

**IMPACT OF *AZOLLA* BIOFERTILIZER ON THE
GROWTH PERFORMANCE OF *LYCOPERSICUM
ESCULENTUM* Linn.**

Dissertation submitted in partial fulfilment of the requirements

For the award of the Degree of Bachelor of Science in
BOTANY

BY

JIGGLE JINSHO

Reg No: AB20BOT006



**DEPARTMENT OF BOTANY
ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM**

2023

CERTIFICATE

This is to certify that the dissertation entitled “**Impact of *Azolla* biofertilizer on the growth performance of *Lycopersicum esculentum* Linn.**” submitted in partial fulfillment of the requirements for the Degree of Bachelor of Science in Botany of Mahatma Gandhi University is an authentic work carried out by JIGGLE JINSHO (Reg No: AB20BOT006) under the supervision and guidance of Dr.Liza Jacob.



DR. LIZA JACOB

Associate Professor

Supervising Teacher

Department of Botany

St. Teresa's college (Autonomous)

Ernakulam



DR. LIZA JACOB

Associate Professor and Head

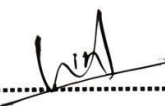

Department of Botany

St. Teresa's College (Autonomous)

Ernakulam

Examiners:

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Place: Ernakulam

Jiggle Jinsho

Date:

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ABSTRACT

For a country to grow and enhance economically, development is required. Sustainable development makes our life better thereby improving the living standards. Increasing population Often drive-up demand for food, which typically results in additional use of chemical fertilizers, Pesticides etc. There is an increasing concern that continuous use of chemical fertilizers on soil Depletes its essential nutrients. Biofertilizers are the substances that contain microbes, which help in Promoting the growth of crops, plants, and trees by increasing the supply of essential nutrients to the plants. *Azolla* is an aquatic heterosporous fern, grows in symbiotic association with *Anabaena azollae*. It aids in nitrogen fixation which increases the fertility of the soil and in turn Enhances the yield. This article describes an effort to enlist and understand the growth response of tomato plant with the application of *Azolla* Biofertilizer

In the present study, a field Experiment was conducted to study the effect of *Azolla* biofertilizer on the growth performance of *Lycopersicum esculentum* Linn.. *Azolla* biofertilizer is used as microbial Inoculant or biofertilizer and promotes the growth and increase the yield by 20-30%. *Azolla* biofertilizer also contains other nutrient i.e., N, P, K. Two Types of growth regimes were compared. From the result it may be concluded that *Azolla* can be recommended as a biofertilizer to Improve the growth and yield of *L. esculentum* Linn.

INTRODUCTION

Sustainable development makes our life better thereby improve the living standards. According to Brundtland definition “Sustainable development is the development that meets the Needs of present without compromising the ability of future Generations to meet their own needs”. The objectives of Sustainable development – provide healthy and productive Life, harmonious with nature, preservation of environmental Resources, preserve cultural diversity, eradicate poverty, reduce disparities in standard of living.

Organic farming is one of the best farming methods to decrease the cost of the production and also to increase the quality of the Product and the product should be free from the chemical Residues and other toxic substances. Organic farming is now gaining importance in India. The Cultivation of crops using biological fertilizers thereby expelled Use of chemicals on fields, improves the soil fertility, increases the crop yield, reduce entry of toxic substances in soil thereby Minimizing pollution and wastage. Organic farming can solve Many of these problems as this system helps to maintain soil Productivity and effectively control pest by enhancing natural Processes and cycles in harmony with environment. It includes processes like crop rotation, green Manure, biological pest control, organic waste management. It Sustains the health of soil, ecosystem, and people.

The fertilizers can be produced naturally or industrially on a large scale. Modern agriculture uses pesticides, herbicides, Fungicides, and harmful chemical fertilizers to produce foods for consumption. The foods which are

produced by Conventional methods are harmful for human health Respectively. The use of chemicals and pesticides on fields Increases the toxic substances that seep into the soil and reduces the soil fertility. Thus, the land will become barren Since the nutrients are been lost from the soil, no cultivation Can take place. Their negative effects on the environment are Manifested through soil erosion, water shortages, salination, Soil contamination, genetic erosion, etc.

Bio fertilizers are the formulates obtained from microorganisms Which when applied to soil mobilizes the soil nutrients and thereby ensure proper growth and development of plants. They don't disturb the microbial population that exists in the Soil. The microorganisms in soil mainly restore the soils natural Nutrient cycle and build soil organic matter. Using bio-Fertilizers, healthy plants can be grown, while enhancing the sustainability and health of the soil can be restored.

The use of bio fertilizers is one of the most important components of nutrient management, they are cost effective and sources of plant nutrients to supplement with crop plants Are exploited in the production of bio fertilizers. A small dose of biofertilizer is sufficient to produce Desirable results because each gram of carrier of biofertilizers Contains at least 10 million viable cells of a specific strain. Bio fertilizers are Important because they are enhancing biomass production, useful in sustainable agriculture, provide protection against droughts, restore soil fertility, Replace use of chemical fertilizers, Cost-Effective, Eco-friendly.

Azolla is an alga mainly used as Biofertilizer in farmlands. It is a floating aquatic fern belonging to the family Salviniaceae. It mainly grows in freshwater, marshy pond water, contaminated water and natural ecosystem. *Azolla* can symbiotically associate with *Anabaena*. There are several benefits of algae *Azolla*, which include; used as a bio fertilizer in paddy fields, produces nitrogen for the Growth of plants, good source of nutrients, act as a renewable Bio fertilizer, suppress the growth of weeds. *Azolla* is rich in Proteins, essential amino acids, vitamins, and minerals. Several Microorganisms such as algae and various inorganic Compounds can be used in the production of bio fertilizers. The use of *A. filiculoides* also increases organic matter and Potassium contents of the soil. *Azolla* is Very appropriate as a substitute for organic Fertilizer with rapid growth of this plant has organic Productivity. With biofertilizer. We were able to grow Different kinds of vegetables like tomatoes, Potatoes, onions etc. *Azolla* Biofertilizer was collected from local fertilizer store were used in this experiment. Our main objective was to investigate the effect of *Azolla* biofertilizer application on *Lycopersicum esculentum* .Linn growth.

REVIEW OF LITERATURE

Azolla biofertilizer has highest N, P, K content i.e., N -3.68 %, P – 20%, K – 0.15% as Compared to other organic sources experiments Conducted at IRRI, Philippines showed that Conversion of biofertilizer from *Azolla* was rather Quick but further research is required to evaluate the exact potential.

According to Bhat et al (2015) The use of bio fertilizers is one of the most important Components of nutrient management, they are cost effective and sources of plant nutrients to supplement with crop plants Are exploited in the production of bio fertilizers.

According to Ananda raj and Delapierre (2016), A small dose of biofertilizer is sufficient to produce Desirable results because each gram of carrier of biofertilizers Contains at least 10 million viable cells of a specific strain

Representatives of *Azolla* species producing substantial Biomass is seen growing in contaminated water and natural Ecosystems Miranda E. F.et al., (2016)

According to Kannaiyan s, (1995) *Azolla* – Anabaena symbiosis, Because of its independence for carbon and nitrogen forms an Important N input in rice cultivation

The use of *Azolla. filiculoides* also increases organic matter and Potassium contents of the soil. Bhuvaneshwari K et al. (2015).

According to Chittora *et al* (2020), *Azolla filiculoides* has a symbiotic association with *Anabaena azollae*, a nitrogen fixing blue-green algae. Blue green algae are also called as Cyanobacteria which are photoautotrophic and prokaryotic in nature. Cyanobacteria are the most Successful and sustained prokaryotic organism during the Course of evolution

Organic fertilizers can be used in place of chemical fertilizers because they Have been found to improve soil fertility, water holding capacity, and structure, while Chemical fertilizers may degrade the soil's physical properties and fertility. Contradictory Results regarding effect on caffeine content was reported (Gao *et al.*)(2017), (Tabu *et al.*)(2019). It was Reported that there was an increase in amino acid and decrease in catechins in tea due to Increased nitrogen source. Chemical and organic fertilizers' effects on the growth of the tea Plant and the quality of black tea have not yet been studied in Nepal. Thus, this study was performed to fulfill this gap and also this study helps to suggest the better fertilizer and their Proper use to increase the quality and productivity of tea

Tomatoes contain about 80% of lycopene. *Azolla filiculoides* biofertilizer Extract used For Tomato growth along with foliar application; the best Results were shown with 100% *Azolla filiculoides* biofertilizer Ashraf Hanafy *et al.* (2019)

MATERIALS AND METHODS

The first step was to select the plant type. The plant selected was *Lycopersicum esculentum* Linn. (Tomato).

The other material used are:

- Growbags
- Sand and soil
- Coir peat
- *Azolla* Biofertilizer

Preparation of potting mixture (control)

The potting mixture for the experiment without *Azolla* Biofertilizer was prepared by using sand, soil, coir peat. The seeds were sown in the nursery bed of soil and after attaining a minimum height they were transplanted into growbags with three saplings in each bag. The growbags contained a mixture of sand and soil in 1:1 ratio and also coir peat.

Preparation of potting mixture (with *Azolla* Biofertilizer)

The potting mixture contained sand, soil, coir peat and *Azolla* Biofertilizer. The seeds were sown in the nursery bed of soil and after attaining a minimum height they were transplanted into growbags with three saplings in each bag. The growbags contained sand and soil in 1:1 ratio, coir peat and 300gram of *Azolla* fertilizer.

- Each growbag were watered on the regular basis and were kept in a place with enough sunlight and shade

GROWTH PARAMETERS

Various growth parameters considered for the study were:

1.MORPHOLOGICAL PARAMETERS

- a. General appearance
- b. Shoot Length
- c. Number of Leaves
- d. Number of Branches

2.PLANT BIOMASS

- a. Wet weight of Leaf, Stem and Root
- b. Dry weight of Leaf, Stem and Root

3.PLANT PIGMENTS

- Chlorophyll a
- Chlorophyll b
- Total Chlorophyll
- Carotenoid

CHLOROPHYLL AND CAROTENOID CONTENT

The method of Arnon (1949) was employed for the quantitative estimation of chlorophyll and carotenoids.

80% acetone was prepared. A pre weighed (250 mg) quantity of fresh leaf material was ground into fine paste 10 ml of 80% acetone was added into it. The extract was centrifuged repeatedly till the lechate became colorless. The supernatant was taken together and was made up to 25 ml with 80% acetone. The extract was kept away from direct sunlight. The optical density of the extract was read at 420,490,540,590,659 wavelengths. The samples were analyzed in duplicates.

From the optical densities, the Chlorophyll and Carotenoid contents were calculated using the formula

1. Chlorophyll a(mg/gm)- $12.7(D_{663})-2.69(D_{645}) \times V/1000 \times W$
2. Chlorophyll b(mg/gm)- $22.9(D_{645})-4.68(D_{663}) \times V/1000 \times W$
3. Total Chlorophyll(mg/gm) $D_{652} \times 1000/34.5 \times V/1000 W$

Where D-optical density

V-final volume of 80% acetone (10 ml)

W weight of sample taken (0.25g)

The results obtained were compared with that of control.

OBSERVATION AND RESULT

SHOOT LENGTH OF PLANT WITH *AZOLLA* BIO FERTILIZER AND CONTROL PLANT

SHOOT LENGTH OF PLANT (*AZOLLA* BIO FERTILIZER)

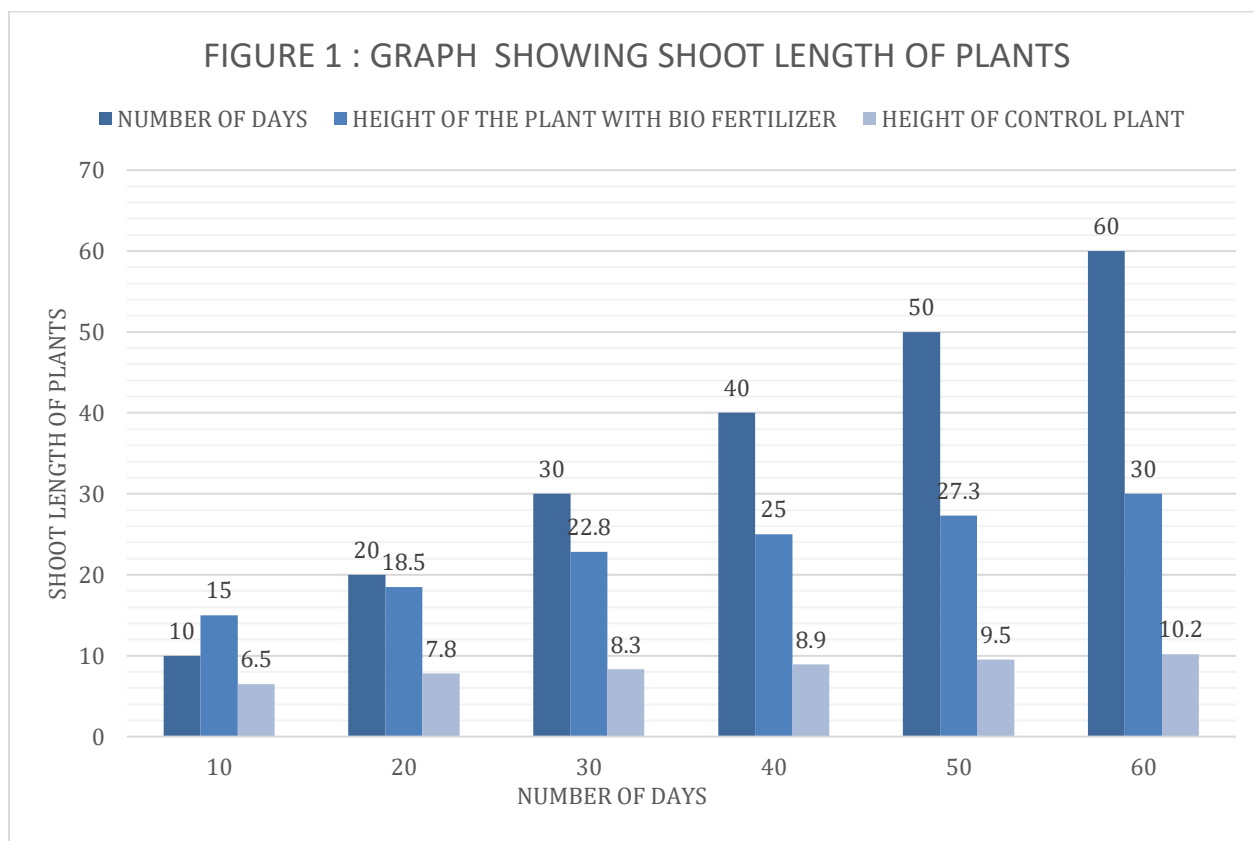
- The shoot length observed on 10th day was 15cm
- The shoot length observed on 20th day was 18.5cm
- The shoot length observed on 30th day was 22.8cm
- The shoot length observed on 40th day was 25cm
- The shoot length observed on 50th day was 27.3cm
- The shoot length observed on 60th day was 30cm

SHOOT LENGTH OF CONTROL PLANT

- The shoot length observed on 10th day was 6.5cm
- The shoot length observed on 20th day was 7.8cm
- The shoot length observed on 30th day was 8.3cm
- The shoot length observed on 40th day was 8.9cm
- The shoot length observed on 50th day was 9.5cm
- The shoot length observed on 60th day was 10.2cm

Table No.1

NUMBER OF DAYS	HEIGHT OF THE PLANT WITH BIOFERTILIZER	HEIGHT OF CONTROL PLANT
10	15 CM	6.5CM
20	18.5 CM	7.8CM
30	22.8 CM	8.3CM
40	25 CM	8.9CM
50	27.3 CM	9.5CM
60	30 CM	10.2CM



NUMBER OF LEAVES OBSERVED ON PLANTS

NUMBER OF LEAVES OF PLANT WITH *AZOLLA* BIOFERTILIZER

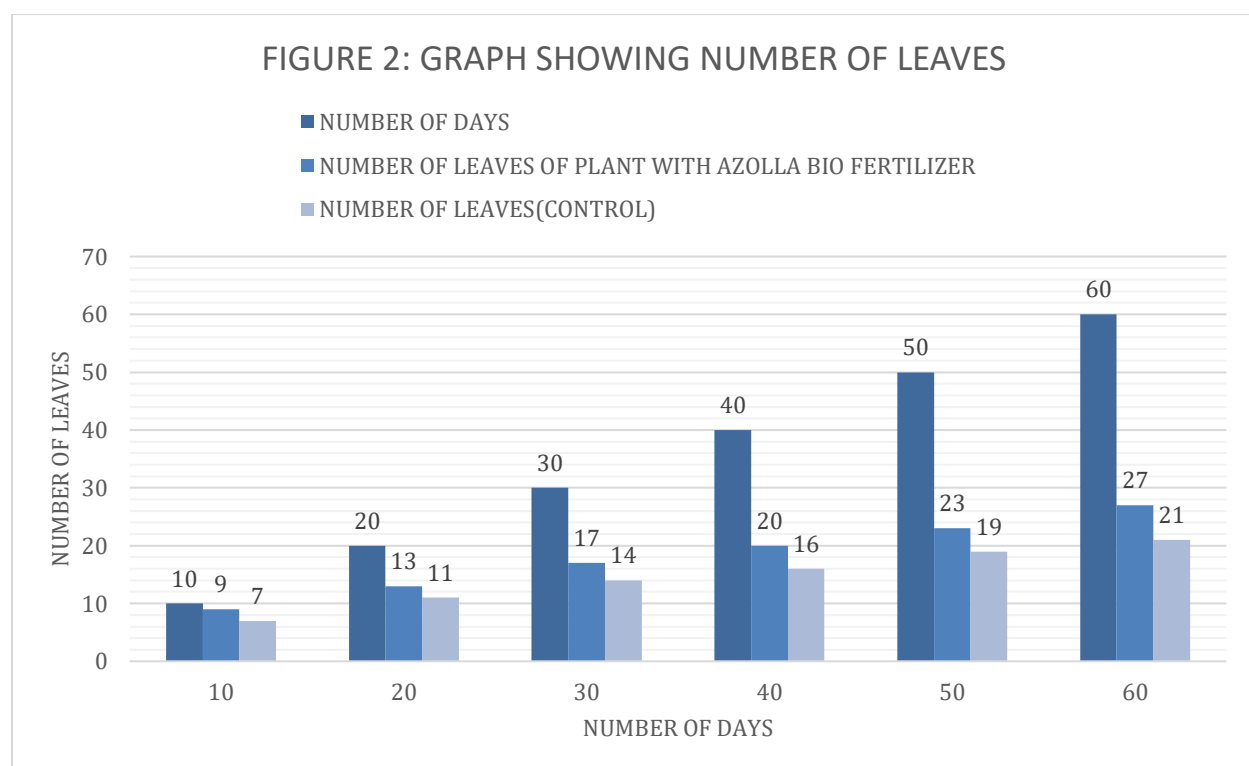
- Number of leaves observed on 10th day was 9nos
- Number of leaves observed on 20th day was 13nos
- Number of leaves observed on 30th day was 17nos
- Number of leaves observed on 40th day was 20nos
- Number of leaves observed on 50th day was 23nos
- Number of leaves observed on 60th day was 27nos

NUMBER OF LEAVES OF CONTROL PLANT

- Numbers of leaves observed on 10th day was 7nos
- Numbers of leaves observed on 20th day was 11nos
- Numbers of leaves observed on 30th day was 14nos
- Number of leaves observed on 40th day was 16nos
- Number of leaves observed on 50th day was 19nos
- Number of leaves observed on 60th day was 21nos

Table No.2

NUMBER OF DAYS	NUMBER OF LEAVES OF PLANT WITH AZOLLA BIOFERTILIZER	NUMBER OF LEAVES(CONTROL)
10	9	7
20	13	11
30	17	14
40	20	16
50	23	19
60	27	21



MORPHOLOGICAL CHARACTERS

PLANT WITH *AZOLLA* BIOFERTILIZER

- Height of the plant: 23.1cm
- Number of branches: 6 numbers
- Number of leaves: 27 numbers
- Root Length: 26.1 cm

CONTROL PLANT

- Height of the plant: 9cm
- Number of Branches: 5 numbers
- Number of Leaves: 21 numbers
- Root Length: 3.5cm

Table No.3

FEATURES	AZOLLA	CONTROL
Height of the plant	23.1cm	9cm
No:of Branches	6nos	5nos
No:of Leaves	23nos	21nos
Root Length	26.1cm	3.5cm

WET WEIGHT

PLANT TREATED WITH *AZOLLA* BIOFERTILIZER

- Wet weight of whole plant ;3.69gm
- Wet weight of root: 0.949gm
- Wet weight of shoot: 2.179gm

CONTROL PLANT

- Wet weight of whole plant: 1.809gm
- Wet weight of root: 0.08gm
- Wet weight of shoot: 1.269gm

Table No.4

PLANT- PART	AZOLLA	CONTROL
Whole plant	3.69 gm	1.809 gm
Root	0.949 gm	0.08 gm
Shoot	2.179 gm	1.269 gm

DRY WEIGHT

PLANT TREATED WITH *AZOLLA* BIOFERTILIZER

- Dry weight of whole plant: 2.53gm
- Dry weight of root: 0.15gm
- Dry weight of shoot: 0.469gm

CONTROL PLANT

- Dry weight of whole plant: 0.281gm
- Dry weight of root: 0,009gm
- Dry weight of shoot: 0.12gm

Table No.5

PLANT PART	AZOLLA	CONTROL
Whole plant	2.53gm	0.281gm
Root	0.15gm	0.009gm
Shoot	0.469gm	0.12gm

AMOUNT OF PLANT PIGMENT

PLANT TREATED WITH *AZOLLA* BIOFERTILIZER

- Chlorophyll a (mg\gm): 1.892
- Chlorophyll b (mg\gm): 1.421
- Total Chlorophyll (mg\gm); 3.25
- Carotenoids (mg\gm): 0.931

CONTROL PLANT

- Chlorophyll a (mg\gm): 1.081
- Chlorophyll b (mg\gm); 1.01
- Total Chlorophyll (mg/gm): 2.12
- Carotenoids (mg\gm): 0.53

Table No: 6

PLANT PIGMENTS	AZOLLA	CONTROL
Chl.a (mg\gm)	1.892	1.081
Chl.b (mg\gm)	1.421	1.01
Total Chl (mg\gm)	3.25	2.12
Carotenoids(mg\gm)	0.931	0.53

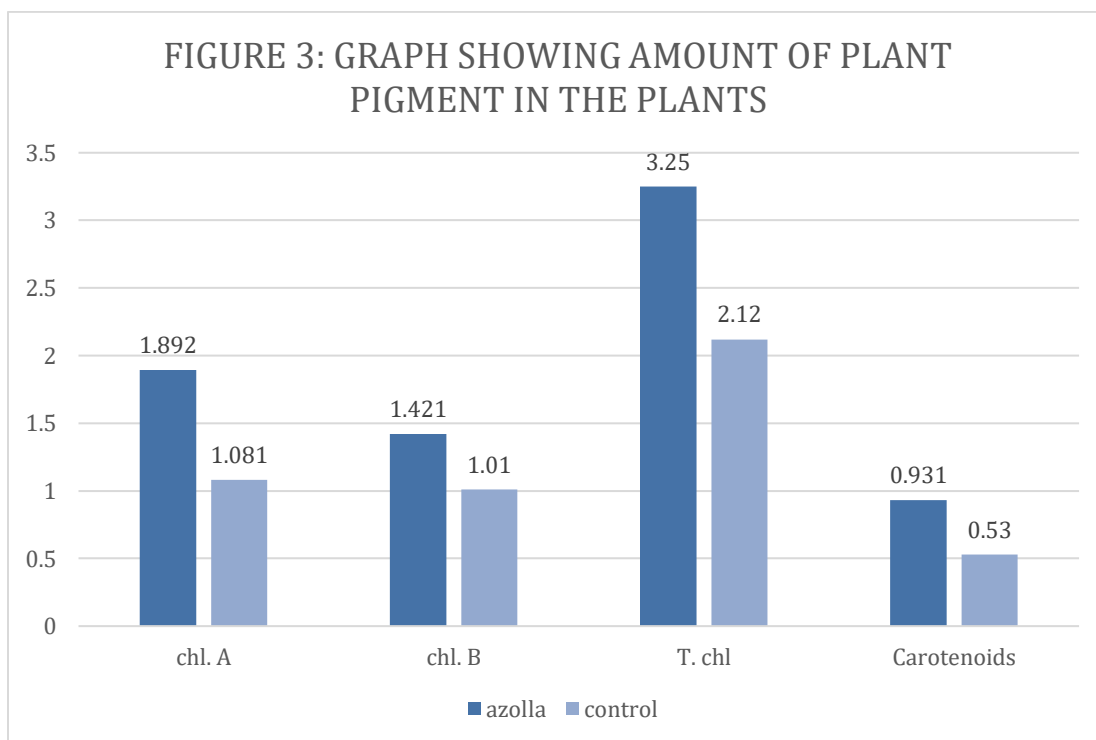




PLATE NO: 1



PLATE NO: 2 (PLANT WITH *AZOLLA* BIO FERTILIZER)



PLATE NO:3 (CONTROL PLANT)



PLATE NO:4 (FLOWER AND FRUIT PRODUCED BY PLANT WITH *AZOLLA* BIO FERTILIZER)

DISCUSSION

In this work a comparative study was conducted between two sets of tomato plants with and without applying *Azolla* fertilizer. Tomato plants grown using *Azolla* biofertilizer showed faster rate of growth when compared to the control plants. Plant growth parameters observed in the study consists of plant height, number of leaves, branches and the flowering stage. The observations showed that the treatment using biofertilizer significantly improved the growth and development of *L.esculentum* Linn.

In the present study, the organic fertilizers had the significant influence on the plant growth, yield and quality of studied tomato varieties and the results are presented in table Nos. 1 and 2 and Table no: 1 and 2 showed the significant differences between effects of fertilizers on tomato plant height and number of leaves. The main result showed that *Azolla* biofertilizer are more effectively promoted plant growth, number of fruits and height of plant with other fertilizer treatments. It can be concluded that *Azolla* biofertilizer can be recommended as a fertilizer to improve tomato fruit quality and yield.

Tomato contains many health promoting compounds and are easily integrated as a nutritious part of a balanced diet (Marti et al., 2016). In addition to consuming the fresh fruits, consumers use tomatoes in processed products such as soups, juices, and sauces (Krauss et al., 2006; Li et al., 2018b). The nutritional importance of tomatoes is largely explained by their various health-promoting compounds, including vitamins, carotenoids, and

phenolic compounds (Raiola et al., 2014; Liu et al., 2016; Marti et al., 2016; Li et al., 2018b). Tomato has been recently gaining attention in relation to the prevention of some human diseases. This interest is due to the presence of carotenoids and particularly lycopene, which is an unsaturated alkali compound, appears to be an active compound in the prevention of cancer, cardiovascular risk and in slowing down cellular aging (Gerster, 1997; Di Cesare et al., 2012; Abdel-Monaim, 2012). Lycopene is found in fresh, red-ripe tomatoes as all-trans (79–91%) and cis- (9–21%) isomers (Shi et al., 1999; Boileau et al., 2002; Abdel-Fattah and Al-Amri, 2012). In the case of this experiment, organic fertilizer was used as an alternative to the chemical fertilizer. When organic fertilizer was used, it resulted in faster growth rate

The purpose of this study is to have a healthier and cheaper alternative fertilizer for *L.esculentum* Linn. The study aims to determine the function of the organic fertilizers that was prepared from easily available sources for the faster and easier growth of *L.esculentum* Linn.

SUMMARY AND CONCLUSION

Application of *Azolla* Biofertilizer, considerably improved the growth performance of *L. esculentum* Linn. Compared to chemical fertilizers *Azolla* biofertilizer gives better result as it has excellent C & N ratio. The application of biofertilizer also increases organic matter content in the soil which in turn triggers the growth and multiplication of soil microbes. It is cost effective and eco-friendly so it can be directly applied as a biofertilizer. The result of the present study shows that *Azolla* biofertilizer had a significant impact on soil microbial C&N value.

Bio fertilizers from microorganisms can replace chemical fertilizers to increase crop production. In principle, bio fertilizers are less expensive and are more environments friendly. Bio fertilizers mainly help in nitrogen fixation process. *Azolla* extract has a good fertilizing capacity and is used in paddy fields respectively. By continuous harvesting, high protein yields can be obtained from *Azolla* cultures, without the need for nitrogen fertilization. Blue green algae in *Azolla filiculoides* are good utilizes of solar energy and provide organic matter to the soil. Promotion of nitrogen fixation by inoculation of highly efficient *Azolla filiculoides* and improvement of soil management and cropping practice is very important to increase the yield of tomato plants especially in Asia. Evaluation of nitrogen fixation is important to understand the application of *Azolla* as a bio fertilizer respectively. The extract acts as a desirable biological fertilizer supplement to chemical fertilizer for organic farming. The extract is nontoxic, harmless, non-flammable and effective to all plants for attaining better germination, growth and yield. Nitrogen available in the soil was also increased due to bio fertilizer treatments over the use of 100% N treatment (control). Earlier studies have

shown that incorporation of fresh or dry *Azolla* into soil increased significantly the soil organic matter, which in turn upon its decomposition by the soil microorganisms had released the macro and micronutrients into soil, leading to increase the soil available N, P and K. In this study, the use of *Azolla* extract applied in different concentrations improved root and shoots length, number of leaves, chlorophyll and carotenoid content. Among the different crops, vegetables are most affected one by the use of chemical fertilizers and pesticides. From the present study the potential of *Azolla* biofertilizer in enhancing the vegetative growth and crop yield of *L.esculentum Linn.* could be proved.

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