tomato.

Reddy *et al.* (2018) opined that the higher plant height (51.00 cm) was recorded with the application of 80 kg N/ha + inoculation with *Azotobacter* + *Azospirillum* in tomato.

Application of *Azotobacter* + recommended dose of NPK and *Azospirillum* + recommended dose of NPK (160:90:90 kg/ha) through chemical fertilizers gave better plant growth and fruit yield in Tomato (Kamal *et al.*, 2018).

Kumar *et al.* (2019) investigated the effect of organic nutrients on certain growth and yield attributes of bitter melon. Results of the experiment revealed that the application of Vermicompost @5t/ha and sea weed extract 3% along with *Azospirillum* @2 kg/ha gave

maximum vine length (223.01 cm), number of primary branches (13.39), number of leaves (419.62) and fruit yield (1.17 kg /plant).

Cyanobacteria

Application of cyanobacterial bio-fertilizers to the rice fields continuously for at least 3-4 consecutive seasons maintain cyanobacterial inoculums, high crop yield and reduces the input of chemical fertilizers (Vaishampayan *et al.*, 2001). Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing the rice growth and yield as a natural biofertilizer (Song *et al.*, 2005).

Gurung and Prasad (2005) had found an increase in the grain yield of paddy crop by 14% and straw yield by 10% in field conditions on exclusive inoculation of cyanobacteria. However, *Azolla* and cyanobacterial combination resulted in considerable increase in grain, straw and N content of soil.

Supplementing heterocystous cyanobacteria in soil significantly increased rice growth and improved soil properties (Saadatnia and Riahi, 2009). The use of full dose of synthetic fertilizer, adding cyanobacteria, *Calothrix ghosei*, *Hapalosiphon intricatus* and *Nostoc* sp. along with one third N + P + K synthetic fertilizer, enhanced wheat production in pot experiments (Karthikeyan *et al.*, 2007).

The inoculation of *Nostoc entophyllum* and *Oscillatoria angustissima* with different doses of chemical fertilizer promoted seed germination, growth and metabolic process of peas (Osman *et al.*, 2010).

Among the different cyanobacterial genus, *Anabaena*, *Nostoc* and *Nodularia*, *Anabaena* showed more positive effect on most vegetative characters of tomato plant (Shariatmadari *et al.*, 2011).

Some cyanobacterial species (*Anabaena* sp. Ck1 and *Chroococciopsis* sp. Ck4) were shown to release both cytokinin and IAA, and inoculation of wheat with these species resulted in enhancement of seed germination, shoot length, tillering, number of lateral roots, spike length and grain weight of plant (Hussain and Hasnain, 2011).

Alam *et al.* (2014) opined that the combined application of 50% NPK, FYM and BGA increases the plant height (137.2 cm), weight of 1000 grains (23.833 gm) and yield (20% than control). The application of cyanobacteria with lower level of applied Urea-N was effective in enhancing the growth attributes of rice plant.

Phosphobacteria

Zahir *et al.* (1998) reported that in maize both the qualitative and quantitative characters were significantly increased by phosphate solubilizing microorganisms.

Combined inoculation of *Rhizobium* and *Phosphobacteria* (*Bacillus megaterium* and *Pseudomonas striata*) for red gram, black gram, green gram and Bengal gram increased the grain yield and maximum grain was recorded when combination of rhizobial strain and *Phosphobacteria* with full dose of N and P in red gram was used (Kannaiyan, 1999).

Jain and Trivedi (2005) found that the combined application of *Rhizobium* and PSB resulted in higher seed yield, oil yield and protein content in soybean. Mohan Kumar *et al.* (2005) reported that application of 100 per cent NPKS along with Zn, B, Mo in combination with *Rhizobium* and PSB has improved nodulation and seed yield of soybean.

The higher net returns (Rs. 45,201 ha⁻¹) and B: C ratio (2.66) of groundnut was recorded by the application of FYM (7.5 t ha⁻¹) + *Rhizobium* + PSB + 3% Panchagavya equivalent to 25 kg N ha⁻¹ (Ravikumar, 2009).

Application of biofertilizers along with organic and inorganic inputs under protected conditions resulted in highest fruit yield per vine and total fruit yield per hectare when 75% RDF+ 75% FYM + *Azotobacter* + *Phosphobacteria* + *Trichoderma* was applied (Anjanappa *et al.*, 2012).

According to Selvakumar *et al.*, (2012), the combined application of *Rhizobium*+ *Phosphobacteria* resulted in significant increase in nodule (80.2) in groundnut.

The chilli cultivars (Arka lohit, Arka jwala and Arka anamika) and Brinjal inoculated with *Phosphobacteria* found to increase crop growth and yield (Rao *et al.*, 2014).

Kumari *et al.* (2015) have shown that the morphological parameters like length of plant, number of tillers and yields increased when combined inoculation of *Azotobacter* sp., *Phosphobacteria* and 100% RDF was done in paddy plants.

According to McCarty *et al.* (2017), the plant height (101.6cm), no. of tillers (16 /plant), no. of spikes (14/plant), spike length (12.6 cm) and no. of grains (46/spike) in Wheat was maximum when liquid based *Phosphobacteria* was applied.

The application of biofertilizers like *Azospirillum* and *Phosphobacteria*, inorganic fertilizers has significant and vital effect on growth parameters of *cucurbits* (Kumar *et al.*, 2018).

Tomato growth, yield and quality parameters such as TSS, ascorbic acid and lycopene contents were higher in plants grown with *Phosphobacteria* and *Azospirillum* (Mukesh Kumar and Kuldeep Kumar, 2019).

Panchagavya

Beulah (2001) reported that, the quality parameters *viz.*, crude fibers, protein, ascorbic acid, carotene content and shelf life were also higher under organic manure applied with panchagavya spray in rice.

Panchagavya was tested for different crops such as turmeric, paddy, onion, ginger, sugarcane, banana, vegetables and curry leaf and it was found that it enhances the growth, vigour of crops, resistance to pest and diseases and improvement in keeping the quality of vegetables and fruits (Natarajan, 2002).

Emily (2003), reported that in *Withania somnifera* (L.) Duna, the plant height, dry matter production and leaf area index increased significantly due to spraying panchagavya at 4 % level. Sanwal *et al.* (2005) noted that foliar spray of panchagavya and amritpani at 10 % and use of natural growth promoters resulted in higher dry matter content, pH, ascorbic acid content, total soluble solids, reducing sugars and total sugars, but lower fiber contents in stems and flowers of broccoli.

Yadav *et al.* (2006a and 2006b) reported an increased yield of crop plants with panchagavya application due to enhancement in the biological efficiency of crop plants.

Vennila *et al.* (2008) investigated that application of 100% recommended dose of fertilizer along with panchagavya spray (2%) significantly increased the okra plant height (131.7 cm) and dry matter production (5.90 g plant⁻¹).

Sudhakar (2008) reported that application of panchagavya was found to be cost effective when used at 2000 L ha⁻¹ in different combinations than jeevamruta used on N basis. It was observed that application of panchagavya is one of the cheap and efficient organic substitute for other organic manures like vermicompost in integrated approach for better crop yield and profitability, besides improving the nutrient status of soil.

Ravi Kumar (2009) conducted a field trial at farmer's field, Chintamani during *kharif* 2008 to know the performance of groundnut under integrated organic nutrient supply systems. The results indicated that pod and haulm yields were significantly higher (2304 kg ha⁻¹ and 2695

kg ha⁻¹, respectively) with the application of FYM (7.5 t ha⁻¹) + *Rhizobium* + PSB + 3% Panchagavya.

Panchagavya amended with seaweed extract has biofertilizer potential in increasing the yield of some crop plants (Sangeetha, 2009).

Kumawat *et al.* (2009) also reported that application of panchagavya at both branching + flowering stages resulted in significant higher net return (55,376 ha⁻¹) as well as B: C ratio (2.66) over application of panchagavya either at branching or flowering in Groundnut.

Sangeetha and Thevanathan (2010) noted that spraying the seedlings with seaweed based panchagavya increased the activities of all the three antioxidant enzymes namely superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) in the leaves.

Gore and Sreenivasa (2011) found that panchagavya is an efficient plant growth stimulant that enhances the biological efficiency of crops.

Increased root nodules might be due to the growth enzymes present in panchagavya which favoured rapid cell division and multiplication as reported by Patil *et al.* (2012).

Number of nodules (85.32) and yield parameters like number of pods per plant (22.41), pod weight (11.63 g plant⁻¹), pod yield (2298 kg ha⁻¹), harvest index (38.53), 100 kernels weight (41.98 g) and shelling percentage (70.09 %) were recorded and found to be significantly higher in groundnut under application of foliar spray of panchagavya combined with neem leaf extract (Choudhary *et al.*, 2014).

According to Rao *et al.*, (2015), the use of vermicompost and panchagavya showed maximum plant height (106 cm) and an enhanced yield of chilli fruit crop. The use of panchagavya has enhanced not only the quantity of the yield, but, it has produced chillies without any addition of chemical either in the form of fertilizer or in the form of pesticides.

Ramesh *et al.* (2015) have studied the influence of organic and inorganic fertilizers on growth, yields and early blight disease of tomato. The spray of panchagavya showed less incidence of early blight (22.80%).

Research conducted by Choudhary *et al.* (2017), have revealed that growth attributes of blackgram *viz.*, plant height (35.89 cm), dry matter/plant (7.20 gm/plant) and leaf area index (1.92) and yield attributes of seed yield (801 kg/ha), straw yield (1735 kg/ha) and biological yield (2536kg/ha) were significantly higher with the application of 4% panchagavya.

According to Rajesh and Jayakumar (2013), the highest morphological parameters (plant height 78.6cm, fresh weight 96.4 mg/plant, dry weight 25.3mg/plant on seedling, flowering and yield stages respectively) were recorded in *Abelmoschus esculentus* (L.) Moench sprayed with 3% concentration of panchagavya.

Application of foliar spray of 4% Panchagavya resulted in maximum plant height (54.9 cm), number of nodules/plant (15.67), fresh weight (157.57 mg) and dry weight (52.67 mg) of nodule/plant and stover yield (2483 kg/ha) of chickpea (Panchal *et al.*, 2017).

The germination percentage was found to be higher in seeds treated with Panchagavya solution. The number of fruits per plant and chilli yield were significantly higher in the combined application of RDF + Panchagavya + Jeevamrutha + Beejamrutha (Singh *et al.*, 2018).

Rivero *et al.* (2003) reported a similar behavior in the leaves of the tomato plant, possibly because these parts of the plant are exposed to more direct light and UV radiation than other plant sections and therefore, exhibit greater amounts of phenolic compounds.

The phenolic contents of tomato plants consist of hydroxycinnamic acid and flavonoids that are mainly located in leaves (Toor *et al.*, 2005). Lumpkin (2005) reported that the contents of chlorophyll and carotenoids in tomato plants are strongly affected by the incidence of light and that the concentration of these metabolites is increased by exposure to light.

Ramakrishnan and Selvakumar (2012) showed that *Azotobacter* and *Azospirillum* treated plants had the highest chlorophyll and protein contents. The chlorophyll and protein content was increased in combined inoculation of RDF+ 75% FYM + *Azotobacter* sp. + *Phosphobacterium* sp. treatments and it was 35.97µg/g and 8.90 µg/g (Kumari *et al.*, 2015).

Steroidal alkaloids from tomato have several biological activities, including antiviral, anti-fungal, antibacterial, anti-inflammatory, cholesterol lowering and immune potentiating properties (Singh *et al.*, 2013).

Afreen *et al.*, (2016) found the presence of Carbohydrate, Tannins, Flavonoids, Steroids, Saponins and Alkaloids in Tomato leaves.

Sravanthi and Rao, (2015) reported that tomato extract contain higher amount of phenols 46 mg/g, β-carotene (1.23mg/g) and ascorbic acid (0.21mg/g) that possess increased antioxidant activity.

The photosynthetic pigment content such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of *Abelmoschus esculentus* (L.) Moench were increased when 3% panchagavya was sprayed (Rajesh and Jayakumar 2013).

Silver Nanoparticles (AgNPs)

AgNPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and nonlinear optical behavior (Quang *et al.*, 2013). Shet *et al.*, (2015) noted that the Tomato extract took more time for color change indicating slow formation of silver nanoparticles. Tomato synthesized nanoparticles showed least degree of color change, which showed lesser degree of silver nano particle formation.

Morones *et al.* (2005), have reported that the silver nanoparticles synthesized by *S.trilobatum*, *O.tenuiflorum* extracts were found to have higher antimicrobial activity against *S.aureus* (30 mm) and *E.coli* (30 mm).

AgNPs have been used extensively as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications (Nour *et al.*, 2010).

The particle size of the silver nanoparticles was found to be 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm corresponding to *O.tenuiflorum*, *S.cumini*, *C.sinensis*, *S.trilobatum* and *C.asiatica* respectively. SEM determination of the brown color stable samples showed the formation of silver nanoparticles and well dispersed nanoparticles could be seen in the samples treated with silver nitrate (Logeswari *et al.*, 2015).

Rafiei *et al.* (2018) described the effectiveness of AgNPs against Foot and Mouth disease virus (FMDV), which is a contagious and acute infectious disease of domestic and wild ruminants. Jassim *et al.*, (2019) reported the biosynthesis of AgNPs using *Carica papaya* juice as bioreductant. The spherical AgNPs produced has average diameter of 75.68 nm and it was effective as an antibacterial agent against *Escherichia coli*, *Pseudomonas aureus*, *Klebsiella pneumoiae* and *Staphylococcus aureus*.

Abbasi *et al.* (2017) have reported green synthesis of spherical AgNPs of size 7 nm using aqueous extract of dried *Juglans regia* green husk which prevented blood clot within 72 hrs in a dose-dependent manner. Azeez *et al.*, (2017) have reported that AgNPs synthesized using cocoa bean extract displayed LC₅₀ of 44.37µg/ma against *Anopheles gambiae* mosquito larvae.

Kuppusamy *et al.*, (2016) utilized extract of *Commelina nudiflora* to synthesize AgNPs and AuNPs which caused reduction of cell viability and exercised improved cytotoxicity against HCT-116 colon cancer cells with IC₅₀ of 100 and 200 µg/ ml.

Saratale *et al.* (2018) produced AgNPs using extract of *Taraxacum officinale* with elevated cytotoxic activity against human liver cancer cells (HepG₂).

Prabhu *et al.* (2013) demonstrated that AgNPs biosynthesized from leaf extract of *Vitex negundo* inhibited the proliferation of HCT₁₅ colon cell line and induced apoptosis in concentration dependent manner.

The biosynthesized AgNPs showed a significant effect on *Bacopa monnieri* seed germination, induced the synthesis of protein, carbohydrate and decreased the total phenolic content, catalase and peroxidase activities (Krishnaraj *et al.*, 2012).

Salama (2012) reported that the AgNPs concentration of 20, 40 and 60ppm showed stimulation on shoot and root elongation of the bean and corn, but, concentration of 80 and 100 ppm showed inhibition on shoot and root elongation. The AgNPs have both positive and negative effect on root elongation. Suriyaprabha *et al.* (2012) noted that silver nanoparticles have both positive and negative effect on root elongation depending upon plant species. They found that root length increased in barley, but inhibited in lettuce.

Pandey *et al.*, (2014) observed that chlorophyll content increased with increasing concentration of AgNPs. Najafi *et al.* (2014) found that silver nanoparticles improved protein rate of *Phaseolus vulgaris*.

The use of Ag nanoparticles as an alternative to pesticides for control of sclerotium forming phytopathogenic fungi was observed by Nair *et al.* (2010).

Silver nanoparticles, due to their antimicrobial properties have been used most widely in the health industry, medicine, textile coating, food storage, dye reduction, wound dressing, antiseptic creams and possess a number of environmental applications (Gao *et al.*, 2014).

Application of silver nano particles in wheat showed enhanced yield@25ppm (Jhanzab *et al.*, 2015). According to Vannini *et al.* (2013), 10 mg/L of polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs) were found to influence root elongation in *Eruca sativa*. Kaveh *et al.*, (2013) found that the treatment of *Arabidopsis thaliana* plants with 1 or 2.5 mg/L of AgNPs increased the seedling biomass, whereas treatment with higher concentrations were found to decrease the seedling biomass.

The higher germination percentage (73.33%) and highest germination rate (1.59 seeds/day) for watermelon were recorded at 2 mg/ml AgNPs. For zucchini plants, the higher germination percentage (86.67% and 90%) and highest germination rates (1.68 and 1.66 seeds/day) was recorded at 0.5 and 2.5 mg/ml of AgNPs, respectively (Almutairi and Alharbi, 2015).

Germination is important for determining the density of the final plant if plant seeds germinate completely and vigorously (Baalbaki *et al.*, 1990).

SEM image showed that the high density nanoparticle in fresh tomato pomace extract is in the range of 70-80 nm and spherical in shape. These silver nanoparticles showed higher antibacterial activity against *Shigella*, *Pseudomonas* and *Staphylococcus* (Many *et al.*, 2014).

CHAPTER III

MATERIALS AND METHODS

A pot culture experiment was conducted with tomato plant using different organic and biofertilizer treatments. The germination percentage, growth parameters recorded and yield were recorded and statistical evaluations of the data are described in this chapter. Phytochemical and Fluorescence analysis was also carried out using different solvent extracts. Characterization and green synthesis of silver nanoparticles using tomato leaf extract was done.

COLLECTION OF SOIL SAMPLE

Red sandy loam soil samples were taken from Karanampettai of Tirupur District for the pot culture experiment. The soil was cleaned by removing stones and other unwanted materials. Red soil was mixed with sand at the ratio of 5:1 (w/w).

COLLECTION OF SEEDS

The seeds of Tomato (*Lycopersicon esculentum* L. var. PKM 1) were collected from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore.

MORPHOLOGY OF THE PLANT

Lycopersicon esculentum L.

Systematic position

Kingdom: Plantae

Class: Angiosperms

Order: Solanales

Family: Solanaceae

Genus: *Lycopersicon*

Species: *L.esculentum* L.

Common names

Tomato, kamalis and tamatar

Botanical description

1. Tomatoes are native to South America, the plant typically grow to 1-3 meters in height and have a weak stem that often sprawls over the ground.
2. The tomato is consumed in diverse forms, including raw, as an ingredient in several dishes, sauces, salads and drinks.
3. The fruit is rich in lycopene, which has beneficial health effects.



Plate 1: Habit of *Lycopersicon esculentum* L. var. PKM 1

Medicinal uses

- Tomatoes are very healthy as they are good source of vitamin A and C.
- Lycopene is a very important antioxidant that can help prevent the development of many forms of cancer (Polivkova *et al.*, 2010).
- A raw tomato has only about 20% of the total lycopene content found in cooked tomatoes.
- Tomatoes have also been widely used as a natural antiseptic agent, because of its nicotinic acid content.

GERMINATION PERCENTAGE

Seeds of tomato were soaked for 6 hours in different treatments from T₀ to T₉. The seeds were soaked in distilled water. On 7th day, the seeds were tested for germination. The germination percentage was calculated using the following formula (ISTA, 1993).

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

POT CULTURE EXPERIMENT

Pot culture experiment was conducted with the tomato plant. The experiment was carried out in the period from October 2019 to January 2020. 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 5:1(w/w) and filled in pots of 6 kg capacity. A study was undertaken to assess the effect of different organic and biofertilizers on the growth and yield parameters of the test plant.

The seeds were soaked in different organic and biofertilizers for 12 hours. The treatments used for the study are as follows:

Nine treatments (T₁ to T₉) were given as detailed below, which were evaluated against an absolute control (T₀). The readings were taken at every 15 days interval i.e., on 15th day, 30th day, 45th day, 60th Day and 75th Day after sowing the seeds. The fertilizers were used singly and in combination also to observe the change in growth, biochemical and yield parameters.

Treatments

T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria

Organic fertilizers

Organic fertilizers are fertilizers obtained from vegetable matter, human or animal excreta. The organic fertilizers used in the current study are Vegetable compost, Vermicompost and Panchagavya.

Vegetable compost

Vegetable compost is the decomposed organic material obtained from kitchen waste rich in nutrients that is produced when bacteria in soil breakdown the organic wastes. The recommended dose of vegetable compost is 5 tons/hectare. The same dosage was calculated for pot and applied for experimental study.

Vermicompost

Vermicompost is the product obtained by composting organic materials with the help of worms namely earth worms like *Eisenia foetida*. Vermicast is the worm castings produced by these worms after decomposing the organic materials. These vermicompost is an excellent,

nutrient rich organic fertilizer which also acts as a soil conditioner. The process of vermicompost production is known as vermicomposting. The recommended dosage of vermicompost is 5tons/hectare.

Panchagavya

Panchagavya is an organic product which can be used as manure. This is prepared by using five main ingredients cow dung, cow urine, cow milk, ghee and curd. It has the potential to play the role of promoting growth and providing immunity in plant system thereby confers resistance against pest and diseases. 62.5 litre ha⁻¹ of panchagavya is the recommended dose for all the crops.

Biofertilizer

Biofertilizer is a natural product carrying living microorganisms derived from the root or cultivated soil. A small dose of biofertilizer is sufficient to produce desirable results because each gram of biofertilizer contains at least 10million viable cells of a specific strain.

Azospirillum

Azospirillum is known for its nitrogen-fixing and phytohormone production ability. *Azospirillum* helps in fixing atmospheric nitrogen and benefit the host plants by the supply of vitamins and growth hormone. *Azospirillum* inoculation increases the growth, nitrogen uptake and yield in number of crops. The recommended dosage of *Azospirillum* is 5kg/hectare.

Blue-green algae (Cyanobacteria)

Cyanobacteria play an important role in maintaining soil fertility, consequently increasing plant growth and yield as a natural biofertilizer. They are free-living and photosynthetic nitrogen fixers. The recommended dose of cyanobacteria (blue-green algae) is 12.5 kg/hectare.

Phosphobacteria

Phosphobacteria is the organism involved in making the insoluble phosphorus available to the plants. Phosphorus is 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). The recommended dose of phosphobacteria is 10 kg/hectare.

In the growing stages of the plants, the fertilizers were sprayed on the plants and growth was carried out on 15th, 30th, 45th and 60th DAS. The yield parameter was measured on the 60th and 75th DAS.

The infection to the plants by various insects was controlled by spraying neem extract on the leaves of the plants.

Growth parameters

Plant samples were uprooted carefully on 15th, 30th, 45th, 60th and 75th day and the following growth parameters were measured and recorded for all the treatments.

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

Root length (Shukla and Mishra, 1986)

The plants were selected from control and each treatment and washed to get rid of adhering soil particles. Then the length of the root was measured with the help of a scale from root collar point to root tip and expressed in cm.

Shoot length (Shukla and Mishra, 1986)

The shoot length of the plants were measured with the scale from the root collar point to shoot apex and expressed in cm.

Fresh weight of plant (Shukla and Mishra, 1986)

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

Dry weight (Shukla and Mishra, 1986)

The collected plant materials were kept in hot air oven at 70°C for 12 hours. The dry weight was taken using an electronic digital balance and measured in grams.

Number of leaves

The number of leaves of tomato plant in different treatments was recorded at 15 days interval.

YIELD PARAMETER

Number of fruits

The number of fruits forming in the tomato plant in different treatments was recorded once the fruit started.

STATISTICAL ANALYSIS

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

Preparation of leaf Powder

The tomato leaves taken for the phytochemical study were collected, cleaned and air dried, under shade for about three weeks. After drying, the leaves were blended using a household electric blender. This fine powder was analyzed for phytochemical, fluorescent and SNPs characteristics (Plate-2).



Plate 2: Dry powder of *Lycopersicon esculentum* L. var. PKM 1 leaves

Preparation of leaf extract

About 10 g of *Lycopersicon esculentum* powdered sample were weighted separately and different solvents like water, ethanol, petroleum ether, chloroform and benzene was added to it. The solution was kept in a shaker overnight. After 24 hours, the extracts were filtered through Whatmann No.1 filter paper to remove particulate matter and get clear solution and the filtrate was stored at 4°C for further use.



Plate 3: Plant extract of *Lycopersicon esculentum* L. var. PKM 1

PHYTOCHEMICAL ANALYSIS

The leaf powder of tomato was dissolved in various solvents and the preliminary phytochemical tests were carried out using Manju *et al.*, (2013). Phytochemical tests were done to find the presence of the active chemical constituents such as Alkaloid, flavonoids, terpenoids, steroids, protein, carbohydrates, etc in the leaf sample.

Qualitative analysis

The extract obtained was subjected to qualitative chemical tests for identification of various phytoconstituents present in the crude extract. The preliminary phytochemicals analyzed are as follows.

Test for Alkaloids

Mayer's Reagent

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

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 Steroids

Lieberman-Burchard's Test

The extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and five 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

Salkowski Test

Five ml of each extract was mixed with 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown precipitate of the interface indicated the presence of terpenoids.

Test for Phenols

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

Test for Carbohydrates

Molisch's Test

Two drops of Molisch reagent was added to an aqueous or hydrochloric acid solution of the extract and two 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.

FLUORESCENCE ANALYSIS

The leaf powder of tomato plant was extracted in acetic acid, picric acid, H₂SO₄, FeCl₃, 1N HCl, HNO₃, NaOH and H₂O for fluorescence analysis and was observed under ordinary visible light, UV light (254 nm) and UV light of long wavelength (365 nm)

Procedure

- About 500 mg of plant powder was taken into clean dry test tube.
- To each tube, 5ml of different organic solvents such as Hydrochloric acid, Acetic acid, Nitric acid, Sulphuric acid, Acetone were added separately.
- Then, all the tubes were shaken and they were allowed to stand for about 20- 25 minutes.
- The solutions obtained were observed under the visible day light, UV light of short wavelength (254nm) and UV light of long wavelength (365 nm) for their characteristics colour development.

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACT

Preparation of leaf extract

Two samples were taken for testing silver nanoparticle synthesis. About 5g of untreated and tomato plants treated with mixture of biofertilizers were taken for the extract. The samples were transferred into 250 ml conical flask containing 100ml of distilled water and boiled at 60⁰C for half an hour. The extract was then filtered through Whatmann No 1 filter paper to remove the particulate matter and to yet a clear solution.

Synthesis of silver nanoparticles

One mM aqueous solution of silver nitrate was prepared and used for the analysis of silver nanoparticles. One ml of plant extract was added to 20 ml of 1mM aqueous solution of silver nitrate (AgNO₃). The solution was heated for few seconds. Later, the reduction of silver ions was observed by a change in the colour of the solution from yellow to brown.

Characterization of Silver Nanoparticles

The characterization of silver nanoparticles was carried out by different techniques such as SEM, UV-Vis and FTIR analysis.

SEM Analysis

Scanning Electron Microscopy is commonly used method for characterization of silver nanoparticles (Cao, 2004). It is used for morphological characterization at the nanometer to micrometer scale. Sample of SEM was prepared by placing the drop of silver nanoparticle suspension over carbon coated grid then it was dried, examined and photographed in SEM.

EDAX analysis

The sputtering of the sample was done using SC7620 Sputter Coater unit under the nitrogen atmosphere. A small strip of carbon tape was stucked on aluminium stub and a pinch of samples was placed on carbon tape. The stubs were placed in the Sputter coater and sputtered at 10 mV for 60 seconds before FESEM analysis. The elemental analysis and distribution of the particles were carried out using EDAX with SUTW-SAPHIRE model detector.

CHAPTER IV
RESULTS AND DISCUSSION

The experiment was conducted in Tomato (*Lycopersicon esculentum* L. var. PKM 1) with six different organic and biofertilizers and combined treatment application on the growth and yield parameters. All the parameters have been statistically analyzed. Green synthesis of silver nanoparticles using control and combined organic and biofertilizer treatment were tabulated with SEM image. The presence of phytochemicals in tomato leaves were also analysed and the results of the study are given below.

Germination percentage

The higher germination percentage (100%) was obtained in T₄ (*Azospirillum*). The percentage was calculated on 9th day after sowing (DAS) seeds (Table – 1 & Figure – 1).

TABLE 1
INFLUENCE OF DIFFERENT ORGANIC AND BIOFERTILIZERS ON
GERMINATION PERCENTAGE OF TOMATO

Treatment	Percentage (%)
T ₀	60
T ₁	90
T ₂	73
T ₃	69
T ₄	100
T ₅	78
T ₆	50
T ₇	90
T ₈	70
T ₉	80

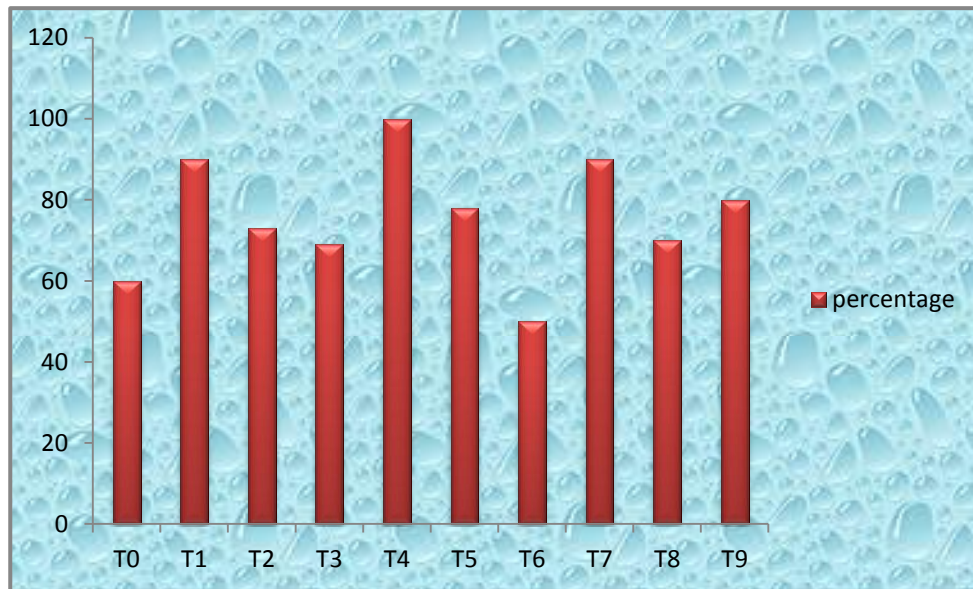




Plate 4: Germination of tomato seeds under various organic and biofertilizers treatments

FIGURE 1

INFLUENCE OF DIFFERENT ORGANIC AND BIOFERTILIZERS ON GERMINATION PERCENTAGE OF TOMATO



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria

Growth Parameters

The growth parameters such as root length, shoot length, fresh weight, dry weight and number of leaves of tomato were analyzed on 15th, 30th, 45th, 60th and 75th day and tabulated (Table 2, 3, 4, 5 & 6) (Fig 2, 3, 4, 5 & 6). Among the treatments, T₅ recorded significantly the highest shoot length of 19.33 ± 4.25cm over the rest of the treatments at 15 DAS. Zodape (2001) have reported that the increase in the yield of wheat by the application of Phosphobacteria might be due to the presence of growth regulator and microelement present in the fertilizer.

TABLE 2
SHOOT LENGTH OF TOMATO PLANT IN CONTROL, ORGANIC AND BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL

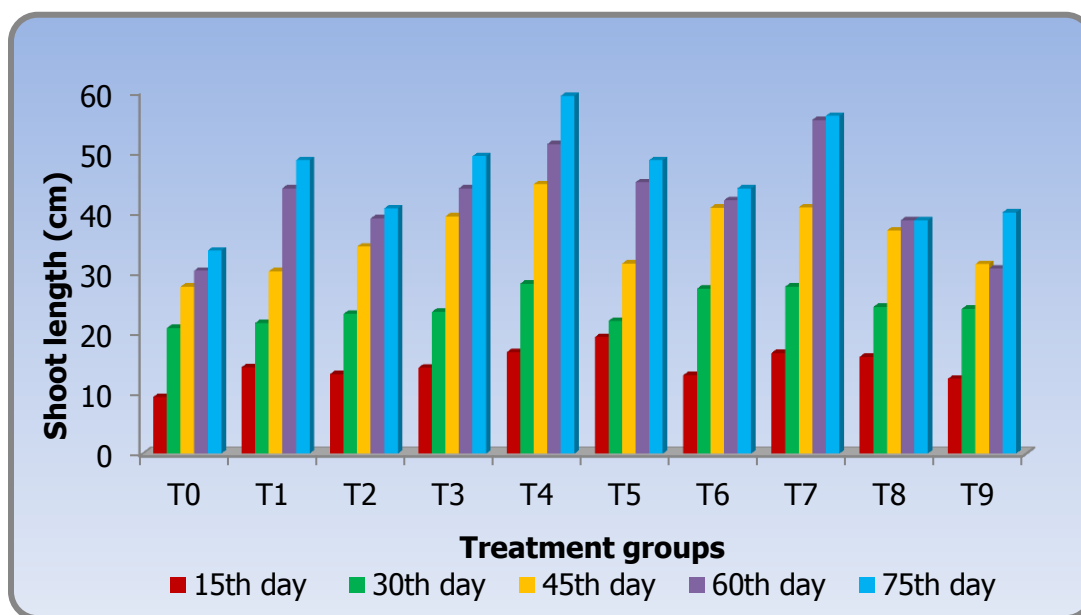
Treatment	Shoot length (cm)				
	15 th day	30 th day	45 th day	60 th day	75 th day
T ₀	9.40±1.15	20.83±2.47	27.67±2.84	30.33±8.14	33.67±7.51
T ₁	14.33±2.08	21.67±3.79	30.23±6.76	44.00±6.08	48.67±6.11
T ₂	13.17±2.47	23.17±3.40	34.33±2.25	39.00±2.65	40.67±3.21
T ₃	14.23±2.91	23.50±4.92	39.33±3.01	44.00±4.00	49.33±3.06
T ₄	16.80±2.25	28.17±6.90	44.67±4.51	51.33±4.16	59.33±4.04
T ₅	19.33±4.25	22.00±1.00	31.50±2.18	45.00±3.00	48.67±4.73
T ₆	13.00±2.88	27.33±4.04	40.77±4.14	42.00±6.08	44.00±4.36
T ₇	16.70±1.47	27.67±3.79	40.83±2.38	55.33±5.51	56.00±3.61
T ₈	16.07±2.10	24.33±2.52	37.00±9.54	38.67±0.58	38.67±3.06

T ₉	12.43±0.98	24.00±2.65	31.43±4.33	30.67±5.86	40.00±4.36
SEd	1.9868	3.1500	3.8780	4.1204	3.7624
CD (P < 0.05)	4.1444	6.5707	8.0894	8.5950	7.8482

The result in the Table 2 clearly indicates that the application of *Azospirillum* gives a positive effect on shoot length on 30 DAS, 45 DAS and 75 DAS and the readings were 28.17±6.90, 44.67± 4.51 and 59.33±4.04 respectively. At 60 DAS, the shoot length was maximum in the combined application i.e., T₇. The use of vermicompost and biostimulant (*Azotobacter* + *Azospirillum*) on the growth and biomass of coriander have been studied by Shirkhodaei *et al.* (2014) and found to be helpful in the development and production of coriander. The minimum shoot length was shown by control (T₀) on 15th, 30th, 45th, 60th and 75th days and the values were 9.40 ±1.15, 20.83±2.47, 27.67 ±2.84, 30.33 ±8.14 and 33.67±7.51 cm.

FIGURE 2

SHOOT LENGTH OF TOMATO PLANT IN CONTROL, ORGANIC AND BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*,
Blue green algae and Phosphobacteria

Root length

Significantly, higher root length was recorded in *Azospirillum* treated tomato plants (14.27±2.40), (21.33±1.53), (22.33±2.52) on the 15th, 60th and 75th day. The combined application of *Azospirillum*+ Cyanobacteria+ Phosphobacteria showed better performance on 15th (12.80±4.93) and 45th (18.10±4.10) day. The effect of organics on the growth, yield and biochemical parameters of chilli have been studied by Singh *et al.* (2014) and they have reported better results by the use of FYM @ 12.5 t/ha + Vermicompost @ 2.5 t/ha + Biofertilizer @ 2.5 Kg/ha *Azospirillum* + PSB. The use of biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirillum* (for nitrogen) and Phosphobacteria, VAM (for phosphorus) to study the response of vegetable crops have shown accumulation of soil enzymes that directly reflects on soil fertility thereby helping in the sustainability of natural soil system (Rao *et al.*, 2014).

TABLE 3

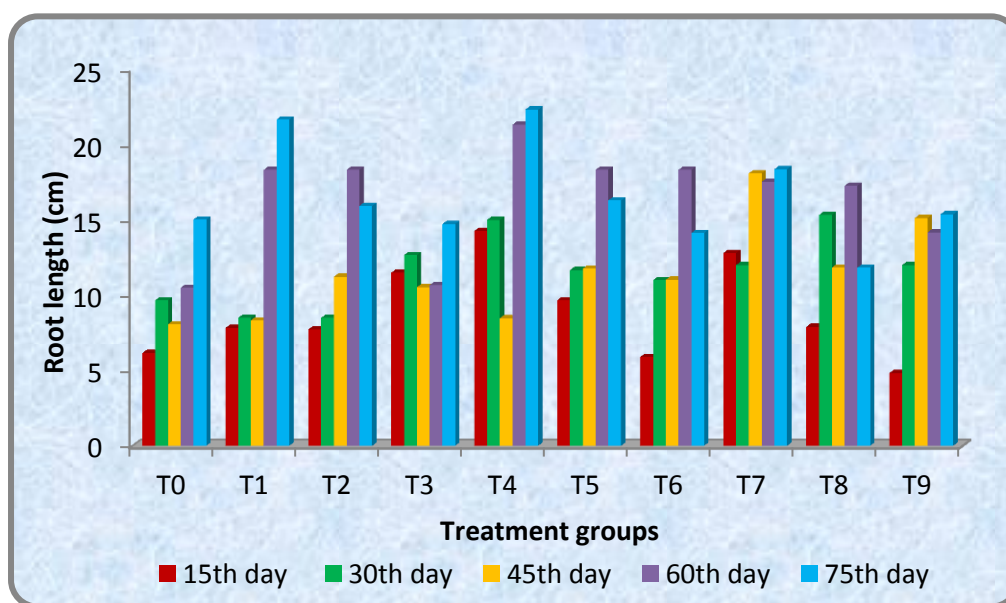
**ROOT LENGTH OF TOMATO PLANT IN CONTROL, ORGANIC AND
BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL**

Treatment	Root length (cm)				
	15 th day	30 th day	45 th day	60 th day	75 th day
T ₀	6.17±1.26	9.67±7.01	8.07±1.10	10.50±2.29	15.03±3.33
T ₁	7.83±2.25	8.50±1.32	8.33±2.08	18.33±1.53	21.67±4.16
T ₂	7.73±0.64	8.50±2.18	11.23±3.80	18.33±2.52	15.93±3.16
T ₃	11.50±1.32	12.67±3.79	10.53±3.40	10.67±1.04	14.73±2.61
T ₄	14.27±2.40	15.00±4.00	8.47±1.55	21.33±1.53	22.33±2.52

T ₅	9.67±2.08	11.67±3.06	11.77±3.29	18.33±2.17	16.30±3.06
T ₆	5.90±3.80	11.00±2.65	11.03±0.75	18.33±1.53	14.13±2.70
T ₇	12.80±4.93	12.00±2.65	18.10±4.10	17.53±1.74	18.37±1.11
T ₈	7.90±3.73	15.33±3.06	11.83±4.65	17.27±0.87	11.83±2.43
T ₉	4.83±2.25	12.00±7.00	15.13±2.80	14.17±2.36	15.37±2.32
SEd	2.2605	3.3425	2.4702	1.5002	2.3196
CD (P < 0.05)	4.7154	6.9723	5.1528	3.1294	4.8387

FIGURE 3

ROOT LENGTH OF TOMATO PLANT IN CONTROL, ORGANIC AND BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria

On 30th day, the root length was maximum (15.33 ± 3.06 cm) in the combined application of Vegetable compost+ Vermicompost+ Panchagavya followed by treated pots (T₄) (*Azospirillum*) (15.00 ± 4.00) cm (Table 3). Earlier Ahirwar and Hussain (2015) have reported an increase in growth, yield and quality of vegetable crops by the application of vermicompost.

Study on various pulses and cereal by Sangeetha and Thevanathan (2010) on the shoot growth, root growth, leaf development and nodule formation showed higher growth rate compared to control by the use of soil amended with dried traditional and seaweed based panchagavya. Earlier Choudhary *et al.* (2014) have shown increase in the yield and quality of groundnut by the foliar application of panchagavya + neem leaf extract at the time of branching and flowering stage. The minimum root length of 8.50 ± 1.32 cm was observed on the 30th day in vegetable compost and vermicompost treated tomato plants. On the 45th day and 60th day, minimum root length was shown by control plant and the readings recorded were 8.07 ± 1.10 and 10.50 ± 2.29 cm respectively.

Number of leaves

The number of leaves was higher in T₁ (Vegetable compost) on 15th day and it was 8.33 ± 0.58 . On 30th day, the number of leaves increased in T₂ (Vermicompost) and T₃ (Panchagavya) 9.67 ± 1.15 . The studies on the effect of vermicompost on the growth and yield of tomato by Gayathri and Vijayalakshmi (2017) have shown that vermicompost increases the quality and quantity of nutrient in the soil, thereby increasing the growth and yield parameters of crop plants. The current study is in accordance with the earlier study by Singh *et al.* (2018) on the growth and yield of tomato.

Rao *et al.* (2015) have compared the yield of chilli when grown with vermicompost as manure and sprayed with panchagavya and without panchagavya and showed better results in terms of length of the plant, number of branches, flowers, number of chilli, length and weight of chilli when foliar application of panchagavya was given. On 45th, 60th and 75th day, it was maximum in T₄ which received *Azospirillum* (13.33±0.58), (25.33±2.52) and (30.67±1.15) (Table 4). Meena *et al.* (2017) have reported combined treatment (75% RDF + 25% FYM + *Azospirillum*) could give a significantly higher morphological as well as maturity parameters in tomato plant.

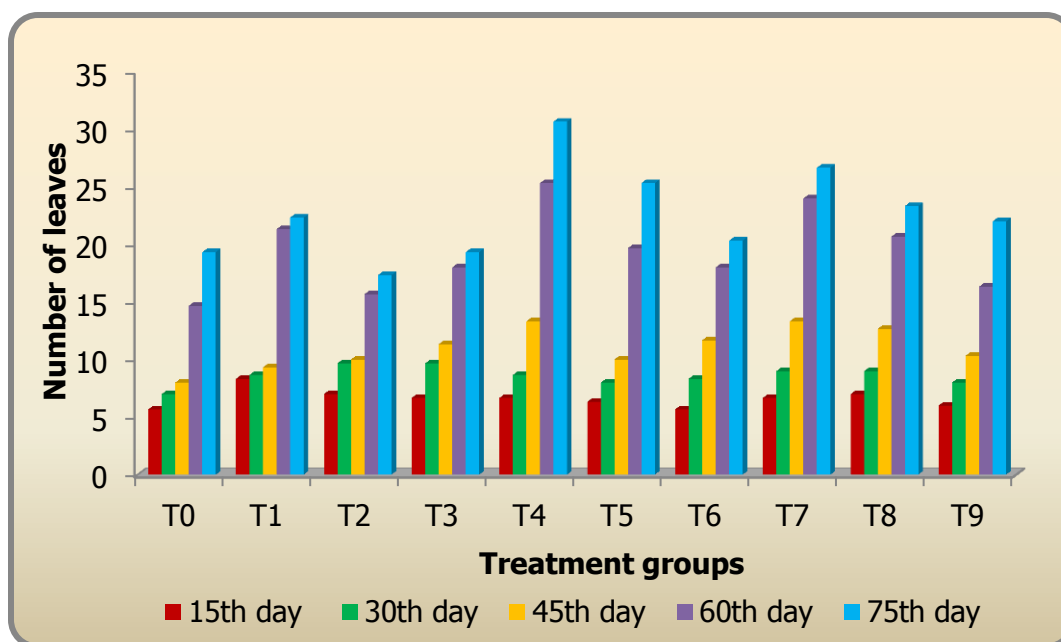
The least number of leaves was observed in control (T₀) plants and the values were 5.67±1.53, 7.00±0.0, 8.00±0.0, 14.67±1.15 and 19.33±3.06 on the 15th, 30th, 45th, 60th and 75th DAS.

TABLE 4
NUMBER OF LEAVES OF TOMATO PLANT PRESENR IN CONTROL, ORGANIC AND BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL

Treatment	Number of leaves				
	15 th day	30 th day	45 th day	60 th day	75 th day
T ₀	5.67±1.53	7.00±0.0	8.00±0.0	14.67±1.15	19.33±3.06
T ₁	8.33±0.58	8.67±1.53	9.33±1.15	21.33±1.15	22.33±2.52
T ₂	7.00±1.00	9.67±0.58	10.00±1.00	15.67±0.58	17.33±2.31
T ₃	6.67±0.58	9.67±1.15	11.33±0.58	18.00±4.36	19.33±4.04
T ₄	6.67±0.58	8.67±1.15	13.33±0.58	25.33±2.52	30.67±1.15
T ₅	6.33±0.58	8.00±0.00	10.00±0.00	19.67±3.51	25.33±4.04
T ₆	5.67±0.58	8.33±0.58	11.67±0.58	18.00±2.00	20.33±3.06
T ₇	6.67±0.58	9.00±1.73	13.33±2.08	24.00±4.00	26.67±1.15
T ₈	7.00±0.00	9.00±0.00	12.67±0.58	20.67±0.58	23.33±3.06
T ₉	6.00±1.00	8.00±1.00	10.33±1.15	16.33±1.53	22.00±2.00
SEd	0.6498	0.8028	0.7888	2.2061	2.2949

CD (P < 0.05)	1.3554	1.6746	1.6454	4.6018	4.7871
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FIGURE 4
NUMBER OF LEAVES OF TOMATO PLANT PRESEN R IN CONTROL, ORGANIC
AND BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria

Fresh weight of the plant

The fresh weight of the plant was higher in T₄ and the values were 2.55±0.52, 17.02±4.84 and 23.07±5.84g on 15th, 60th and 75th day. The use of RDF along with biofertilizers (*Azospirillum* and P-solubilizing bacteria at 2.5 Kg/ha each) could influence the growth and yield of tomato (Singh *et al.*, 2017).

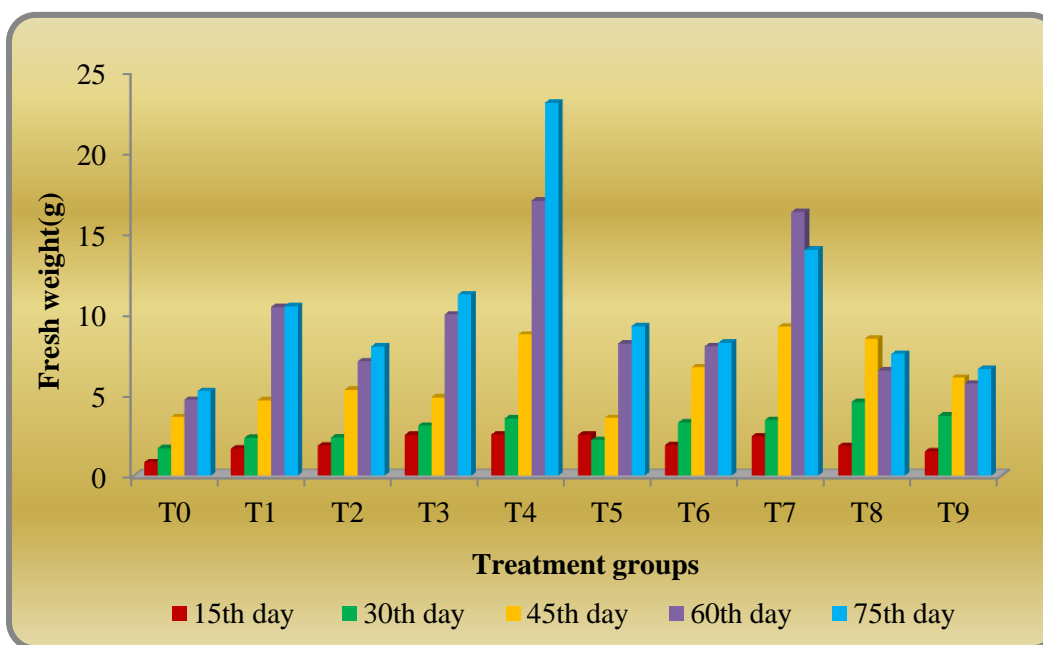
TABLE 5
FRESH WEIGHT OF TOMATO PLANT IN CONTROL, ORGANIC AND
BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL

Treatment	Fresh weight (g)				
	15 th day	30 th day	45 th day	60 th day	75 th day
T ₀	0.83±0.05	1.69±0.43	3.63±0.76	4.70±0.57	5.24±0.78
T ₁	1.68±0.39	2.34±0.67	4.67±2.30	10.44±1.09	10.49±0.87
T ₂	1.86±0.06	2.37±1.01	5.32±1.03	7.08±2.19	8.00±1.54
T ₃	2.52±0.40	3.09±1.08	4.85±1.43	9.98±0.95	11.22±4.57
T ₄	2.55±0.52	3.54±1.47	8.73±0.54	17.02±4.84	23.07±5.84
T ₅	2.54±0.62	2.21±0.13	3.57±0.93	8.18±0.58	9.25±0.53
T ₆	1.89±0.31	3.29±1.36	6.71±1.68	8.01±0.76	8.22±0.45
T ₇	2.43±0.37	3.44±0.31	9.22±1.34	16.32±5.11	13.98±1.89
T ₈	1.84±0.83	4.56±0.65	8.48±0.53	6.52±0.99	7.54±0.53

T ₉	1.50±0.41	3.71±0.33	6.06±2.03	5.70±1.02	6.61±0.92
SEd	0.3717	0.7029	1.1316	1.9933	2.0640
CD (P < 0.05)	0.7753	1.4662	2.3604	4.1580	4.3055

On 30th and 45th day, the fresh weight of the plant was high in the combined application of Vegetable compost+ Vermicompost+ Panchagavya (4.56±0.65g) and *Azospirillum*+ Cyanobacteria+ Phosphobacteria (9.22±1.34g) treatments (Table 5).

FIGURE 5
FRESH WEIGHT OF TOMATO PLANT IN CONTROL, ORGANIC AND
BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria

The use of panchagavya as a novel approach for the sustainable vegetable crops production in Pindar Valley of Uttarkhand has been carried out. Singh *et al.* (2018) have shown that Panchagavya is the best technique to produce residue free crop. It contains macro and micronutrients as well as growth hormones that help in sustainable crop production. Vermicompost incorporation had a significant ($P<0.05$) positive effect on flower numbers, leaf growth and fresh and dry weight of *Petunia hybrida* when compared to both control and peat amended potting media (Chamani *et al.*, 2008).

The minimum fresh weight of the tomato plant was shown by control plants (T₀) and the values were (0.83±0.05), (1.69±0.43), (3.63±0.76), (4.70±0.57) and (5.24±0.78)g on the 15th, 30th, 45th, 60th and 75th DAS.

Dry weight of the plant

Among the treatments from T₁ to T₉, the treatment T₇ which received combined application of *Azospirillum*+ Cyanobacteria+ Phosphobacteria showed maximum dry weight of 0.86±1.20g on 15th DAS. Chittapun *et al.* (2018) have done pot experimental studies on the growth and yield of rice cv Pathum Thani 1 and showed improvement by the use of half of the recommended dose of chemical fertilizer + cyanobacteria as biofertilizer.

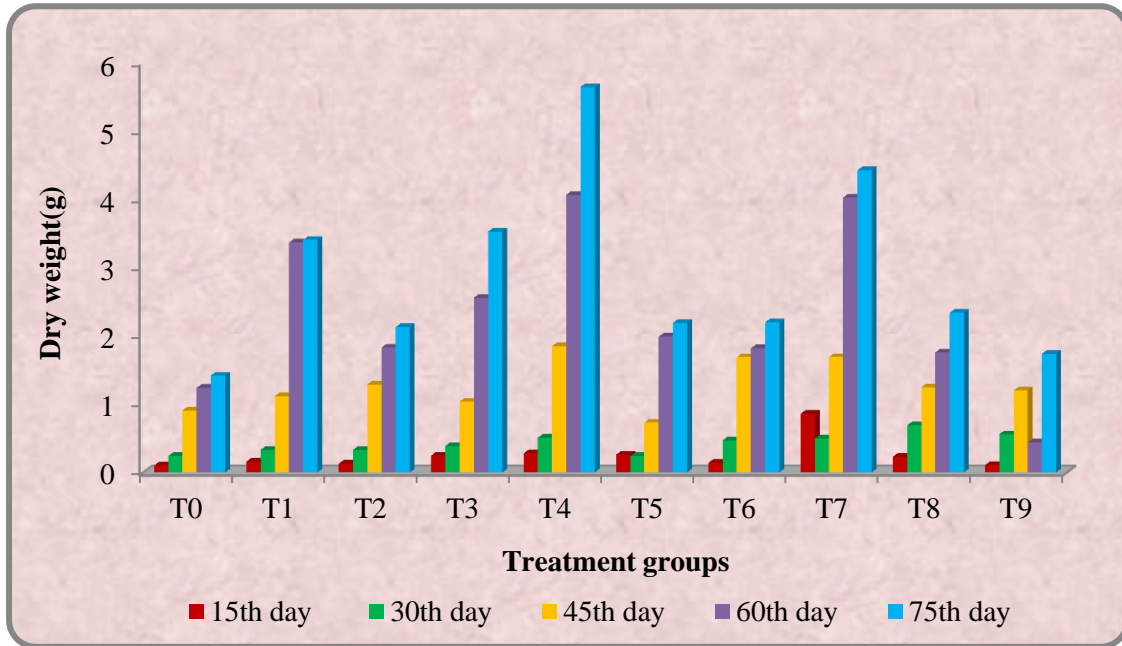
Application of *Azospirillum* gave a positive effect as maximum dry weight on 45th, 60th and 75th DAS and the readings recorded were 1.85±0.13g, 4.07±1.17g and 5.65±1.02g respectively (Table 6). Earlier Tilak (1995) have shown better fixation of nitrogen and production of more grain yield by the inoculation of bio-VAM and *Azospirillum* rather than singly inoculated plants in pearl millet.

On the 30th DAS, dry weight was maximum (0.69±0.14g) in the combined application of Vegetable compost+ Vermicompost+ Panchagavya (T₈).

TABLE 6
DRY WEIGHT OF TOMATO PLANT IN CONTROL, ORGANIC AND
BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL

Treatment	Dry weight (g)				
	15 th day	30 th day	45 th day	60 th day	75 th day
T ₀	0.10±0.03	0.24±0.09	0.91±0.54	1.24±0.24	1.42±0.21
T ₁	0.16±0.05	0.33±0.23	1.12±0.65	3.37±0.37	3.41±0.33
T ₂	0.13±0.09	0.33±0.26	1.29±0.11	1.83±0.89	2.13±1.00
T ₃	0.25±0.01	0.39±0.15	1.04±0.70	2.56±0.68	3.53±1.55
T ₄	0.28±0.08	0.51±0.31	1.85±0.13	4.07±1.17	5.65±1.02
T ₅	0.26±0.10	0.24±0.01	0.73±0.46	1.99±0.18	2.19±0.22
T ₆	0.14±0.07	0.47±0.20	1.69±0.13	1.82±0.14	2.20±0.48
T ₇	0.86±1.20	0.50±0.02	1.69±0.58	4.03±0.63	4.43±0.53
T ₈	0.23±0.14	0.69±0.14	1.25±0.19	1.76±0.56	2.34±0.46
T ₉	0.11±0.03	0.56±0.12	1.20±0.20	0.44±0.36	1.74±0.68
SEd	0.3145	0.1462	0.3527	0.4986	0.6223
CD (P < 0.05)	0.6560	0.3050	0.7358	1.0401	1.2981

FIGURE 6
DRY WEIGHT OF TOMATO PLANT IN CONTROL, ORGANIC AND
BIOFERTILIZERS TREATMENT AT DIFFERENT DAYS INTERVAL



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria



Plate 5: Pot culture experiment showing the growth of tomato plant under various organic and biofertilizers treatments on 15th day



Plate 6: Length of Tomato plant on 15th day



Plate 7: Pot culture experiment showing the growth of tomato plant under various organic and biofertilizers treatments on 30th Day



Plate 8: Length of Tomato plant on 30th Day



Plate 9: Pot culture experiment showing the growth of tomato plant under various organic and biofertilizers treatments on 45th day



Plate 10: Length of Tomato plant on 45th Day

Number of flowers

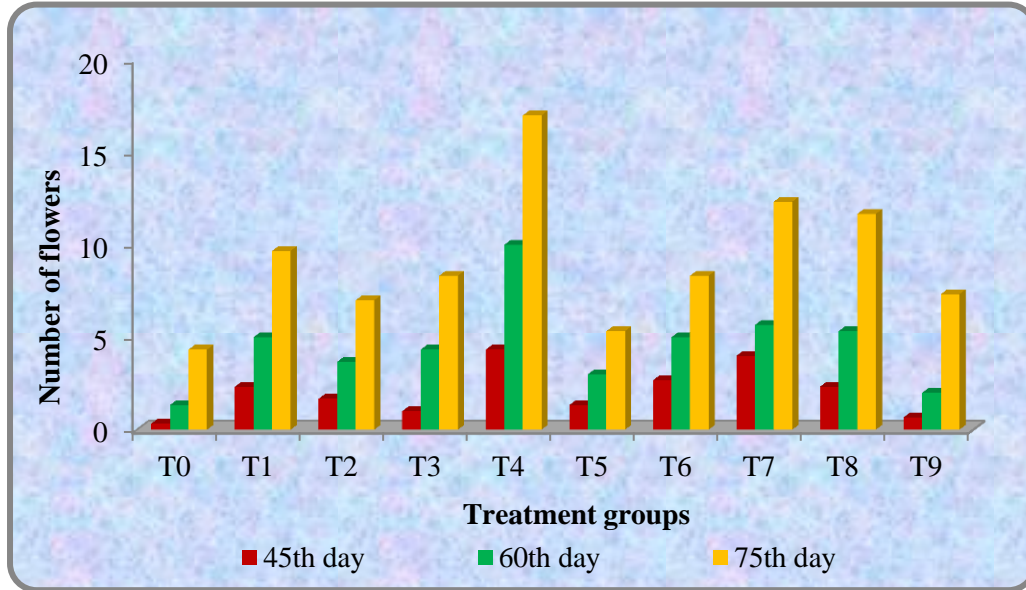
Number of flowers per plant as influenced by application of different biofertilizers and organic fertilizers are presented in Table 7. The number of flowers per plant varied significantly with the treatments. Significantly high number of flowers (4.33 ± 1.53), (10.00 ± 1.00) and (17.00 ± 2.00) was noted in treatment T₄ receiving *Azospirillum* on 45th, 60th and 75th day followed by treatment T₇ which received combined application of *Azospirillum*+ Cyanobacteria+ Phosphobacteria (4.00 ± 1.00), (5.67 ± 1.15) and (12.00 ± 2.52).

TABLE 7
NUMBER OF FLOWERS FORMED IN TOMATO PLANT IN CONTROL, ORGANIC AND BIOFERTILIZERS TREATMENT POTS ON THE 45TH, 60TH AND 75TH DAS

Treatment	Number of flowers		
	45 th day	60 th day	75 th day
T ₀	0.33±0.58	1.33±0.58	4.33±0.58
T ₁	2.33±0.58	5.00±2.00	9.67±4.04
T ₂	1.67±0.58	3.67±0.58	7.00±1.00
T ₃	1.00±0.00	4.33±1.15	8.33±3.06
T ₄	4.33±1.53	10.00±1.00	17.00±2.00
T ₅	1.33±0.58	3.00±1.00	5.33±1.53
T ₆	2.67±1.15	5.00±1.00	8.33±1.53
T ₇	4.00±1.00	5.67±1.15	12.33±2.52
T ₈	2.33±1.15	5.33±1.15	11.67±3.51
T ₉	0.67±0.58	2.00±1.00	7.33±1.53
SEd	0.7149	0.9189	1.9437
CD (P < 0.05)	1.4913	1.9169	4.0544

FIGURE 7

NUMBER OF FLOWERS FORMED IN TOMATO PLANT IN CONTROL, ORGANIC AND BIOFERTILIZERS TREATMENT POTS ON THE 45TH, 60TH AND 75TH DAS



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria

The study by Sahu *et al.* (2012) on cyanobacteria, as potential biofertilizer have shown that the cyanobacterial fertilizers helps in increasing the soil organic matter, stabilizes the soil, releases growth promoting substances, solubilizes the insoluble phosphates and improves the physic-chemical properties of soil, thereby creating an environment friendly agro-ecosystem for farmers. Earlier Mali *et al.* (2018) have concluded that when radish is supplemented with vermicompost and PSB resulted in higher growth and yield of radish.

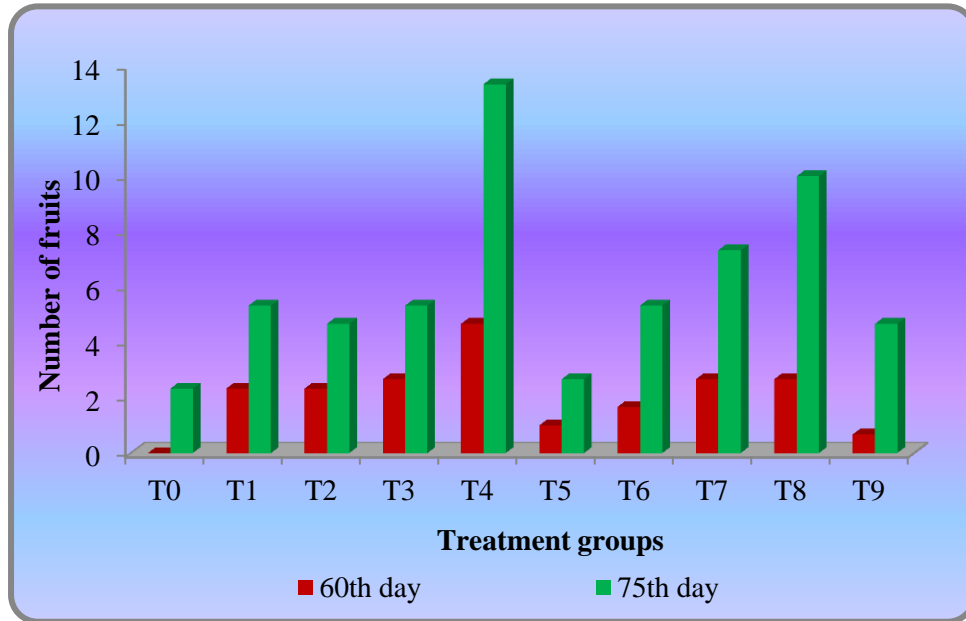
Yield Parameters

The yield parameter of tomato was analyzed on 60th and 75th day and the results are tabulated in Table 8. Number of fruits was more in plant treated with *Azospirillum* (T₄) on the 60th (4.67±0.58) and 75th (13.33±3.06) DAS. Meena *et al.* (2014) have shown a significant increase in fruit weight of tomato cv PUSA SHEETAL by the application of *Azospirillum* at 2 kg/ha than the control plant.

TABLE 8
NUMBER OF FRUITS FORMED IN TOMATO PLANT IN CONTROL, ORGANIC AND
BIOFERTILIZERS TREATMENT POTS ON THE 60TH AND 75TH DAS

Treatment	Number of fruits	
	60 th day	75 th day
T ₀	-	2.33±0.58
T ₁	2.33±1.53	5.33±1.53
T ₂	2.33±0.58	4.67±1.53
T ₃	2.67±1.53	5.33±4.16
T ₄	4.67±0.58	13.33±3.06
T ₅	1.00±0.00	2.67±1.15
T ₆	1.67±0.58	5.33±2.08
T ₇	2.67±0.58	7.33±1.53
T ₈	2.67±1.15	10.00±3.00
T ₉	0.67±0.58	4.67±0.58
SEd	0.7149	1.8074
CD (P < 0.05)	1.4913	3.7702

FIGURE 8
NUMBER OF FRUITS FORMED IN TOMATO PLANT IN CONTROL, ORGANIC AND
BIOFERTILIZERS TREATMENT POTS ON THE 60TH AND 75TH DAS



T₀ – Control

T₁ – Vegetable compost

T₂ – Vermicompost

T₃ – Panchagavya

T₄ – *Azospirillum*

T₅ – Blue green algae

T₆ – Phosphobacteria

T₇ – *Azospirillum*+ Blue green algae+ Phosphobacteria

T₈ – Vegetable+ Vermicompost+ Panchagavya

T₉ – Vegetable, vermicompost, Panchagavya, *Azospirillum*, Blue green algae and Phosphobacteria



Plate 11: Pot culture experiment showing the growth of tomato plant under various organic and biofertilizers treatments on 60th day



Plate 12: Fruits of Tomato on 75th day

Preliminary Phytochemical Analysis of leaf of *Lycopersicon esculentum* L. var. PKM 1

Preliminary phytochemical analysis of leaf extracts (ethanol, chloroform, petroleum ether, benzene and water) of tomato showed the following results.

In the present study, the phytochemical analysis was carried out to detect the active constituents such as alkaloids, flavonoids, steroids, saponins, terpenoids, amino acids, phenols and carbohydrates (Table – 9). Studies by Omodamiro and Amechi (2013) have revealed the presence of carotenoids, phenols, tannin, flavonoids, saponin, phytates in tomato extract. The daily consumption of tomato increases these phytoconstituents in our body thereby helping in preventing or suppressing the oxidative stress related diseases.

TABLE 9
QUALITATIVE PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACT OF TOMATO
USING DIFFERENT SOLVENTS

Test for	Ethanol	Chloroform	Water	Petroleum ether	Benzene
Alkaloids	-	+	-	-	-
Flavonoids	+	+	-	+	+
Terpenoids	+	+	-	+	-
Steroids	+	+	-	+	-
Saponins	-	-	+	-	+
Aminoacids	-	-	+	-	-
Tannin	-	+	-	-	-
Phenols	+	+	+	+	+
Carbohydrate	+	+	-	-	-

‘+’ - Present ‘-’ - Absent

In *L. esculentum* L., among the five solvents used for extraction, ethanol, chloroform, petroleum ether, benzene and water showed the presence of phenols. Alkaloids were observed only in chloroform extract. Flavonoids, terpenoids and steroids were observed in ethanol, chloroform and petroleum ether extract. Flavonoid was present in benzene extract also. Saponins

was present in water and benzene extract. Aminoacid present in water extract and tannin present in chloroform extract. Carbohydrate was observed in ethanol and chloroform extract.

Oz and Kafkas (2017) have studied the phytochemicals in fruits and vegetables and reported that the effect of the processing treatments undergone by food eaten by humans largely depends on the phytochemicals present in them. So, it is necessary to understand the phytoconstituents of fruits and vegetables. The health benefits of these fruits and vegetables are highly dependent on its antioxidant property.

FLUORESCENCE ANALYSIS OF *Lycopersicon esculentum* L. var. PKM 1

The leaf powder of tomato plant was extracted in acetic acid, H₂SO₄, 1N HCl, HNO₃ and H₂O. The fluorescence analysis of the leaf extracts were observed under ordinary visible light and also under UV light short wavelength (254 nm) and long wavelength (365) recorded in Table- 10. The fluorescence analysis of leaf powder of *L.esculentum* showed different colour with the treatment of chemicals. The leaf powder in treatment with acetic acid, sulfuric acid and water showed dark green colour under visible light and leaf treated with hydrochloric acid showed dark green colour under UV long wavelength.

When the leaf powder was treated with hydrochloric acid, acetic acid and H₂SO₄, it showed fluorescent green under UV light short wavelength (254) and in water under long wavelength (365 nm). The leaf powder showed dark brown under UV long wavelength in treatment with acetic and sulfuric acid respectively. Pale blue and Yellowish green was observed under visible light when the leaf powder was treated with HCl and HNO₃ respectively. The leaf powder showed light green and orange colour in leaf powder treated with nitric acid and water under UV light short wavelength. Pale orange colour was found in leaf treated with nitric acid under UV long wavelength.

Table 10: Fluorescence analysis of *Lycopersicon esculentum* L. var. PKM 1

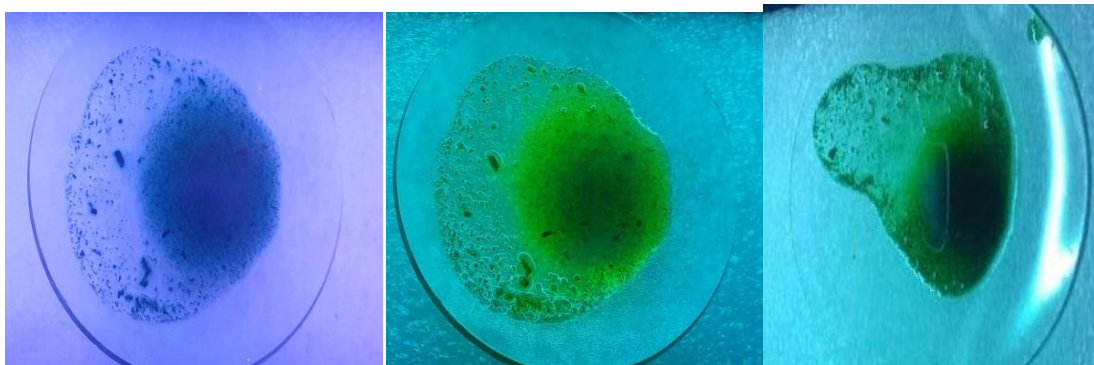
S.No	Treatment with chemical reagent	Visible light	UV Fluorescence	
			254nm	365nm
1	Hydrochloric acid	Pale blue	Fluorescent green	Dark green
2	Acetic acid	Dark green	Fluorescent green	Dark brown
3	Nitric acid	Yellowish green	Light green	Pale orange
4	Sulphuric acid	Dark green	Fluorescent green	Dark brown
5	Water	Dark green	Orange	Fluorescent green

Visible light

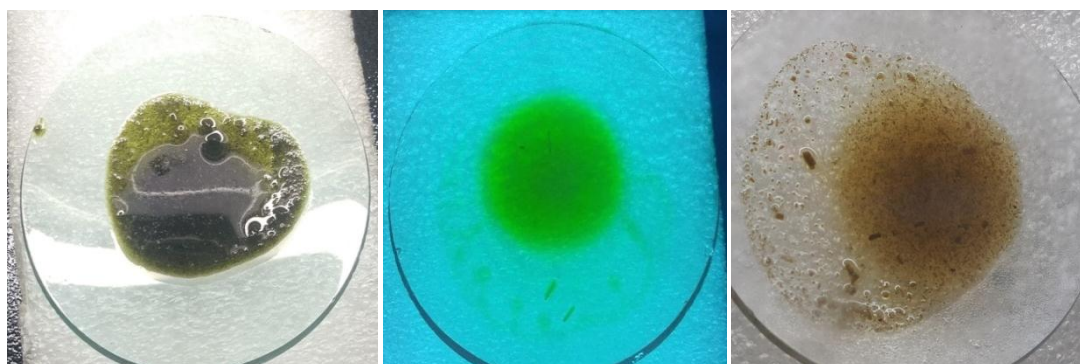
Short wavelength

Long wavelength

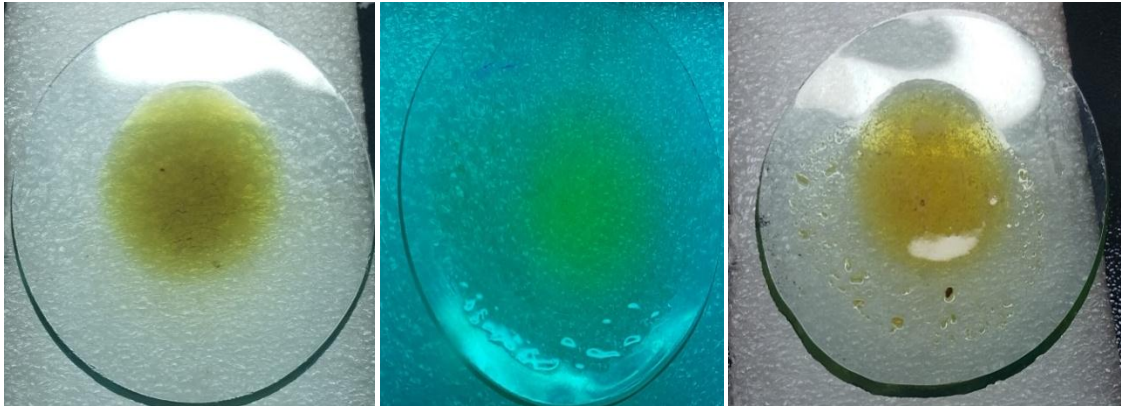
Hydrochloric acid



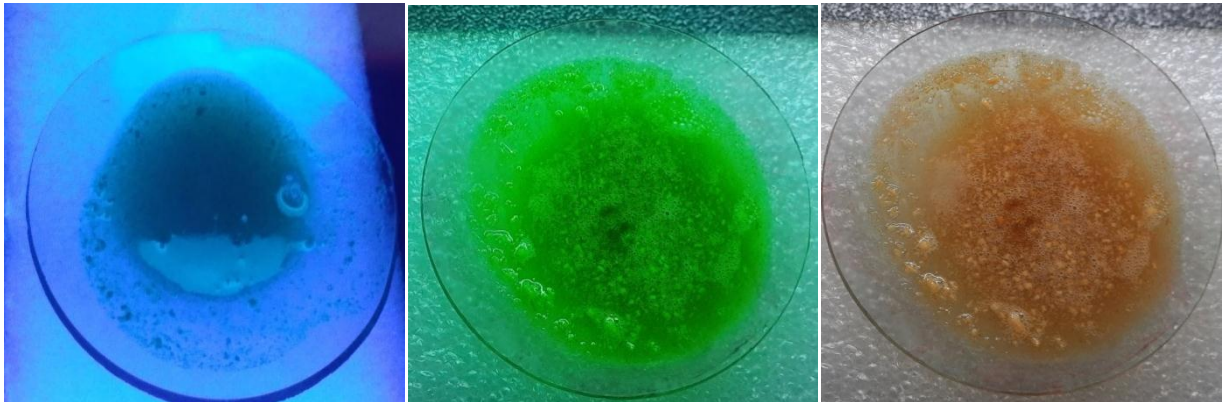
Acetic acid



Nitric acid



Sulphuric acid



Water



Plate 13: Fluorescence analysis of leaf powder extracts of *Lycopersicon esculentum* L. var. PKM 1 under visible light and UV light using different chemical reagents and water

SYNTHESIS OF SILVER NANOPARTICLES

For the synthesis of the silver nanoparticles, colour change was noted by virtual observation in tomato leaf extract incubated with aqueous solution of silver nitrate. The solution was stirred for 2 min. The reduction process Ag^+ to Ag^0 nanoparticles was followed by the color change of the solution from light brown and then brownish red colour, this exhibit the formation of silver nanoparticles. There is increase in intensity of absorption peaks after regular intervals of time and the colour intensity increased with the duration of incubation.

Farghaly and Nafady (2015) have utilized the leaf extract of Rosemary for the biosynthesis of silver nanoparticles (AgNPs). The extract is used as both reducing as well as stabilizing agent. They have shown that the green synthesis of AgNPs from Rosemary leaves is inexpensive and eco-friendly. They have also studied the effect of AgNPs on the growth of wheat and tomato plants.



Plate 14: Photograph showing synthesized AgNPs in solution

Characterization of Silver nanoparticles

Generally, nanoparticle synthesis involves physical, mechanical and chemical methods. But these are very expensive and harmful (Mali *et al.*, 2018). So, with an aim to develop a clean, nontoxic and eco-friendly technologies, various biological sources has been used for nanoparticle formation. The presence of amino groups, proteins, carbohydrates and carboxylic groups play a vital role in the formation of nanoparticles. Synthesized nanoparticles are important in different fields and so could benefit mankind.

Nanoparticles are generally characterized by their size, shape, surface area and dispersity (Jiang *et al*, 2009). The common techniques of characterizing nanoparticles are as follows:

SEM image

SEM was used to view the morphology and size of silver nanoparticle. SEM image showed the high density nanoparticle synthesized by tomato plant extract and were relatively spherical in shape. This confirmed the development of silver nanostructures.

SEM image (Plate) of the sample of control tomato plant confirms the existence of very small and uniformly spherical nanoparticles. It showed that the silver nanoparticles (AgNPs) were spherical in shape with an average size between 13.16 nm to 33.49 nm. This SEM HV is 25kV that implies stability and activate more conductivity in the view field of 2.08 μ m.

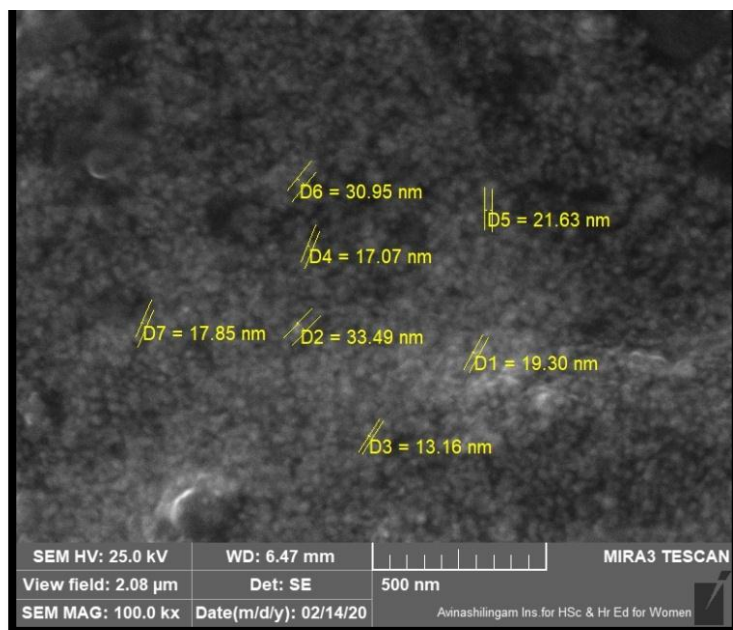


Plate 15: SEM image of the sample of control tomato plant

The SEM image of tomato plant treated with mixture of organic and biofertilizers showed the nanoparticle in the range of 14.03nm -64.22 nm. The SEM HV is 30.0 kV that implies spherical in shape and high conductivity. This size of particle confirms the presence of nanoparticle.

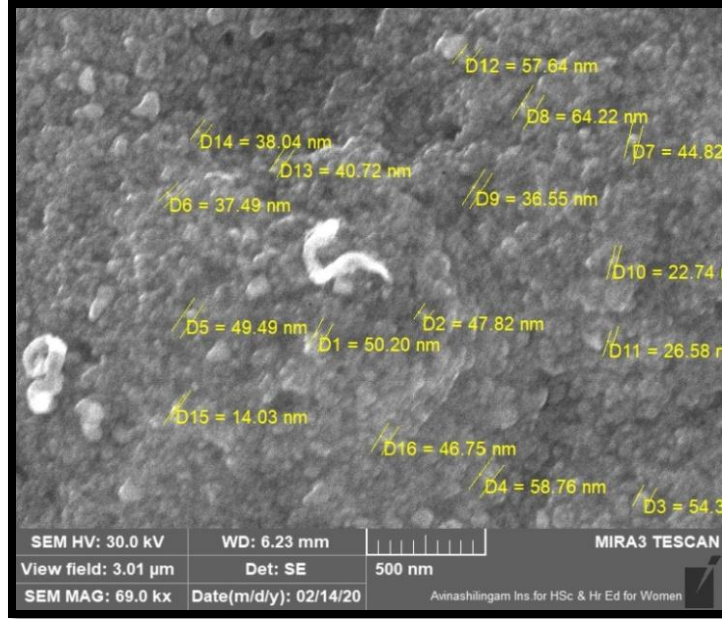


Plate 16: SEM image of tomato plant treated with mixture of organic and biofertilizers

EDAX

Analysis through Energy Dispersive X-ray(EDX) spectrometers confirmed the presence of Elemental silver signal of the silver nanoparticles. The vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in KeV. Identification lines for the major Emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified (Fig 9 ; Table 11).

Table 11 Elements present in *Lycopersicon esculentum* L. var. PKM 1 of Control plant

Element	Weight%	Atomic%	Elements present in percentage
C	47.96	70.32	62%
O	21.81	24.00	20%
Ag	16.22	2.65	4%
Cl	4.64	2.30	6%
Au	1.37	0.61	2%

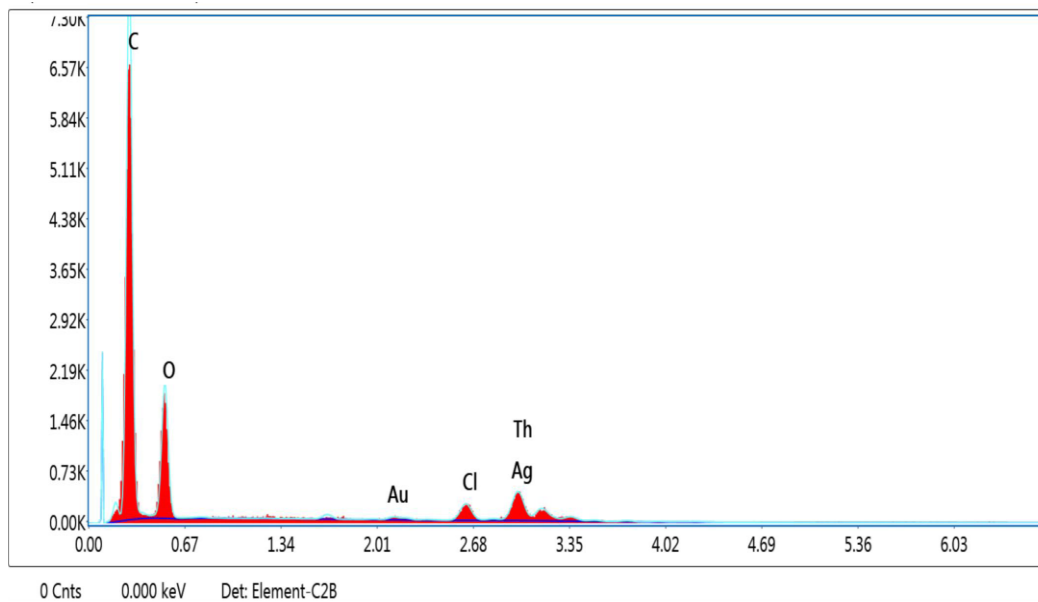


Fig: 9 EDAX spectrums recorded showing sharp peak between 2.8 and 3.0 keV confirming the presence of silver in the sample of *L. esculentum* L. var. PKM 1 of Control plant

Table 12 and Fig 10 showed the presence of silver ions and the amount of silver present in the sample. This indicates the reduction of silver ions to elemental silver.

Applications of Nanotechnology in agriculture can prove to be a boon to mankind. It uses the agricultural inputs more effectively and reduces the by-products that could harm the environment as well as human health (Bhagat *et al.*, 2015). Logeswari *et al.* (2015) have studied the synthesis of nanoparticles from commercially available plant powders. Use of plant materials for nanoparticle biosynthesis is considered to be a Green technology, since it does not involve any harmful chemicals and it could be used immensely in medical field for their efficient antimicrobial activity.

Kumar *et al.* (2017) have studied a simple low cost and eco-friendly approach for the synthesis of AgNPs from Andean blackberry extract.

Tomato is an important vegetable used in our day-to-day life. It can be used as a salad and for various food preparations like juice, soup, sauce, ketchup or puree. The processing by food industry for various products produces large amount of waste at different stages (Many *et al.*, 2014) that could be effectively used to produce eco-friendly nanoparticles.

Table 12 Elements present in *Lycopersicon esculentum* L. var. PKM 1 treated with mixture of organic and biofertilizers

Element	Weight%	Atomic%	Elements present in percentage
C	59.58	73.03	65%
O	26.56	24.45	16%
Ag	10.45	1.43	9%
Cl	2.10	0.87	5%
Au	1.04	0.08	2%
Si	0.28	0.15	2%

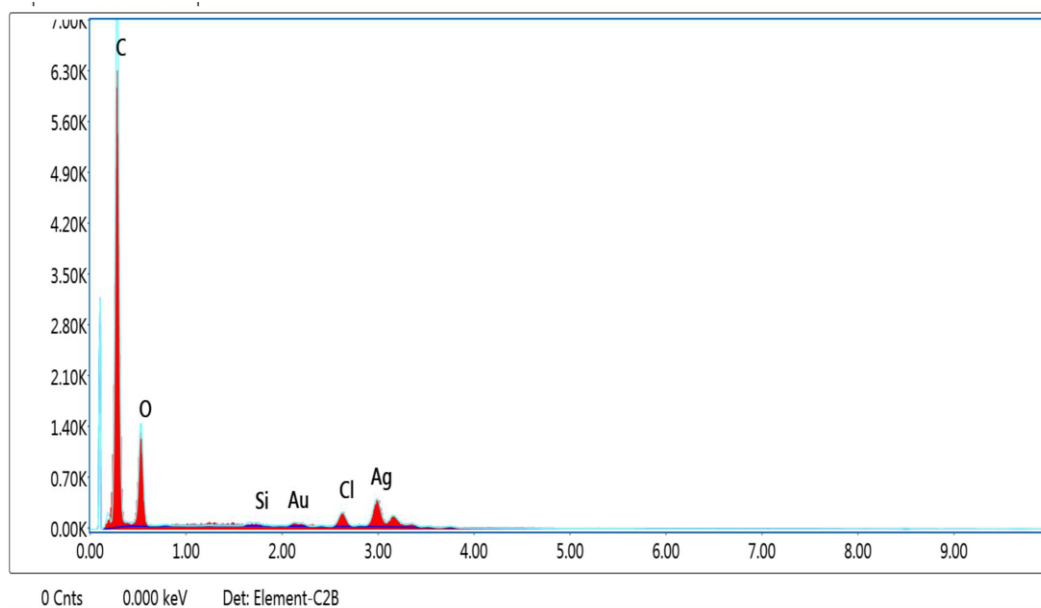


Fig:10 EDAX spectrum recorded showing sharp peak between 2.8 and 3.2 keV confirming the presence of silver in the sample of *L. esculentum* L. var. PKM 1 treated with mixture of organic and biofertilizers

Green synthesis of nanoparticles is an eco-friendly and economical approach (Sithara *et al.*, 2017). Kholoud *et al.* (2010) have studied the synthesis and applications of AgNPs and found that nano-size particles (< 100 nm dia.) are gaining more attention currently for their wide range of application in various industrial fields.

Logeswari *et al.* (2015) have utilized silver nitrate solution for the synthesis of AgNPs from plant extracts.

Nanotechnology due to the production of materials at nano scale level is emerging as a growing field with its application in Science and Technology (Albrecht *et al.*, 2006).

The present study on the green synthesis of Silver Nanoparticles is an initiative to understand its presence in tomato plant that could be further utilized for drug development.

CHAPTER V

SUMMARY AND CONCLUSION

Traditionally, organic manures are used for supplying plant nutrients. The current practice of using synthetic fertilizers may not fulfill and maintain the soil quality needed for sustainable crop productivity. Organic manures such as, vegetable compost, panchagavya and biological inputs like vermicompost and biofertilizers etc. improves soil physical condition. It is not only important in augmenting the crop productivity, but also for maintaining the sustainability in agriculture as an eco-friendly means of soil health management.

The present investigation has demonstrated the improvement in the germination, growth and yield of tomato plant by the application of organic fertilizers and biofertilizers either singly or in combination when compared to control plant.

Growth and germination percentage

The effect of organic and biofertilizers on the seed germination of Tomato was recorded on 7th day. Higher germination percentage was noticed in T₄ (*Azospirillum*) treated tomato plant.

The growth parameters were evaluated on 15th, 30th, 45th, 60th and 75th day. The shoot length was significantly higher in T₅ (Blue green algae) on 15th day. On 30th, 45th and 75th day it was higher in the application of *Azospirillum* (T₄). On the 60th day, the shoot length was significantly higher in the combined application of biofertilizers (T₇).

Significantly, higher root length was recorded in *Azospirillum* treated tomato plants on the 15th, 60th and 75th day. The combined application of *Azospirillum*+ Cyanobacteria+ Phosphobacteria showed better performance on 45th day. The root length was higher in the combined application of Vegetable compost+ Vermicompost+ Panchagavya on the 30th day. These results clearly show that the application of organic fertilizers or biofertilizers either singly or in combination increase the growth of the tomato plant.

The number of leaves was significantly higher in T₁ (Vegetable compost) on 15th day and on 30th day, the plants treated with Vermicompost (T₂) and Panchagavya (T₃) showed maximum number of leaves. On the 45th, 60th and 75th day, the number leaves were found to significantly increases in tomato plants treated with *Azospirillum* (T₄).

The fresh weight of the plant was higher in T₄ (*Azospirillum*) on 15th, 60th and 75th day. On 30th and 45th day, the combined application of Vegetable compost+ Vermicompost+ Panchagavya and *Azospirillum*+ Cyanobacteria+ Phosphobacteria treatments showed

significantly higher fresh weight when compared to control plants and plants treated with individual organic and biofertilizers.

Similarly, the dry weight of the plant was found to be significantly higher in combined application of *Azospirillum*+ Cyanobacteria+ Phosphobacteria (T₇) on the 15th DAS. On the 30th DAS, dry weight was high in the combined application of Vegetable compost+ Vermicompost+ Panchagavya (T₈) and on 45th, 60th and 75th DAS; the application of *Azospirillum* gave a positive effect as maximum dry weight content.

In the case of flowers, significantly higher number of flowers was noted in *Azospirillum* treatment (T₄) on 45th, 60th and 75th DAS. In accordance with the flower formation, the development of fruit was also higher in tomato plants treated with *Azospirillum* (T₄) on the 60th and 75th day. In control plant, there was no fruit formation on the 60th DAS. Minimum number of fruits was formed in control plant on the 75th DAS.

The phytochemical screening using different solvent extracts of tomato leaves such as ethanol, chloroform, water, petroleum ether and benzene showed the presence of phenols in all the extracts. Alkaloid was observed only in chloroform extract. Flavonoids, terpenoids and steroids were observed in ethanol, chloroform and petroleum ether extracts. Flavonoid was present in benzene extract also. Saponins was present in water and benzene extract. Aminoacid was observed in water extract and tannin present in chloroform extract. Carbohydrate was observed in ethanol and chloroform extract.

The results of the screening studies carried out in the tomato plant for the various phytoconstituents clearly shows the presence of primary metabolites such as carbohydrate and aminoacids and secondary metabolites such as flavonoids, terpenoids, steroids, alkaloids and saponins.

The fluorescence analysis of leaf powder extracts of tomato plant showed a color range from pale blue to dark green under visible light and fluorescent green under UV light of 254 nm and dark green to dark brown under UV light of 365 nm wavelength in different chemical reagents such as Hydrochloric acid, Acetic acid, Nitric acid, Sulphuric acid and Water.

Green synthesis is an ecofriendly and cost effective procedure for large scale production of Silver nanoparticles. In the present study, silver nanoparticles were synthesized from the aqueous leaf powder extract of *Lycopersicon esculentum* L. var PKM 1. Characterization of silver nanoparticles at optimum condition was done using SEM analysis. This showed the

presence of silver nanoparticles at a higher range in tomato plants treated with mixture of organic fertilizer and biofertilizer rather than the control plant. EDAX analysis showed the presence of various elements at different concentration levels. The elements present in the control tomato plant were Carbon, Oxygen, Silver, Chlorine and Gold. Apart from these elements, the mixture of organic fertilizer and biofertilizer treatment showed the presence of Silicon at 2% level. The percentage of silver in tomato plants treated with mixture of organic and biofertilizers was found to be higher (9%) when compared to the control plant (4%). The percentage of Gold was equal (2%) in both control plants and plants treated with mixture of organic and biofertilizers. The presence of silver in the tomato plants treated with mixture of organic and biofertilizer shows that the plant could be used for the production of particles at nano scale that has a wide range of application in industry.

CONCLUSION

As a boon to farmers, application of biofertilizer play an important role in improving the supply of nutrients, organic carbon, accumulation of soil enzymes and future productivity of crop plants. It is suggested that by using these natural fertilizers the Indian farmer will be benefitted financially and the people will be benefitted by getting organically produced vegetables. Now-a-days, the safety of the environment and the global demand for pesticides residue free food is an increasing concern that has evoked keen interest in using eco-friendly products that are easily biodegradable for crop production and which do not leave any harmful toxic residue besides conserving nature. Organic fertilizers when applied at appropriate dose acts as a growth promoter for the crop by retaining the soil fertility.

The phytochemicals offer many health benefits when consumed as part of the usual human diet because the presence of variety of secondary metabolites contribute to the biological and pharmacological effects.

Green synthesis of the nanoparticles is a promising technology, which is eco-friendly and economical. These biologically synthesized silver nanoparticles could be of immense use in medical field.

In the present study, the application of various organic fertilizer and biofertilizer separately and in combination gave significant increase with regard to growth and yield of tomato plant when compared to the control. The increase in vegetative growth might be due to the vital role played by the presence of microorganisms that increase the fertility of the soil.

Therefore, application of organic fertilizers such as vegetable compost, vermicompost and panchagavya and various biofertilizers such as *Azospirillum*, blue-green algae and phosphobacteria is suggested to be used in vegetable crop 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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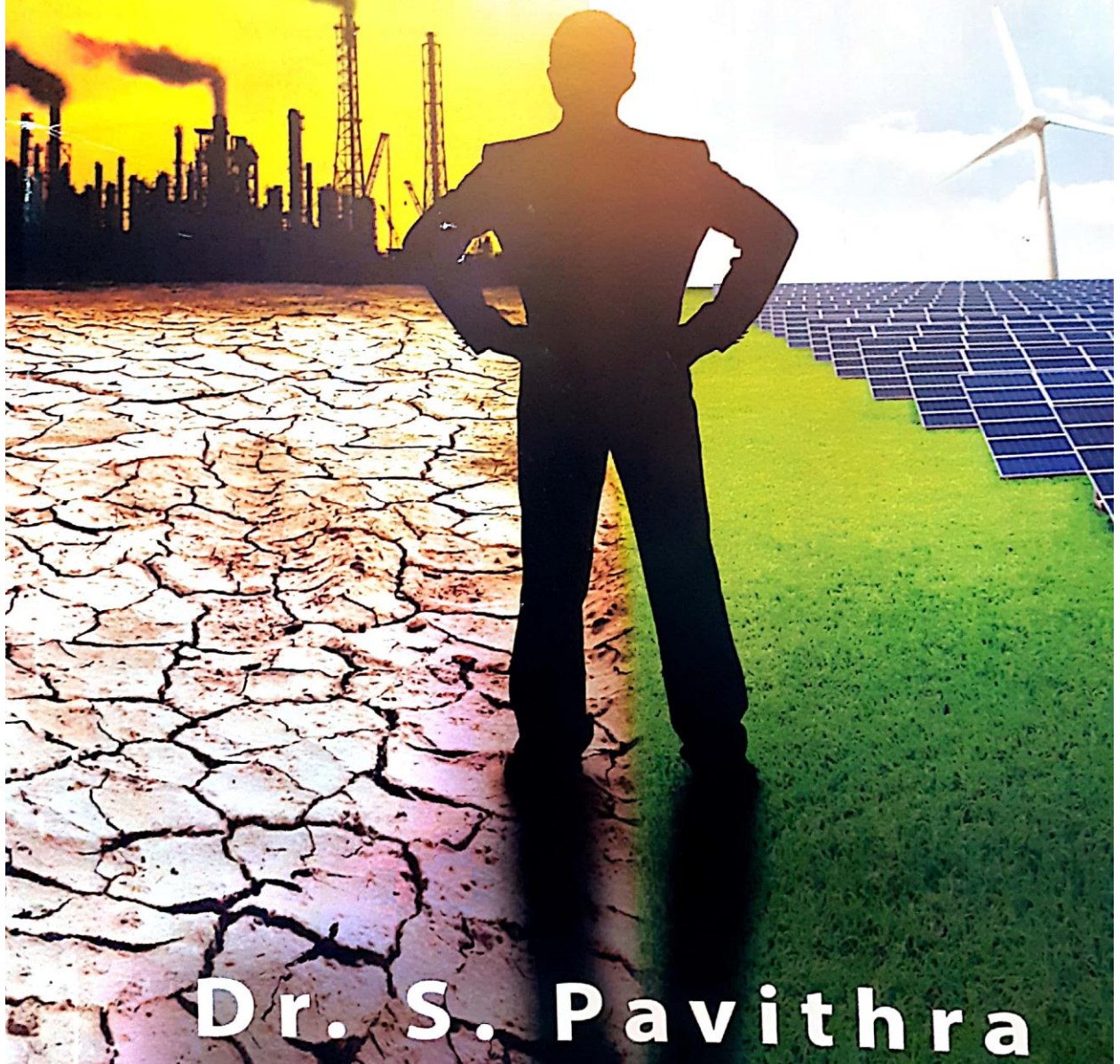
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Appendix



BUSINESS **— AND —** **ENVIRONMENT**



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**A REVIEW ON AGRICULTURAL SUSTAINABILITY FOR A
CLEAN ENVIRONMENT**

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Sustainable agriculture is farming sustainably without compromising the ability of future generations to meet their needs. It provides a potential solution to enable agricultural systems to feed a growing population within the changing environmental conditions. The biological activity of the soil is suppressed by synthetically compounded fertilizers and pesticides. Sustainable agriculture systems rely on crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes, etc to maintain soil fertility and productivity. The better way to manage pests, weeds and diseases is by the use of natural, biological and cultural controls.

Organic farming is a method of crop and livestock production involving fertilizers, genetically modified organisms, antibiotics and growth hormones without the use of pesticides. Bio-fertilizers are defined as biologically active products or microbial inoculants containing one or more beneficial bacteria fungal strains in easy to use and economical carrier materials which add, conserve and mobilize crop nutrients in the soil. Organic fertilizers contain organic compounds that increase soil fertility directly or by their decay. In other words, Bio-fertilizer is a substance that contains living microbes which when applied to soil colonizes the rhizosphere and increases the primary nutrient availability thereby promoting the growth of the plant.

Physical participation of degradation results in fragmentation, thus increasing the surface area of action, turnover, and aeration. On the other hand, biochemical changes in the degradation of organic matter are carried out by microorganisms through enzymatic digestion, enrichment by nitrogen excrement and transport of inorganic and organic materials. The benefits of vermicomposting in the recycling of organic wastes, viz., animal wastes¹, crop residues², industrial wastes^{3,4,5}, etc., have been reported at a laboratory scale, and very little attention has been given to undertake such studies in field conditions to know the success of the method.

VERMICOMPOST

In Latin, *vermis* means worms. Vermicomposting is the process of conversion of organic materials (usually wastes) into a humus-like material with the help of worms very quickly and efficiently. Compost contains

plant nutrients (N, P, and K) in variable amounts. Among various sources of organic matter, vermicompost has been recognized to possess potential as soil amendments⁶.

Earthworms play a vital role in plant growth and productivity. The selection of earthworm species is an important step in vermicomposting. Among many earthworm species, only a few are suitable for vermicomposting of organic wastes. *Eisenia foetida* is the widely used earthworm species for vermicomposting due to its potential to convert compostable organic materials such as agricultural wastes and animal manures. Animal wastes are an important resource that is used to supplement organic matters and improve soil conditions.

The main benefits of vermicomposting are: it produces natural organic fertilizers; cost-effective and safety to the environment. Compost helps in the optimization of nutrient management and the land application of compost may contribute to increasing soil organic matter thereby reducing soil erosion. Organic manure helps to improve the physical condition of the soil and provide adequate amounts of necessary nutrients for the soil productivity⁷.

Bio-fertilizers are microbial preparations containing living cells of different microorganisms that can mobilize plant nutrients in the soil from unusable to a usable form. It is considered environmentally friendly, plays a significant role in crop production, helps to build up the lost micro-flora and improves soil fertility⁸. Bio-fertilizers are substances that make use of microorganisms to increase the fertility of the soil. They are taken from plants or animal wastes along with the microbial mixtures. It produces the growth-stimulating substances like auxin, gibberellins, and cytokinins which contribute towards the vigorous growth of the plant⁹. The microorganisms require organic matter for their growth and activity in soil and provide valuable nutrients to the plants¹⁰.

Bio-fertilizers such as VAM, Phospho-bacteria, and Vegetable compost have potential practical applications, which contribute very much in increasing crop productivity through increased biological nitrogen fixation, increased availability or uptake of nutrients through phosphate solubilization

or increased absorption, stimulation of plant growth or by the rapid decomposition of organic residues¹¹. Microorganisms also produce the growth-promoting substances which greatly influence seed germination, root growth, and proliferation¹². They are cost-effective, eco-friendly and renewable sources of nutrients and as such, they play a vital role in maintaining long-term soil fertility and sustainability¹³. Microorganisms help in increasing the nutrient level of the plants and allow them to grow in a healthy environment. They are also environment-friendly and do not cause pollution of any sort. Bacteria, fungi, cyanobacteria, etc. are the main source of bio-fertilizers. They can be cultured and used for inoculating seed or soil or both under ideal conditions to increase the nutrient availability to plant. Among this, mycorrhiza is an important one in the agriculture field for the cultivation of many crops¹⁴. Many bio-fertilizers contain living microorganisms, which provide direct or indirect beneficial effects on plant growth and crop yield through different mechanisms¹⁵. They are suggested as an alternative to mineral fertilization and for reducing environmental pollution¹⁶.

The nitrogen plays an important role in the life cycle of a plant. It is the main plant mineral nutrient needed for chlorophyll production and other plant cell components (proteins, nucleic acids, and amino acids). Crop yield is affected by plant N status. Nitrogen is an essential component in all vital processes of plants. Nitrogen plays an important role in most of the physiological processes. It imparts dark-green color to plants, promotes leaves, stem and other vegetative part's growth and development. Moreover, it also stimulates root growth. Nitrogen produces rapid early growth, improves fruit quality, enhances the growth of leafy vegetables, and increases the protein content of fodder crops. It encourages the uptake and utilization of other nutrients including potassium, phosphorus, and controls the overall growth of the plant.

VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI

VAM - Vesicular Arbuscular Mycorrhizal is a fungus that penetrates the cortical cells of a vascular plant. Vegetable inoculated with vesicular-arbuscular mycorrhizal fungi shows a considerable increase in the growth and yield, because

of improved uptake of phosphorus and other mineral nutrients particularly in phosphorus-deficient soil. Vesicular Arbuscular Mycorrhizal fungi belong to the Phylum Glomeromycota. They are symbionts with terrestrial plant roots. It is now generally recognized that they not only improve the phosphorus nutrition of the host plant but also its growth, which may result in an increase in resistance to drought stress and some diseases. Therefore, VAM fungi offer great potential for sustainable agriculture and the application of VAM fungi to agriculture has been developed. Therefore, these can be used in modern sustainable agriculture particularly as a bio-control agent.

Mycorrhizal fungi possess distinct symbiotic associations with plant roots. The mycorrhizal fungi help in mobilizing the macro and micronutrients from adjacent soil to the root system through the hyphal network. The symbiotic association between a fungus and the roots of a vascular plant is termed as mycorrhiza. They are an important component in soil. VAM fungi inhabit a variety of ecosystems including agricultural lands, forests, grasslands, and many stressed environments, colonize the roots of most plants, including bryophyte, pteridophyte, gymnosperms, and angiosperms¹⁴. A study has been undertaken to evaluate the role of VAM fungi in the growth of groundnut crop plants¹⁷.

PHOSPHO-BACTERIA

Phospho-bacteria (Phosphate solubilizing bacteria-PSB) is one of the most useful plants soil microorganisms, widely used as bio-fertilizer. PSB helps in the uptake of insoluble phosphate by plants. PSB plays an important role in the enhancement of plant growth by improving the texture of the soil by adding organic matter to the soil, solubilizing the insoluble phosphorus in soil^{18,19}. To compensate for phosphorus deficiency in the soil, phosphate fertilizer is being used widely. However, the increased use of chemical fertilizers causes soil contamination. In such a case, PSB helps in the utilization of unavailable native phosphates²⁰. They possess the ability to convert insoluble compounds of phosphorus into available phosphates thereby enhancing the nutrient availability to plants. Crop improvement could be achieved by the application of phosphate solubilizing bacteria

improve solubilization of fixed soil phosphorus and applied phosphates, resulting in higher crop yields.

Phosphorus solubilizing bio-fertilizers are carrier-based preparations containing living cells of microbes like bacteria, fungi, and actinomycetes which may help in increasing crop productivity by way of helping in the solubilization of insoluble phosphorus, stimulating plant growth by providing hormones, vitamins and other promoting substances²¹. The most essential growth-promoting factor that induces root elongation, proliferation and changes the root architecture is phosphate, without which normal crop maturity is not possible²².

VEGETABLE COMPOST

Vegetable waste generally consists of carrot, potato, brinjal, cauliflower, coriander leaves, tomato, lemon, lady's finger, and onion. Vegetable waste is considered as a good source of organic material. It is relatively easy to decompose vegetable waste when compared to garden waste which is usually high in lignin due to the wood waste included in it²³. It is differentiated from green waste, which includes biodegradable waste from gardens and municipal parks²⁴. Vegetable waste provides good amount of nutrients for the growth of microorganisms, they are neither pathogen nor concerned with human health. However, they produce strong odors while decomposing. The high moisture content of vegetable waste causes it highly expensive for disposal. The vegetable waste containing lettuce and onion waste has high amounts of Sulphur (0.2% and 0.7%) respectively. Their moisture content is 96.2% and 91.1% respectively. The breaking down involves aerobic processes and carried out by bacteria, fungi, insects, and animals that inhabit the soil. These organisms make use of complex nutrients of the compost and release the essential minerals and elements into soil. It is available easily for crops and enhance their yield and provide healthy and nutritious food to the mankind²⁵. Vegetable compost is a biodegradable material generated in large quantities from various sources like domestic, industries, municipalities and markets. These

wastes can be treated for bio-energy production through fermentation under controlled conditions or else used for composting. Vegetable waste compost application to agriculture is done towards the development of management systems and to maximize agronomic benefit and reduce the environmental risk²⁶.

Plants are an essential resource for humans because they are the backbone of life on Earth. Humans obtain many products directly or indirectly from plants^{27,28}. The plants produce several chemical compounds that have biological functions as well as produce defense against insects, fungi and anthophagous mammals. A minimum of 12,000 such compounds are isolated until now, which is considered to be only 10% of the total availability²⁸. Numerous plant species contain active ingredients such as alkaloids, phenols, tannins, cryogenics, and terpenoids²⁹.

Plants are an important source of many natural products such as essential oils, natural dyes, pigments, waxes, resins, tannins, alkaloids, amber, and cork. Phytochemical is a natural bioactive compound present in plants that along with the nutrients and fibers act as a defense mechanism to protect the plants from various diseases. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design³⁰. The main bioactive components include alkaloids, tannins, flavonoids, and phenolic compounds³¹.

PANCHAGAVYA

Panchagavya also contains phosphate solublizing microorganisms. Panchagavya has played an important role in providing pest and disease resistance, resulting in an overall increase in yields^{32,33}. Panchagavya possesses the properties of fertilizers and biopesticides³⁴. Panchagavya is one of the most important and beneficial manure. It induces large leaves and enhances the high level of photosynthesis. In the groundnut, it influences the high level of nodulation and it increases the height of the plant. It increases the sugar content and... Panchagavyais a combination of five

products namely cow dung, cow urine, milk, ghee, and curd of a cow. Of these, the three direct constituents are cow dung, urine, and milk; and the two derived products are curd and ghee. The consequences of panchagavya applications are superior growth, yield, and quality of crops. It provides most of the macronutrients and micronutrients, vitamins, required amino acids, growth-promoting substances and beneficial microorganisms for growth of the plant³⁵.

AZOSPIRILLUM

Azospirillum is a gram-negative, microaerophilic, non-fermentative and nitrogen-fixing bacteria belonging to the Rhodospirillaceae. It is a free-living, plant-growth-promoting bacterium (PGPB) capable of affecting growth and yield of numerous plant species, many of agronomic and ecological significance. They are found in the soil around plant roots and root surfaces³⁶. It also produces growth-promoting substances like Indoleacetic acid (IAA), gibberellins and promotes root proliferation^{37,38}. It helps in increasing the rootlet density and root branching thereby resulting in an increased uptake of mineral and water. *Azospirillum* is found to be very effective in increasing 10- 15% yield of cereal crops and fixing N₂ up to 20-40 Kg N/ha³⁹. Positive growth response of inoculated plants could be due to the provision of nutrients especially nitrogen and growth-promoting hormones by *Azospirillum* and enhanced uptake of phosphorus and other nutrients due to mycorrhizal colonization⁴⁰.

Bio-fertilizer and organic amendments are cost-effective nutrient sources that could serve as an alternative to chemical fertilizers and improve crop production in low-input agriculture. These increase the soil organic carbon and stimulate microbial activity thus providing N and P to the soil. Soil microorganisms play an important role in recycling the nutrients and providing them for plant growth⁴¹. Their population and activities are reflected upon by the quality of soil, as well as, environmental conditions of the soil⁴². Bio-fertilizer helps to increase crop production by increasing Biological Nitrogen Fixation, increased availability or uptake of nutrients through solubilization or increased absorption, stimulation of plant growth

through hormonal action or antibiosis, or by decomposition of organic residues⁴³.

CONCLUSION

At present, environmental sustainability is an issue that needs much attention. The current review is based on the use of organic wastes and microorganisms for a sustainable environment. Bio-fertilizers help in increasing the availability of plant nutrients thereby maintaining the fertility of the soil. Microorganisms do the role of nitrogen fixation, solubilize insoluble phosphates and synthesize biomass for manuring of crops like rice. Bio-fertilizers are economical, renewable and eco-friendly, but couldn't replace chemical fertilizers. Sustainable crop production depends on the rational use of chemical fertilizers and pesticides along with organic manures for better soil health. Microbial organisms can be multiplied faster and utilized. Farmers can be trained to cultivate microorganisms on a large scale and utilize for the growth of plants. The production of bio-fertilizers using organic wastes and microorganisms is the safer and cheaper method for India.

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