Effect of bio-stimulants on performance of strawberry (*Fragaria* × *anana*ssa Duch.) cv. Festival under organic cultivation

A thesis
Submitted to the

Uttar Banga Krishi Viswavidyalaya

In partial
Fulfilment of the requirements for the award of the Degree of

MASTER OF SCIENCE (HORTICULTURE)
in
FRUIT SCIENCE
by

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(H-2020-012-M)

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2022

THIS THESIS IS DEDICATED TO MY BELOVED GRANDPARENTS

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CERTIFICATE I

This is to certify that the work recorded in the thesis entitled, **"Effect of bio-stimulants on performance of strawberry (*Fragaria* × *ananassa* Duch.) cv. Festival under organic cultivation"** submitted by **Miss Sudarshana Bhujel (H-2020-012-M)** in partial fulfillment of the requirements for the degree of M.Sc. (Horticulture) in Fruit Science of the Uttar Banga Krishi Viswavidyalaya, Pundibari, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the study reported in the thesis have not so far been submitted for any other degree or diploma.

The assistance and help received from various sources during the course of investigation have been duly acknowledged.

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CERTIFICATE –II

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Minor Subject	:	Post Harvest Technology
Name of the Chairman	:	Dr. Nilesh Bhowmick
Designation of the Chairman	:	Professor
Degree to be awarded	:	M.Sc. (Horticulture) in Fruit Science
Year of Award of Degree	:	2022
Name of the University	:	Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar,West Bengal

ABSTRACT

Strawberry (*Fragaria* × *ananassa* Duch.) a man made hybrid is one of the world's most delicious and refreshing soft fruits. It is an aggregate fruit under the Rosaceae family and a cultivated octaploid having chromosome number 2n=8X=56. The demand of strawberry fruit has been gradually increasing for both domestic and foreign markets. Strawberry has been promoted as a cash crop among farmers in rural regions of northern parts of West Bengal in recent years by the different government and non-government organizations. People are becoming more health concerned due to the use of chemical fertilisers and the vulnerability to illnesses. Popularizing demand for organic fruits is resulted in potential increment of income for producers. The existing agro-climatic condition is highly suited for strawberry cultivation in the northern parts of West Bengal. A considerable area is under organic cultivation in these locality. However, the soils are mostly acidic in nature, with a coarse texture and low in fertility. Since, bio-stimulants are compounds that improve plant growth, resilience to water, and tolerance to abiotic stress and thereby, reducing the need for fertilisers. Considering this fact and its importance, the present experiment was conducted in strawberry cv. Festival using foliar spraying of four kinds of bio-stimulants with a frequency of one spray (30 DAP) and two sprays at (30 days after planting and two sprays at 30 and 60 days after planting) at a concentration of 4mll^{-1} and 4gl^{-1}, comprising of nine treatments including one control (water spray at 30 and 60 days after planting) and all the treatments were replicated thrice in Randomized Block Design (RBD) at Directorate of Cinchona and Medicinal Plants Mungpoo, Dargeeling,West Bengal. It was found that the foliar application resulted maximum plant height (21.30cm), root length (22.07cm), flowering duration (98.33 days), yield (650.10 g per plant) with humic acid @ 4gl^{-1} applied at 30 and 60 days after planting, whereas, earliest flower bud emergence (82.32 days) was noticed in application of humic acid @ 4gl^{-1} at 30 DAP and fruit diameter (3.60cm), fruit weight (20.75g) was recorded maximum in seaweed extract @ 4mll^{-1} applied at 30 and 60 DAP. Based on the results, it can be concluded that foliar applications of humic acid at 4 gl^{-1} at two distinct intervals 30 DAP and 60 DAP proved to be the most suitable treatment for boosting flowering, fruit production and fruit quality attributes of strawberry. The findings suggested that the usage of humic acid has also resulted overall yield and quality attributes of strawberry.

Keywords: Strawberry, organic, bio-stimulant, flowering, fruiting

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ACKNOWLEDGEMENT

Better than a thousand hollow words, is one word that brings peace and great achievements. Words are very poor substitute to express one's emotions and feelings, there are no other alternative to give vent to one's sentiments, particularly on an occasion like this, my acknowledgements are many times more than what I am expressing here.

Graceful as He always is, I shall forever be thankful to God for He has always showered His blessings and love upon me, little would I prosper without His unbound grace.

It is my proud privilege to express my deepest sense of gratitude to my venerable and belovedChairman of my advisory committee **Dr. Nilesh Bhowmick**, Professor, Department of Pomology and Post Harvest Technology, for his learned counsel, arduous and meticulous guidance, kind treatment during the course of investigation and constructive criticism have installed in me the spirit of confidence and deep sense of respect and gratitude. The keen interest, abundant because of which I have been able to successfully complete the work assigned to me.

Mere words can never suffice the expression of the sense of my insolventness and gratitude to the other members of my advisory committee **Dr. Aditi Chakraborty, Dr.Binayak Chakraborty and Dr.Mutum Preema Devi (Dept. of PPHT)** for their priceless advice.

I would like to express my special thanks of gratitude to **Dr. Samuel Rai**, Director and other staff of the **Directorate of Cinchona and Other Medicinal Plants**, Mungpoo, Darjeeling, West Bengal for allowing me to conduct the experiment in the strawberry polyhouse during COVID-19 pandemic and for providing me with all the facility that was required.

I avail this opportunity to convey my deepest sense of regards to **Dr. Sarad Gurung In Charge, RRS, Hill Zone, Kalimpong, UBKV, Sri Akashdeep Thapa, Farm Manager of Dalapchand Farm, Darjeeling KVK** and all the respected teachers of Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari for their unconditional support, guidance and suggestion encouragement and care.

It's my fortune that I have been blessed with wonderful batchmates **Umrasong Mog, Debidatta Barman, Sajitha Siril, Sanasam Angousana**. I emphatically extend my hearties thank for their constant support and for making my moment enjoyable and memorable.

I am lucky to have brilliant and even dedicated galaxy of my beloved juniors **Diya, Apoorva, Tejaswini, Sahil, Gururaj** who were always willing to help me at the time of my research work.

I would like to convey my sincere thanks to all my seniors **Santosh da, Bidya di,** *Novin da, Nim da, Saidiksha di, Vivek da, Sukanya di, Swadesh da, Sindhu akka and Dr. Santanu Layek, Assistant Horticulturist, DCMP, Mungpoo, West Bengal for their help and cooperation in completing my research work..*

*I avail myself of this opportunity to express my sincere gratitude with great reverence to **Shri. Nitesh Ch Saha (Technical Asst), Shri. Ananda da, Shri. Saurav da** for their sincere help during my research.*

*I am highly thankful to **Shiva da** for his valuable help during the field work and keeping the record.*

*My words fall short of expressing how much my parents, **Mr. Mani Kumar Bhujel** and **Mrs. Adina Chettri**, who are my pillars of support, have meant to me. They have always been a source of inspiration for me and have shown me unwavering love, support, and encouragement.*

I acknowledge with pleasure the Uttar Banga Krishi Viswavidyalaya for monitoring me to pursue post graduation programme in Faculty of Horticulture, Pundibari.
The presentation that follows is the work assisted by many seen and unseen hands and minds, I am thankful to all of them.

Place: UBKV, Pundibari.

Date: 7/09/22

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SPECIAL ACKNOWLEDGEMENT

I would like to express my special thanks of gratitude to **Dr. Samuel Rai***, Director and other staff of the* **Directorate of Cinchona and Other Medicinal Plants***, Mungpoo, Darjeeling, West Bengal for allowing me to conduct the experiment in the strawberry polyhouse during COVID-19 pandemic and for providing me with all the facility that was required.*

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Abbrevations	**: Description**
%	: Per cent
@	: At the rate of
Cv	: Cultivar
Cm	: Centimeter
Kg	: Kilogram
Mg	: milligram
DAP	: Days after planting
et al.,	: Co-workers
Fig.	: figure
g	: gram
m	: Meter
m^3	: Cubic meter
NPK	: Nitrogen phosphorous potassium
NS	: Non significant
SEm±	: Standard error of mean
LSD	: Least significant difference
g/plant	: Gram per plant
i.e.	: That is
TSS	: Total soluble solids

Chapter I
INTRODUCTION

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.), a manmade hybrid is one of the world's most delicious and refreshing soft fruits. It is an aggregate fruit of Rosaceae family and a cultivated octaploid with chromosome number 2n=8x=56 (Wang *et al* 1996). Its progenitors are the two American diploides *F. chiloensis* and *F. virginiana*. Although China, Mexico and the United States are the top three producers, strawberries are grown on every continent using local varieties and techniques (USDA, 2021). The temperate regions of India, such as Jammu and Kashmir and Himachal Pradesh extensively cultivating strawberry since last two decades or more. However, in recent years, the development of new varieties that can withstand a wide range of climatic conditions and the standardization of new agro-techniques have led to strawberry cultivation in non-traditional provinces of India too (Sharma and Sharma 2004). It is mostly grown in the hills of India. Its primary growing regions are Kashmir Valley, Bangalore, Dehradun, and Nainital (district) in Uttarakhand, Maharashtra, and West Bengal (Anon, 2016). According to Singh *et al* (2008), its cultivation is now becoming more popular in the tropical and subtropical regions of Pune (Maharashtra), Muzaffarnagar, Saharanpur, and Meerut (UttarPradesh), Jalandhar and Gurdaspur (Punjab), Gurgaon (Haryana), Bangalore (Karnataka), and Meghalaya. Currently, strawberry is grown on 6,000 hectares of land throughout India with annual production of around 4.3 thousand metric tonnes for both domestic consumption and export (Annon, 2018).

Chandler, Tioga, Belrubi, Fern, Torrey, Silva, and Pajaro are some of the strawberry cultivars growing successfully in India. It is a good source of minerals (Calcium 14 mg, Iron 0.38 mg, Magnesium 10 mg, Phosphorus 10 mg, Potassium 166 mg, Sodium 1 mg per 100 g of edible portion), vitamins (Vitamin C 56.7 mg, Thiamin 0.02 mg, Riboflavin 0.066 mg, Niacin 0.23 mg, Pantothenic acid 0.34 mg, Vitamin B-6 0.059 mg, Vitamin A 27 IU, Vitamin E 0.14 mg per 100 g of edible portion) amino acids like Isoleucine 0.014 g 100 g^{-1}, Lysine 0.025 g 100 g^{-1}, Methionine 0.001g 100 g^{-1} etc (Sharma, 2002). Strawberry can be processed to make wine, jam, ice cream, jelly, soft drinks, etc. In addition, synthetic strawberry flavours and scents are widely used in a variety of products, including lip gloss, hand sanitizers, confectionery, perfume, and many more.

The demand of strawberry fruit has been gradually increasing for both domestic and foreign markets. It has been promoted as a cash crop among farmers in rural regions of northern parts of West Bengal in recent times by different government and non-government organizations. Prevailing agro climatic conditions is believed to be congenial for growing strawberry in open field in the areas like Darjeeling, Kalimpong, Jalpaiguri, Alipurduar, Coochbehar districts of the state. It is currently farmed on about 10,000 bighas with the productivity of 1,000kg bigha^{-1} (Rai, 2020).

Although strawberry are considered a temperate fruit, but can be grown in subtropical climates. In temperate and subtropical countries, strawberry is frequently cultivated in both protected and open conditions, with mean day temperature ranging from 22–25°C and night temperatures 7–13°C. It thrives in humid or dry regions with a typically need of 12-hours of sunshine. Well-drained, medium loam soil rich in organic matter is best suited for strawberry cultivaton. The soil should be some what acidic with a pH ranging from 5.7 to 6.5 (Anon, 2017).

Continuous use of chemical fertilisers without any use of organic manures or biofertilisers has resulted in a decline in soil health in terms of physical and chemical properties, a decline in soil microbial activities, a reduction in soil humus, increased pollution of soil, water, and air with significant reduction in crop growth and yield. Excessive use of chemical fertilisers and pesticides, particularly at fruiting stage is very harmful and serious concern for human health. Further, it may also affect the bio-environmental and conventional agricultural systems, which can be remedied through organic farming. Protecting the environment and soil health as well as the negative effects of intensive fruit production are urgently needed (Dobrzyński *et al* 2014).

People are becoming more health concerned as a result of the use of chemical fertilizers and the vulnerability to illnesses. Hence, demand for organic fruits is growing day by day, resulting in increased income for producers. One of the most essential techniques for sustainable agriculture is the utilization of environmentally friendly bio-products. Using bio-regulators to increase production is a popular horticultural practice (Latimer, 1992). In horticulture, biologically derived substances have been employed to avoid or mitigate abiotic and biotic stress too. Many of these materials are natural products that do not contain chemical or plant growth regulators (Russo and Berlyn, 1991). They are known as bio-stimulant.

A large variety of bio-stimulants, which are applied to crops, have arrived on the market (Boehme *et al* 2004). According to Crouch *et al* (1992), they can be divided into three categories based on their source and content: humic substances, hormone-containing products, and amino-acid-containing products.

Bio-stimulant is a combination of peptides and free amino acids, produced by chemical and/or enzymatic hydrolysis of organic matrix of plant or animal origin, with a wide range of composition (Maini, 2006). With the understanding of bio-stimulants and their promise for sustainable agriculture production, a large number of bio-stimulant products are being introduced to the market which require further research. The nutritional health of strawberry plants is critical for achieving the desired levels of productivity and overall fruit quality. The rate of mineral nutrients (macro and micro) utilized during its entire production cycle was found to be positively connected with growth, yield and quality indices. Strawberry cultivation in the modern world is frequently done in the protected systems viz. greenhouse, plastic tunnel etc. and in conjunction with soil less technology.

In the hill zone of West Bengal, strawberry cultivation is becoming more and more popular, but marketing and financial success are suffering since the fruit doesn't have the demanded quality and size. The soil of Darjeeling is coarse textured, acidic in nature with low in fertility (Anon, 2012). Since bio-stimulants are very helpful to improve plant growth, resilience to water and tolerance to abiotic stress while reducing the need for fertilisers, these compounds are effective at modest quantities, encouraging the smooth operation of the plant's critical activities and allowing for high yields and high-quality products. Considering this fact an experiment was conducted to study the impact of different bio-stimulant on growth, flowering and fruiting characteristics of strawberry at the Directorate of Cinchona and other Medicinal Plants, Mungpoo, Darjeeling,West Bengal with the following objectives–

i) To study the influence of different bio stimulants on growth parameters of strawberry under organic cultivation.

ii) To study the influence of different bio-stimulants on flowering and fruiting characteristics of strawberry under organic cultivation.

iii) To study the time of application of different bio-stimulants on strawberry under organic cultivation.

Chapter II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The present investigation entitled **"Effect of bio-stimulants on the performance of strawberry (*Fragaria* × *ananassa* Duch.) Cv. Festival under organic cultivation"** was conducted during September 2021 to June 2022. This chapter discusses several major reviews of literature that are pertinent to this research.

Al-Shatri *et al* (2016) investigated the effects of four dosages (0, 2, 4, and 8 gL^{-1}) of seaweed extract (Alga 600) applied with fertigation on nutrient absorption of strawberry cv. Albion. The macro and micronutrient content of strawberry leaves was improved by 8 gL^{-1} seaweed extract.

Soppelsa *et al* (2020) used calcium chloride alone and in conjunction with a commercial product containing a seaweed extract to spray on the tree canopy of "Jonathan" apple. The seaweed extract improved apple quality by increasing reddish colour (+32 percent colour index) and final anthocyanin content in the fruit skin. After 160 days of storage, both bio-stimulants considerably reduced the occurrence of "Jonathan spot" by 20%.

Soppelsa *et al* (2019) used different classes of bio-stimulants at commercial dosages on strawberry plants seven times from pre flowering to berry maturation. Chitosan treatment considerably enhanced pulp hardness by 20%, whereas alfalfa- and seaweed-treated fruits had a high nutritional value (e.g., phenolics content) (18%–20% higher as compared to control).

The effect of foliar spray of seaweed extract (1.0 or 2.0 mlL^{-1}) on strawberry growth, chlorophyll, mineral content and various fruit-quality metrics as well as yield was studied by El-Miniawy *et al* (2013). The most effective treatment for improving fruit growth and quality, of strawberry cv. Sweet Charlie was three foliar spray of 2.0 mlL^{-1} seaweed extract.

Abourayya (2020) studied the effect of soil application of humic acid and foliar spray of milagro bio-stimulant on three-year-old uniformly grown Nonpareil almond. They applied the bio-stimulant at the start of the growth season. The soil application of 30 g humic acid with 30 mlL^{-1} milagro per tree as foliar spray was the most promising treatment as a new non-chemical, low-cost, and environmentally safe fertilisation technique for improving growth and nutritional status of Nonpareil almond under Nubaria conditions.

Eshghi and Garazhian 2015 found that foliar treatment of humic acid notably at 600 and 900 mgL^{-1}, significantly enhanced growth yield and fruit quality of strawberry cv. Paros.

Ullah *et al* (2014) investigated the effect of humic acid (1.5, 3.0, and 4.5 mlL^{-1})on strawberry cv. Chandler. Humic acid sprayed at 30 days after transplantation at 3mlL^{-1} resulted in more fruits (28.3) per plant, total carotenoids (17.84 mgg^{-1}) and total lycopene content (0.0244mgg^{-1}) whereas, at 4.5mlL^{-1} humic acid spray, the least percent of fruit waste was reported.

Aminifard *et al* (2012) applied fulvic acid (0, 25, 100, 175 and 250 $mgkg^{-1}$) to examine its influence on pepper antioxidant activity and fruit quality. Fulvic acid administered at 25 $mgkg^{-1}$ resulted in the greatest carbohydrate, lycopene and carotene levels whereas, the control had the lowest values. Fruit quality in terms of total soluble solids and titratable acidity was favourably improved by fulvic acid treatments.

A dilute combination of humic and fulvic acids (Zymo) derived from earth worm humus were sprayed from bloom to fruit maturity in weekly interval. Long-term humic acid treatment improved fruit quality by lowering the frequency of deformed and rotting fruits while also boosting sugar content significantly (Neri *et al* 2002).

The influence of bio-stimulants on the nutritional condition and fruit yield of 'Kent' mango was investigated by Lobo *et al* (2019). Bio-stimulants comprising soluble nutrients, L-amino acids, free amino acids and Lithothamnium algae extract improve the nutritional condition of the mango 'Kent' and boosted the fruit yield significantly.

Shafeek *et al* (2016) studied the effect of humic acid by ground drenching and spraying on cucumber plants cultivated in a plastic house. It was recorded that increasing humic acid levels enhanced cucumber growth, fruit yield and quality significantly over control.

El-Hoseiny *et al* (2020) investigated the effects of individual and combined application of humic acid (potassium humate: 0.15%, 0.30%, 0.45%) and boron (boric acid: 300, 600 mgL^{-1}) on the development of "Zebda" mango trees in Egypt. The largest observable effect was seen with the application of 0.30% humic acid + 600 mgL^{-1} boric acid where the annual tree productivity and fruit quality was improved significantly over control.

Citrus fruit harvested from trees sprayed with 0.5 mlL^{-1} ‘Primo’ (amino acid) exhibited highest increase in fruit physical and biochemical quality characteristics (Khan *et al* 2022).

During the two consecutive seasons of 2009-2010 and 2010-2011, researchers investigated the effect of foliar spraying of seaweed extract (1.0 or 2.0 mlL^{-1}) with different number of applications on strawberry growth, chlorophyll and mineral content of leaves, fruit-quality parameters as well as yield. Foliar spraying of seaweed extract at 2.0 $mlL^{-1}$for three times was shown to be the most effective treatment to boost the plant growth, fruit quality and yield of strawberry cv. Sweet Charlie (El-Miniawy *et al* 2014).

For each apple cultivar, forty trees were treated with seaweed based leaf extracts (brown algae, *Fucus spp*) that included mineral nutrients at 3.8, 3.5, and 5.9 $kgha^{-1}$ $year^{-1}$ of N, P_2O_5, and K_2O, respectively (Malaguti *et al* 2001). In the cultivar Mondial Gala, foliar sprays boosted red colour intensity and spread, but not in Fuji.

Tomato plants were given varying concentrations of Humic acid treatment in the soil and on the leaves (0, 10 and 20 mlL^{-1}). The study found that humic acid sprays with a concentration of 20 mlL^{-1} might be used successfully to improve tomato growth and production (Yildirim2007).

Fulvic acid at 80–160 mgL^{-1} increased plant vigor, root length, yield and quality of tomato significantly (Zhang *et al* 2021).

The impact of soil and foliar application of humic and amino acids on strawberry (*Fragaria* x *ananassa*) cv. Festival was examined by Shehata *et al* (2011). The findings of this study revealed that strawberry grown in compost treated plots with humic acid had much longer plants and heavier fruits when compared to mineral fertiliser.

Green algae and amino acids were investigated as bio-stimulants on three hot pepper cultivars in a field experiment, carried out by Zamljen *et al* (2021). Although the impact of both was primarily cultivar specific, amino acids were shown to be superior to algal extract.

Zewail (2014) had conducted an experiment to investigate the effect of seaweed and amino acid on common bean plant (*Phaseolus vulgaris* L.). The obtained results clearly showed that application of seaweed at 2 mlL^{-1}combined with the amino acids at 4 mlL^{-1} increased the growth and yield attributes in terms of plant height, leaves per plant, total leaf area per plant, dry weight of shoots, specific growth rate, number of pods per plant pod weight as well as photosynthetic pigments and total chlorophyll content at the age of 65 days after sowing.

Mufty and Taha (2021) investigated the effects of foliar spray of humic acid and seaweed extract at three concentrations on growth, flowering and yield of strawberry cultivars Albion and Rubygem. The results revealed that Rubygem had a greater leaf dry weight, root dry weight, number of flowers, yield per plant as well as nitrogen and phosphorus content in the leaf.

Seaweed extract (0.1-0.5%) were used as foliar sprays in combination with minimal to no wetting agents. While drench application had no impact on the physiological condition of the plant, foliar application promoted photosynthetic recovery in grapevines under drought stress (Frioni *et al* 2021). Similar findings was also reported in sour orange by Spann *et al* (2011).

Seaweed foliar spray at 1.33 gl^{-1} improved TSS, fructose, sucrose, and health-related compounds in strawberry cv. Rubygem and cv. Kabarla while also raising the quercetin content in both the cultivars (Kapur *et al* 2018).

Brown seaweed extracts at high dosage (33% solution with tap water) were able to improve iron absorption with favourable effects on plant growth, yield and physiological status of strawberry after a single drench treatment (Spinelli *et al* 2010).

Foliar spray of seaweed extract applied 2 or 3 times at weekly interval in almond increased K uptake leaf area and over all biomass accumulation as reported by Saa *et al* (2015).

Spinelli *et al* (2010) reported that effect of seaweed (extracted from brown algae) drenched at 33.3% (10 mL of product in 20 mL tap water) increased rhizosphere acidification significantly with consequent higher iron uptake, increased vegetative growth, leaf chlorophyll content, stomata density, photosynthetic rate and yield.

Irani *et al* (2021) observed that foliar spray of amino acid in grapevine @ 0.5% increased berry size, fruit yield, total soluble solids significantly while decreased titratable acidity. Further, it was also helped to increase chlorophyll, ABA, proline, nutrients, soluble carbohydrate, proteins and ROS scavenging enzymes activities (GPX and CAT) significantly over control.

Garde-Cerdán *et al* (2014) noticed that foliar spray of amino acid in grapevine @ 0.075% increased total amino acid concentration (30%) and yeast assimilable nitrogen (58%) significantly.

Mosa *et al* (2021) studied the effect of foliar application of amino acid in apple @ 25-100 ppm which increased leaf nutrient and chlorophyll concentrations, fruit set (9%–24%), fruit size (14%–21%) and fruit yield (75%–162%) significantly; while reduced the fruit drop (20%–31%). Further, it also increased fruit carbohydrate concentration (7%–46%) and flesh firmness (18%–79%) significantly over control.

Foliar application of humic acid and fulvic acid in apricot @ 5gL^{-1}was found effective to increase the antioxidant activity in apricot at harvest (60%–220%) (Tarantino *et al* 2018).

Similarly, foliar application of humic acid in grapevine @ 0.58–0.73 gL^{-1} was reported to increase berry weight, berry volume, bunch weight, fruit yield per vine (23%–32%) and soluble solids content in berry juice at harvest (5%–12%) as compared to control (Popescu *et al* 2018).

El-Razek *et al* (2012) reported that foliar or drench application of humic acid in peach @ 0.25−0.5% increased the fruit weight (31%–78%), fruit yield per tree (31%–78%), soluble solids content in fruit juice (18%–51%), skin anthocyanins (47%–88%), and decreased titratable acidity in fruit juice (0.43 %) at harvest.

In strawberry foliar spray of humic acid @ 0.025−0.100 gL^{-1} had increased fruit weight (41%–169%), fruit yield per plant (55%–116%), vitamin C content (1%–2%), red colour intensity of fruit skin (765%–796%), leaf P, K, Ca, Mg concentration and decreased total antioxidant capacity (8%–24%) as reported by Aghaeifard *et al* (2016).

El-Hoseiny *et al* (2020) reported that foliar application of humic acid in mango @ 0.15−0.45% increased vegetative growth, tree fertility, fruit yield per tree (24%–192%), fruit weight (5%–12%) and soluble solids content of fruit juice (5%–11%) at harvest. While treating the pomegranate fruits with humic acid @0.5–1.5%, at harvest.

Kamal *et al* (2017) reported that fruit yield per tree (18%–64%), fruit weight (13%–50%), number of fruits per tree (8%–39%), percent of fruit juice (5%–10%), soluble solids content of fruit juice (14%–19%) at harvest was increased significantly over control by humic acid treatment which significant reduction in titratable acidity (8%–19%).

According to Mansy *et al* (2004) foliar application of the seaweed extract to strawberry cultivars Elkat and Salutin in Poland affected both yield and quality.

According to Norrie and Keathley (2006) grape cv. Thompson seedless was applied with *Ascophyllum nodosum* seaweed extract. With 4 and 8 sprays @ 2 Lha^{-1}, increased the quantity of berries per bunch, the size of the berries, and the number of primary bunches per plant. The yields of treated plots were greater the next year than those of the control, which was attributable to an increase in berry weight and size.

Giuseppe *et al* (2005) studied the effect of humic acid foliar application on grapes cv. Italia. They noticed that when compost humic acid (20 mg/l) was sprayed on grape vines, the total soluble solids increased to (16.3 °B), the °Brix/acidity ratio increased to (43.9), and the acidity level decreased to (0.37 g/100 ml of juice)

Fathy *et al* (2010) studied the impact of humic acid treatments on the growth, yield, and fruit quality of 'Canino' apricots. They discovered that 2.9% humic acid + 10% NPK applied as a foliar spray had the greatest effects on fruit set (27%) and fruit yield (31.4 kg/tree).

In peach trees, El-Boray *et al* (2016) found that the use of bio-stimulants increased shoot growth. This was brought on by the increased availability of organic or inorganic nutrients in addition to the direct uptake of carbon, which prevented photorespiration and improved plant-growth responses.

Thanaa *et al* (2016) examined the effects of foliar sprays of seaweed extract + amino acids on the vegetative characteristics of the 'Anna' apple tree, either alone or in combination. They found that, in both seasons, the application of 2 ml/l seaweed extract + 0.5 ml/l amino acids produced the most significant shoot length and leaf area.

Chapter III

MATERIALS AND METHODS

MATERIALS AND METHODS

The study **“Effect of bio-stimulants on performance of strawberry (*Fragaria* × *ananassa* Duch.) cv. Festival under Organic Cultivation”** was conducted from September 2021 to June 2022. In this chapter, experimental tools, procedures and statistical design employed through out the research have been discussed.

3.1 Experimental Site

This experiment was conducted at the Directorate of Cinchona and Other Medicinal Plants, Mungpoo, Darjeeling, West Bengal during September 2021 to June 2022. The area lies under the northern hill zone of West Bengal at 26°58'17''N latitude and 88°22'16''E longitude (measured with geographic coordinate system). Altitude of the area was 1200 ft above MSL. Darjeeling area is having an acidic soil with coarse texture and low fertility level (Anon, 2012). Average temperature was maximum 30°C and minimum 08°C with an average rainfall of 4500 mm per annum.

Table 3.1.1. Chemical properties of the experimental soil

Particulars	**Values**	**Methods Employed**	**Reference**
Ph	5.41	The soil pH was determined in1:2:5 soil pH Meter	Black (1965)
Organic Carbon	0.73%	Walkley and Black’s rapid titration method (Jackson, 1973)	Jackson (1973)
Available N	392.66 $kgha^{-1}$	Alkaline; $KMNO_4$ method developed	Subbiah and Asija (1956)
Available P	20.10 $kgha^{-1}$	Brays’s method-Bray and Kurtz	Jackson (1973)
Available K	320.83 $kgha^{-1}$	Flame photometer method	Black (1965)

Table 3.1.2. Mean meteorological data during the period of field experimentation (2021-2022).

Month-Year	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)
	Maximum	Minimum	Maximum	Minimum	
Sep-2021	28.5	20.6	86	72	152.3
Oct-2021	25.4	18.6	87	80	323.8
Nov-2021	22.8	12.0	75	56	3.2
Dec-2021	18.5	9.2	69	58	17.6
Jan-2022	16.6	8.6	68	61	3.9
Feb-2022	15.3	8.3	80	69	53.9
March-2022	25.5	15.9	76	50	1.5
April-2022	25.6	18.2	83	70	63.7
May-2022	26.0	19.2	84	77	151.7
June-2022	25.4	20.5	92	87	271.4

Source: Integrated Agromet Advisory Services, Kalimpong observatory, Uttar Banga Krishi Viswavidyalaya.

3.2 Methodology

3.2.1. Experimental Method

Strawberry cv. Festival were chosen from the polyhouse at the Directorate of Cinchona and Other Medicinal Plants, Mungpoo, Darjeeling,West Bengal. During September, 2021 53 ft × 33 ft raised beds were made under the polyhouse with a gap of 30 cm between the beds. In those beds strawberry plants were planted at a spacing of 45cm × 30cm. During the preparation of beds FYM @ 25 kg per $6m^2$ (individual bed size) was applied.

Time of planting: Last week of September (29/09/2021)

Planting material used: Runner plantlets

Plant spacing: 45 cm × 30 cm.

Type of bed: Raised beds.

Gap between beds: 30 cm among the adjacent beds

Size of the individual bed: 6m× 1m ($6m^2$)

Plants per beds: Twenty (20)

Number of beds: 9×3: 27 (Twenty seven)

Total plants under the experiment: 27× 20: 540

Method of application of biostimulants : Foliar application

Number of foliar treatments: Nine (09)

3.2.2. Treatment details:

Sl.	Treatments
T_1	Control (Water spray) at 30 + 60 DAP
T_2	Seaweed extracts - $4mll^{-1}$ at 30 DAP
T_3	Humic acid - $4gl^{-1}$ at 30 DAP
T_4	Fulvic acid - $4gl^{-1}$ at 30 DAP
T_5	Amino acid - $4mll^{-1}$ at 30 DAP
T_6	Seaweed extracts - $4mll^{-1}$ at 30 + 60 DAP
T_7	Humic acid - $4gl^{-1}$ at 30 + 60 DAP
T_8	Fulvic acid - $4gl^{-1}$ at 30 + 60 DAP
T_9	Amino acid - $4mll^{-1}$ at 30 + 60 DAP

***DAP=Days after transplanting**

No. of replication: Three (03)

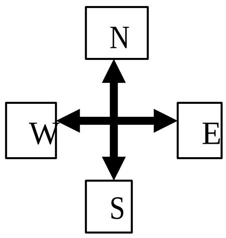
Design of experiment: Randomized Block Design (RBD)

Table 3.2.3 Sources of bio-stimulant

The bio-stimulant source included the following:

Sl.	Name of bio-stimulant	Source
1	Seaweed extracts	Sagarika made by IFFCO it contains 28% seaweed extract (Red & Brown Algae)
2	Humic acid	Tata rally gold GR made by Rallis India Limited it contains Humic acid-28.9%
3	Fulvic acid	Shilajit Double Power (Fulvic acid) made by Hifield Organics Inc. It contains Fulvic Acid 80%
4	Amino acid	Syngenta Isabion made by Arzignano, Italy it contains amino acid 62.5%

Table 3.2.4 Lay out of Experimental plot



Road

Entrance gate

T_1 R_1	T_4 R_2	T_5 R_3
T_8 R_1	T_6 R_2	T_7 R_3
T_2 R_1	T_3 R_2	T_9 R_3
T_5 R_1	T_9 R_2	T_2 R_3
T_9 R_1	T_7 R_2	T_4 R_3
T_6 R_1	T_5 R_2	T_8 R_3
T_4 R_1	T_8 R_2	T_1 R_3
T_3 R_1	T_1 R_2	T_6 R_3
T_7 R_1	T_2 R_2	T_3 R_3

Cinchona plantation

3.2.5. Cultural practices

During field preparation, farmyard manure was applied as fertiliser at a rate of 25 kg per bed.The polyhouse was manually weeded at every 20 days interval. Sprinkler irrigations were given as and when needed. The field is kept weed free by applying plastic sheets. To prevent disease, insect and pest infestation, there commended organic fungicides and pesticides were applied.

3.3.1. Observation recorded

Plant height, number of leaves, length of the shoots, root length, number of runners per plant, days taken to emergence of first flower bud, flowering and fruiting duration, fruit set percent, total number of fruits per plant, yield, fresh weight of shoot and root was measured in Directorate of Cinchona and Other Medicinal Plants, Mungpoo, Darjeeling. Just after harvesting, ripened fruits were brought with all protective measures to the department of Pomology and Postharvest Technology, Uttar Banga Krishi Viswavidyalaya, pundibari, Coochbehar for the estimation of fruit quality attributes. The details of the observations are as follows-

3.3.2. Physical parameters

Observation on different aspects of plant growth, flowering and fruiting parameters were recorded.

3.3.2.1. Plant height (cm): The average height of plants was measured using a measuring scale from the crown level to the tip of the main leaves at the intervals of 30 days (Rana, 2001).

3.3.2.2. Number of leaves: Data on the number of leaves was recorded from 10 plants of two rows in the centre of each bed and results was expressed as a leaf number per plant (Rana, 2001).

3.3.2.3. Length of the petiole (cm): The petiole length of ten randomly selected plants was measured from the tallest tips to the end of the crown using measuring scale at intervals of 30 days and their mean values was calculated there after (Thokchom,2019).

3.3.2.4. Root length (cm): Root lengths of ten random plants was measured from the crown end to the tip of the longest root with the help of centimeter scale in cm and their average was calculated (Thokchom, 2019).

3.3.2.5. Number of runners/plant: The average number of runners produced by each plant was recorded after harvest of the fruits.

3.3.2.6. Days taken to emergence of first flower bud: The period between the date of planting to date of first flower opening was recorded for calculating the days taken to produce first flower (Kidmos *et al* 1996).

3.3.2.7. Flowering duration: From the opening of first flower to last one for individual plant was recorded to determine the duration of flowering. It was expressed in terms of days.

3.3.2.8. Duration of fruiting: The duration of fruiting periods was recorded i.e., from the first fruit set to the last fruit setting. It was expressed in terms of days.

3.3.2.9. Fruit set percentage (%): The following equation was used to compute the fruit set percentage:

$$Fruit\ set\ percentage\ (\%) = \frac{\text{Number of set fruits}}{\text{Total number of opened flowers}} \times 100$$

3.3.2.10. Total number of fruits/plant: Total number of fruits per plant was recorded at the time of harvesting from 10 plants and results was expressed as number of fruits per plant.

3.3.2.11. Fruit weight (g): To measure the average fruit weight, 10 fruits from each replication were randomly chosen and weighted on an electrical weighing balance. Then average fruit weight was calculated and expressed in gram.

3.3.2.12. Fruit diameter (cm): The length of ten randomly chosen fruits from each replication was measured with a vernier calliper and the value was expressed in centimetre.

3.3.2.13. Yield (g/plant): The total fruit production in each treatment was recorded from 10 plants per plot and yield per plant was calculated in g per plant.

3.3.2.14. Shoot fresh weight (g): The fresh weight of shoot of ten randomly selected plants were weighted through weighing balance and their average was recorded and expressed in grams (g) (Thokchom, 2019).

3.3.2.15. Root fresh weight (g): Ten random plants was uprooted and their weights was measured with the help of weighing balance in grams (g) and their average was calculated (Thokchom, 2019).

3.3.2.16. Shoot dry weight (g): After washing the uprooted plants, shoots was placed in a hot air oven for 48 hours at 80°C. Thereafter, the shoot dry weight of each plant was recorded and represented in gram per plant (Thokchom, 2019).

3.3.2.17. Root dry weight (g): Roots of uprooted plant was washed and kept in hot air oven for 48 hours at 80°C and there after dry matter weight per plant was recorded as root dry matter gram per plant (g/plant) (Thokchom, 2019).

3.3.3. Bio-chemical parameters

3.3.3.1. Total soluble solids (ºBrix): By using a hand refractometer made by ERMA having 0-30 ºB scale, the total soluble solids (TSS) of the fruit samples was measured and the result was expressed in °Brix (^{o}B) following the method described by Ranganna (1986). It was adjusted at 0 ^{o}B at 20^{o} C using a temperature correction table. It was measured for ten fruits for each replication and average was calculated there after.

3.3.3.2. Total sugar (%): Ten gm of freshly collected fruit sample was taken and it was crushed with luke warm water with the help of mortar and pestle. 100ml of volumetric flask was taken and volume made up to 100 ml. Then 20 ml of juice was taken in a volumetric flask and 1-2 ml of conc. HCl was added into it. On the next day, a drop of phenolphthalein indicator was added into it. Sample was taken in the burette. Then 100 ml of conical flask was taken, added 2 ml of fehlings solution A and 2 ml of fehlings solution B and volume made upto 50ml, then added 1-2 drop of methylene blue indicator into it. Titration was done by continuous heating of the flask and pouring the sample from the burette drop by drop. Finally brick red colour given the end point (Ranganna,1986).

3.3.3.3. Reducing sugar(%): Ten gm of freshly collected fruit sample was taken and it was crushed with luke warm water with the help of mortar and pestle. 100ml of volumetric flask was taken and volume made up to 100 ml. Sample was taken in the burette. Then 100 ml of conical flask was taken, added 2 ml of fehlings solution A and 2 ml of fehlings solution B and volume made upto 50ml, then added 1-2 drop of methylene blue indicator into it. End point was determined by the brick red colour (Ranganna, 1986).

3.3.3.4. Titratable acidity (%): A 10g sample was weighed. The sample was diluted to 100ml with distilled water. Using a standard solution of 0.1 N sodium hydroxide, the sample was titrated to the end point. The end point was determined using phenolphthalein indicator. 1 ml of phenolphthalein indicator was added to the sample and titrated to the faint pink end point. The volume of 0.1N sodium hydroxide used was recorded (Ruck, 1969).

3.3.3.5. Ascorbic acid content (mg/100g): Utilizing the visual titration technique with 2, 6-dichlorophenol-indophenols, the ascorbic content of fruit pulp was assessed. A 20g of the fruit sample was smashed with 3 percent metaphosphoric acid, and then filtered it. 5 ml of the juice was taken and mixed with 25 ml of 3 percent metaphosphoric acid. A 5ml aliquot was collected and titrated against the dye (Ranganna, 1986).

Statistical Analysis

For statistical interpretation, analysis of variance for each parameter was performed using Proc Glm of Statistical analysis System (SAS) Software (Version 9.3). Means separations for different parameter were performed using Least Significant Difference (LSD) test ($P \leq 0.05$). Normality of residuals under the assumptions of ANOVA was tested using Kolmogrov-Smirnov test using Proc- Univariate procedure of SAS (Version 9.3).

Data Transformation:

Data transformation is the most successful corrective technique when the variation and the mean are functionally connected. With this method, the original data are transformed onto a new scale, creating a new data set that is intended to meet the variance homogeneity requirements.

The guidelines shown below were helpful to choose the transformation scale for percentage statistics derived from recent data that is most appropriate.

Rule1: Percentage data that falls between the range of 30 to 70% need not be modified.

Rule 2: Only percentage data in the ranges of 0–30% or 70–100%, but not both, should be transformed using the square root method.

Rule 3: For percentage data that does not fall within any of the ranges mentioned in Rules 1 or 2, the arcsine transformation or transformation should be employed. (Gomez and Gomez, 1983).

Chapter IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The investigation was carried out at the Directorate of Cinchona and Other Medicinal Plants, Mungpoo, Darjeeling,West Bengal.

The experimental data generated over the course of the investigation is presented and discussed using the following headings and sub-heads:

4.1. Effect of different bio-stimulants on growth parameters

4.1.1. Plant height (cm)

Data presented in Table 4.1 revealed that the plant height varied significantly among the different bio-stimulants. Humic acid applied at the rate of $4gL^{-1}$ at 30 and 60 DAP had the maximum plant height T_7 (21.30cm) followed by T_8 (20.31 cm), T_6 (18.98 cm) and T_9 treatment (18.84 cm).

Humic acid has the potential to boost hormonal activity in plants and improve plant growth (Serenella *et al* 2002). The increase in growth might be attributable to the impact of application of humic acid as reported by (Canellas *et al* 2000). The increased absorption of the three main plant nutrients, nitrogen (N), phosphorus (P), and potassium (K), is one of the stimulative effects of humic compounds on plant height. The need for applying NPK fertiliser is decreased when the soil contains sufficient humic compounds (Chen *et al* 2004).

Similar findings were validated by Arancon *et al* (2004), who discovered that humic acid bio-stimulant significantly boosted plant height in strawberry.

Table 4.1. Effect of bio-stimulants on vegetative growth and flowering behaviour of strawberry plants.

Treatments	Plant height (cm)		Length of petiole (cm)		Increment (%) of shoot petiole	Number of leaves		Root length (cm)	Number of runners/plant	Days taken to emergence of first flower bud	Flowering duration (Days)
	30 DAP	**60 DAP**	**30 DAP**	**60 DAP**		**30DAP**	**60DAP**				
T_1	16.32^{e}	18.25^{c}	11.52^{b}	12.26^{d}	6.42^{c}	4.75^{f}	7.81^{d}	18.34^{e}	6.93^{f}	102.05^{ab}	76.67^{e}
T_2	16.75^{de}	18.31^{c}	11.66^{b}	13.04^{cd}	11.44^{a}	5.28^{de}	8.00^{d}	19.55^{de}	9.17^{d}	97.82^{bc}	80.55^{de}
T_3	18.30^{c}	18.82^{bc}	13.03^{a}	13.09^{cd}	0.46^{f}	5.90^{bc}	8.30^{bcd}	19.76^{cde}	10.36^{c}	82.32^{e}	85.75^{cd}
T_4	17.86^{cd}	18.61^{c}	13.01^{a}	13.06^{cd}	0.38^{f}	5.59^{cd}	8.23^{cd}	18.83^{de}	10.27^{c}	87.55^{de}	82.55^{cde}
T_5	16.53^{de}	18.28^{c}	11.64^{b}	12.80^{cd}	9.96^{b}	5.07^{ef}	8.00^{d}	18.60^{e}	7.93^{e}	108.85^{a}	79.29^{de}
T_6	18.98^{abc}	18.93^{bc}	13.23^{a}	13.44^{bc}	1.58^{e}	6.00^{bc}	8.73^{bc}	21.96^{ab}	12.10^{b}	93.35^{cd}	94.24^{ab}
T_7	20.07^{a}	21.30^{a}	13.98^{a}	15.58^{a}	11.83^{a}	6.80^{a}	9.65^{a}	22.07^{a}	14.63^{a}	98.82^{bc}	98.33^{a}
T_8	19.84^{ab}	20.31^{ab}	13.91^{a}	14.52^{ab}	4.38^{d}	6.09^{b}	8.99^{ab}	21.40^{abc}	12.10^{b}	94.35^{bcd}	96.30^{a}
T_9	18.31^{bc}	18.84^{bc}	13.17^{a}	13.24^{cd}	0.53^{f}	5.88^{bc}	8.41^{bcd}	20.34^{bcd}	11.13^{c}	86.55^{de}	88.30^{bc}
SEm±	0.50	0.53	0.36	0.37	0.18	0.16	0.22	0.56	0.30	2.66	2.44
LSD($P \leq 0.05$)	1.52	1.60	1.08	1.13	0.55	0.48	0.71	1.69	0.90	7.98	7.32

**Means with same letter are not significantly differs from each other*

**T_1= Control (Water spray) at 30 + 60 DAP;T_2=Seaweed extracts - 4mll^{-1} at 30 DAP;T_3= Humic acid - 4gl^{-1} at 30 DAP;T_4= Fulvic acid - 4gl^{-1} at 30 DAP;T_5= Amino acid - 4mll^{-1} at 30 DAP;T_6= Seaweed extracts - 4mll^{-1} at 30 + 60 DAP;T_7= Humic acid - 4gl^{-1} at 30 + 60 DAP;T_8= Fulvic acid - 4gl^{-1} at 30 + 60 DAP;T_9= Amino acid - 4mll^{-1} at 30 + 60 DAP

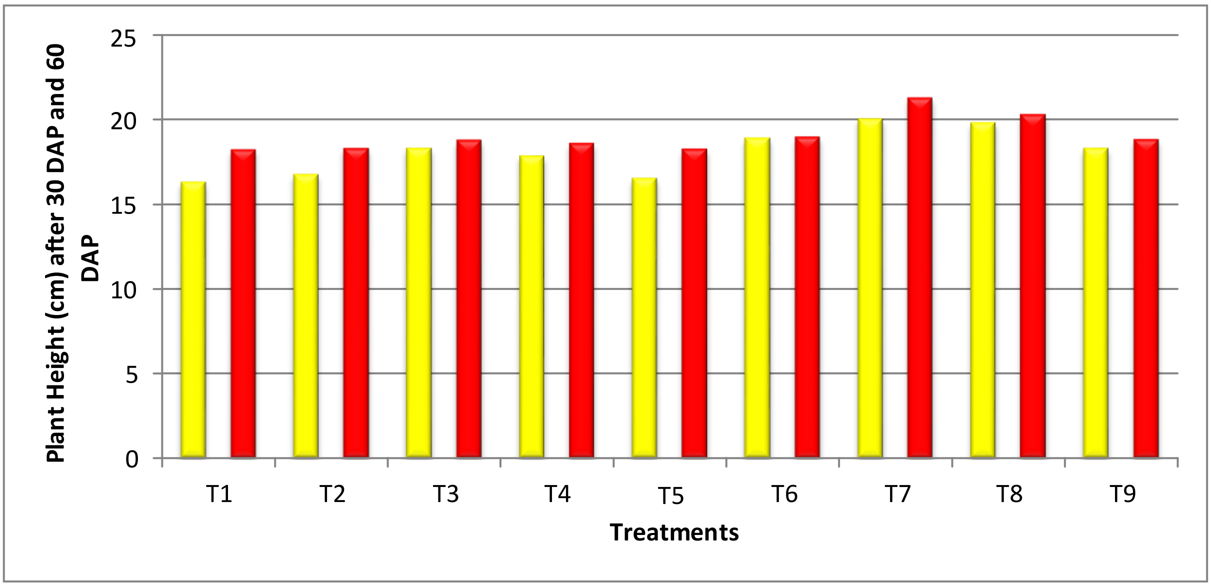


Fig 4.1.1. Effect of different bio-stimulants on plant height after 30 DAP and 60 DAP of strawberry cv. Festival

4.1.2. Number of leaves

It was evident from the data presented in Table 4.1 that all the treatments showed significant variation in terms of number of leaves per plant from initial stage to harvesting of the crop. T_7 had the maximum number of leaves (6.80 after 30 days and 9.65 after 60 days of transplanting) which was statistically superior to all other treatments. T_1 had the least number of leaves (4.75 after 30 days and 7.81 after 60 days after transplanting).

The increase in number of leaves per plant is due to humic acid's potential to boost hormonal activity in the plants along with improvement in plant growth, plant height, nutrient absorption and stress tolerance (Serenella *et al* 2002).

Dursun *et al* (2002) found maximum number of leaves in plants when treated with humic acid.

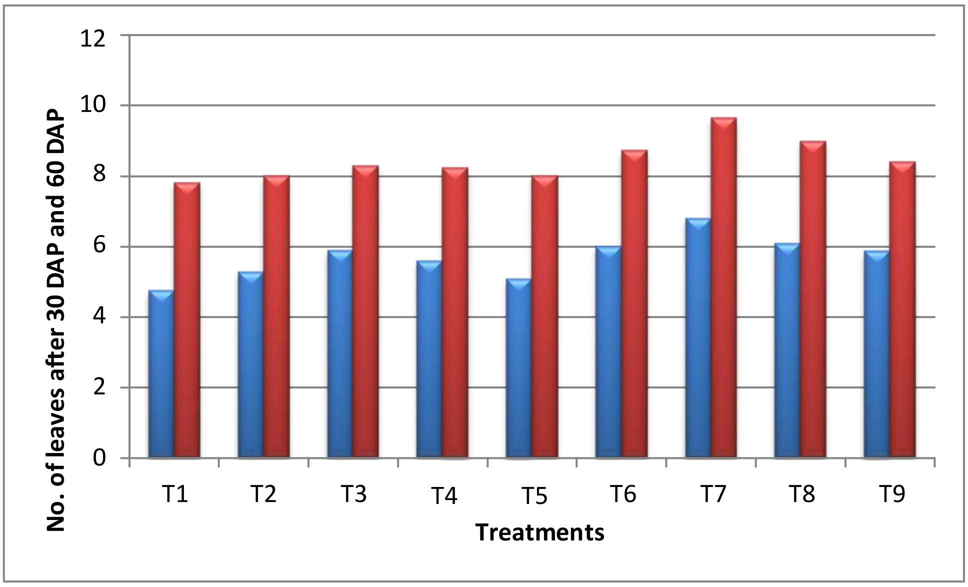


Fig 4.1.2. Effect of different bio-stimulants on number of leaves after 30 DAP and 60 DAP of strawberry cv. Festival.

4.1.3. Length of the petiole (cm)

Petiole length was varied from 15.58 cm (T_7)-12.26cm (T_1) due to application of different bio-stimulants (Table 4.1). Strawberry cv. Festival treated with 4gL^{-1} Humic acid sprayed 30 and 60 days after transplanting (T_7) had the longest shoots after 30 (13.98cm) and 60 days after transplanting (15.58cm) which is statistically similar with T_8 and T_6 with corresponding value 14.52 cm and 13.44 cm after 30 and 60 days after transplanting respectively. T_7 also had the highest percent of petiole increment.

Humic acid is effective fertiliser transporters and activators for foliar applications. Increased plant development mechanisms within the leaves result in an increase in the carbohydrates content of the leaves and stems. These carbohydrates are subsequently translocated into the roots, from where they are partially released into the rhizoplane and rhizosphere to supply nutrients for diverse soil microbes (Baldotto *et al* 2010). Humic acid treatment stimulates the photosynthetic pigments, which enhances plant photosynthesis which as a result increases the length of the petiole Abbas *et al* (2013).

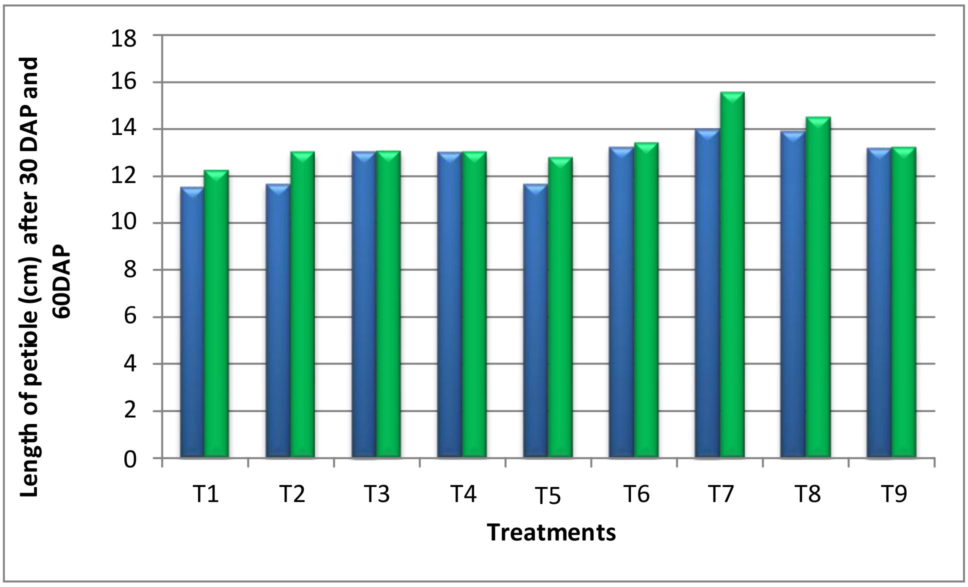


Fig 4.1.3. Effect of different bio-stimulants on length of petiole after 30 DAP and 60 DAP of strawberry cv. Festival.

4.1.4. Root length (cm)

It is evident from the data presented in Table 4.1 that the root length of strawberry was significantly affected when different biostimulants were applied. Strawberry cv. Festival treated with $4gL^{-1}$ humic acid sprayed 30 and 60 days after transplanting (T_7) exhibited a considerable increase in root length of (22.07 cm) was statistically at par with T_6 (21.96 cm) whereas the control (T_1) had the least increase in root length (18.34cm).

The formation of complexes between humic acid and mineral ions, catalysis of humic acid by plant enzymes, influence of humic acid on respiration and photosynthesis, stimulation of nucleic acid metabolism, and humic acid's hormonal activity are a few of the useful assumptions put forth to explain the effect of humic acid on plant growth parameters (Rafeii, 2014).

These findings are consistent with those of Ozlem *et al* (2017). Humic substances have a strong influence on plant root growth and are an excellent plant root growth stimulant. When humic acids are given to soil, they improve root initiation and boost root development as reported by Zandonadi *et al* (2007).

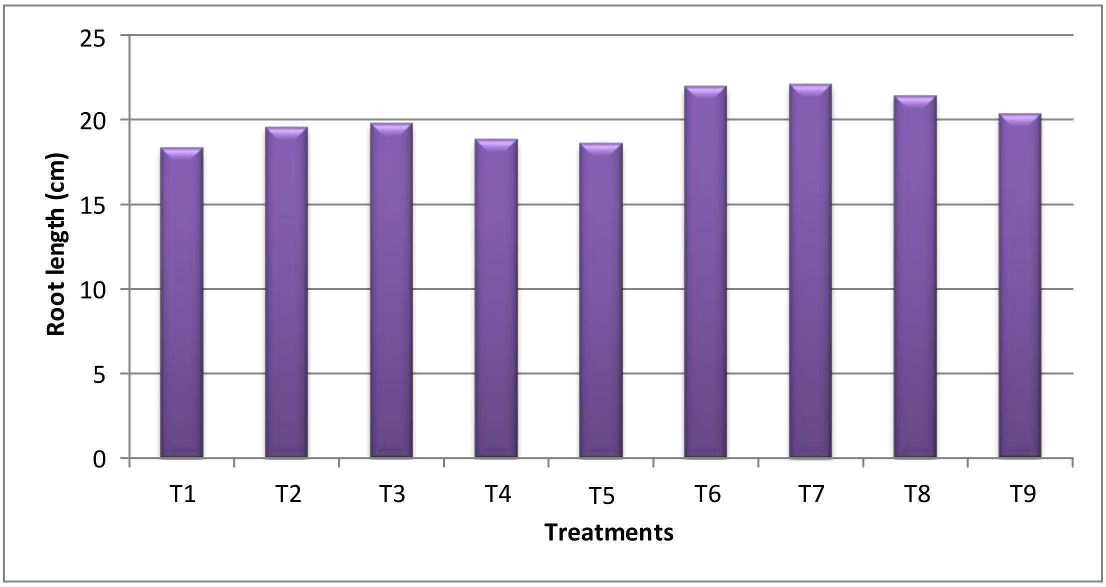


Fig 4.1.4. Effect of different bio-stimulants on root length of strawberry cv. Festival.

4.1.5. Number of runners/plant

The number of runners per plant was considerably influenced by the application of bio-stimulants (Table 4.1). T_7 had the maximum number of runners (14.63) which was statistically superior to all other treatments and had at par with T_6 and T_8 treatment (12.10). On the other hand T_1 had the least number of runners per plant (6.93).

There have been reports of both direct and indirect effects of humic substances on plant development. The indirect effects of humic compounds have been associated to an improvement in the physical, chemical, and biological characteristics of soil. These substances seemed to have the capacity to directly influence plant development via accelerating respiratory processes (Smidova, 1960), increasing cell permeability, triggering hormonal growth responses, or a combination of these activities (Vaughan, 1974).

These findings are in agreement with those of Shafshak *et al* (2011) and Mohamed (2015), who found that addition of humic acid to the plants greatly improved all the plant growth characteristics significantly.

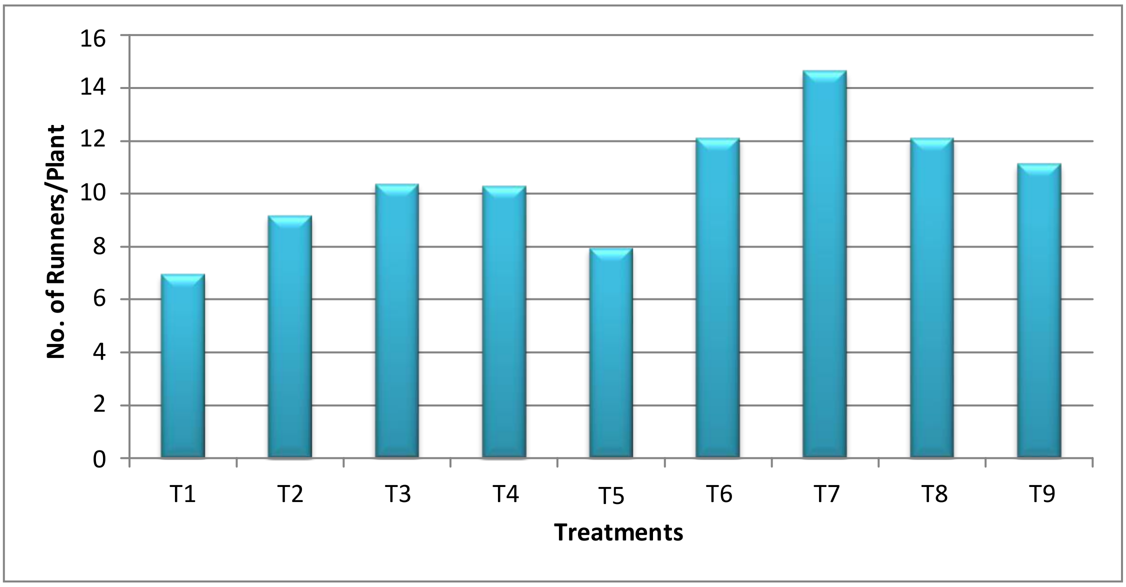


Fig 4.1.5. Effect of different bio-stimulants on number of runners/plant of strawberry cv. Festival.

4.1.6. Days taken to emergence of first flower bud

Days taken to emergence of first flower bud after the spray of different bio-stimulants on strawberry varied greatly and is summarized in Table 4.1. After the initial spray of bio-stimulant, strawberry treated with $4gL^{-1}$ humic acid, T_3 took a minimum days for emergence of first flower buds (82.32 days) which is statistically similar with T_9 (86.55 days). Among all the treatments maximum days taken to emergence of first flower bud after first spray was observed in treatment T_5 (108.85 days) with statistically at par results in T_1 (102.05 days).

Among the effective assumptions put forth to describe the impact of humic acid on plant growth parameters are the formation of complexes between humic acid and mineral ions, catalysis of humic acid by plant enzymes, influence of humic acid on respiration and photosynthesis, stimulation of nucleic acid metabolism, and humic acid's hormonal activity (Rafeii, 2014).

These findings are consistent with those of (Feafel and Mirdad 2014), who demonstrated that plant flowering was improved as a result of increasing the humic acid content, which led to an earlier flowering and an increase in the number of flowers per cluster.

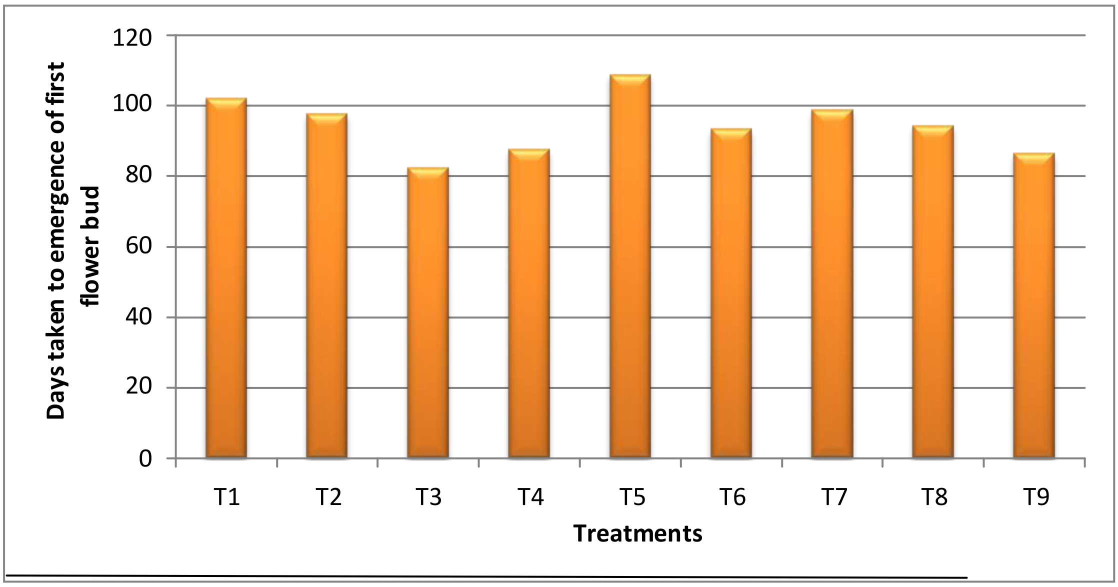


Fig 4.1.6. Effect of different bio-stimulants on days taken to emergence of first flower bud of strawberry cv. Festival.

4.1.7. Flowering duration

The results show that the overall time of flowering was dramatically influenced when different bio-stimulants were used. T_7 had the longest flowering period (98.33 days) which was statistically equal to T_8 (96.30 days), whereas T_1 had the shortest flowering period (76.67days). Furthermore, statistically comparable findings were obtained with treatments T_6 and T_9 (94.24 and 88.30 days respectively). The superior treatment of T_7 recorded an increase of 21.66 days compared to least T_1 treatment (Table 4.1).

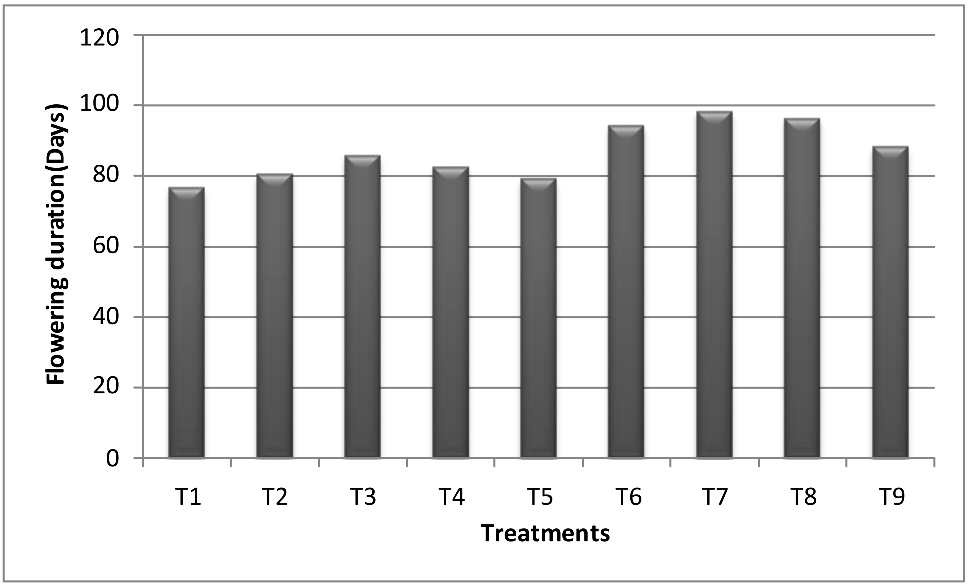


Fig 4.1.7. Effect of different bio-stimulants on flowering duration of strawberry cv. Festival

4.2.1. Duration of fruiting

The results demonstrate that when different bio-stimulants were applied, the overall period of fruiting was drastically altered. T_7 had the longest fruiting time (94.10 days), which was statistically similar to T_8 (89.77days), while T_1 had the shortest duration of fruiting (69.81 days). The treatments T_6 and T_9, were statistically at par with corresponding values of 86.82 and 84.07 days, respectively. The superior treatment of T_7 recorded an increase of 24.29 days compared to least T_1 treatment (Table 4.2).

Table 4.2. **Effect of bio-stimulants on fruiting behavious and yield attributes ofstrawberry**

Treatments	Duration of fruiting (Days)	Fruit set (%)	Total number of fruits per plant	Fruit weight (g)	Fruit diameter (cm)	Yield (g/plant)
T_1	69.81^{e}	65.25(53.91)^{de}	23.2^{f}	16.36^{e}	2.00^{e}	379.55^{d}
T_2	76.27^{de}	74.52(59.74)^{b}	25.4^{ef}	18.23^{cd}	2.70^{d}	463.04^{c}
T_3	78.08^{cd}	72.67(58.54)^{bc}	28.3^{cd}	16.87^{de}	2.00^{e}	477.42^{c}
T_4	76.32^{de}	61.39(51.61)^{ef}	26.6^{de}	17.09^{de}	2.50^{d}	454.59^{c}
T_5	72.20^{de}	64.38(53.39)^{de}	24.7^{ef}	18.23^{cd}	2.00^{e}	450.28^{c}
T_6	86.82^{b}	57.60(49.40)^{f}	27.9^{cd}	20.75^{a}	3.60^{a}	578.92^{b}
T_7	94.10^{a}	80.78(64.09)^{a}	32.9^{a}	19.76^{abc}	3.30^{bc}	650.10^{a}
T_8	89.77^{ab}	60.62(51.16)^{ef}	32.4^{ab}	19.96^{ab}	3.50^{ab}	646.70^{a}
T_9	84.07^{bc}	68.51(55.90)^{cd}	30.2^{bc}	19.10^{bc}	3.20^{c}	576.82^{b}
SEm±	2.27	2.68	0.78	0.51	0.07	14.79
LSD ($P \leq 0.05$)	6.82	5.69	2.35	1.55	0.23	44.36

**Figures in the parenthesis are the angular transformed value*

***Means with same letter are not significantly differs from each other*

***T_1= Control (Water spray) at 30 + 60 DAP;T_2=Seaweed extracts - 4mll^{-1} at 30 DAP;T_3= Humic acid - 4gl^{-1} at 30 DAP;T_4= Fulvic acid - 4gl^{-1} at 30 DAP;T_5= Amino acid - 4mll^{-1} at 30 DAP;T_6= Seaweed extracts - 4mll^{-1} at 30 + 60 DAP;T_7= Humic acid - 4gl^{-1} at 30 + 60 DAP;T_8= Fulvic acid - 4gl^{-1} at 30 + 60 DAP;T_9= Amino acid - 4mll^{-1} at 30 + 60 DAP

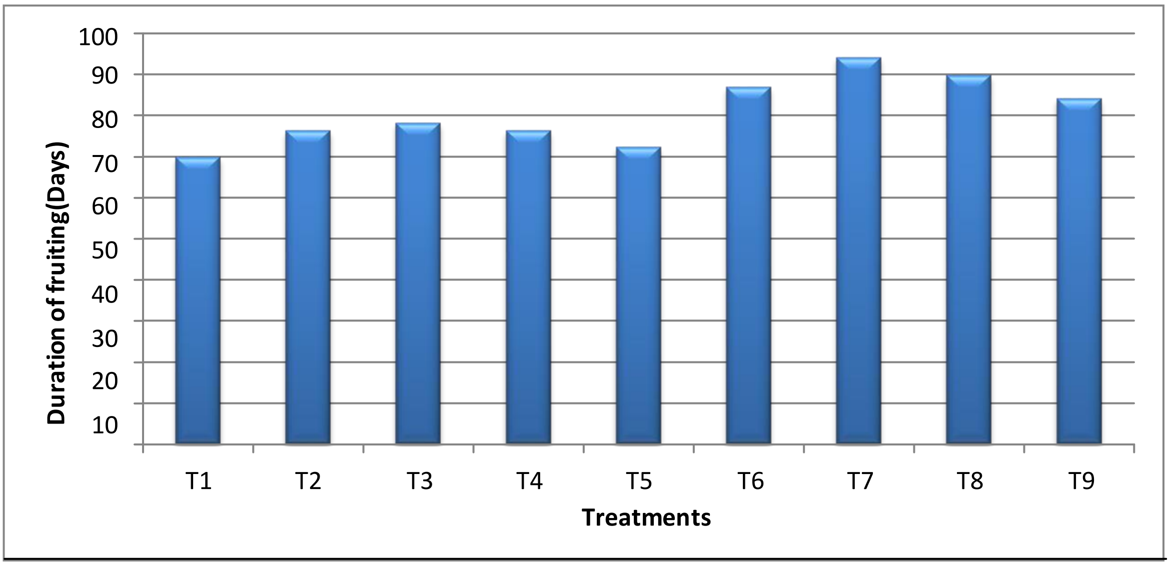


Fig4.2.1. Effect of different bio-stimulants on duration of fruiting of strawberry cv. Festival

4.2.2. Fruit set percent (%)

The data on the percent of fruit set as presented in Table 4.2 revealed that maximum percent of fruit set (80.78%) was observed in T_7 whereas T_6 recorded the minimum percent of fruit set (57.60%). It was observed that fruit set percent was significantly differs among different treatments and record is highest (80.78%) with humic acid at $4gL^{-1}$ applied at 30 and 60 days after transplanting (T_7) followed by (74.52%) seaweed extract @ $4gL^{-1}$ applied at 30 days after transplanting (T_2).

The possible role for increasing higher fruit set percent by humic acid may be due to the vegetative development which have resultedin an increase in the quantity of flowers and fruits. Zhang *et al* (2000) reported that humic acid contains cytokinins, and their application results in increased levels of auxin and endogenous cytokinin, perhaps improving plant development and increasing the fruit set percentage of plant.

The present experimental findings were also supported by the findings of (Kishore *et al* 2021)

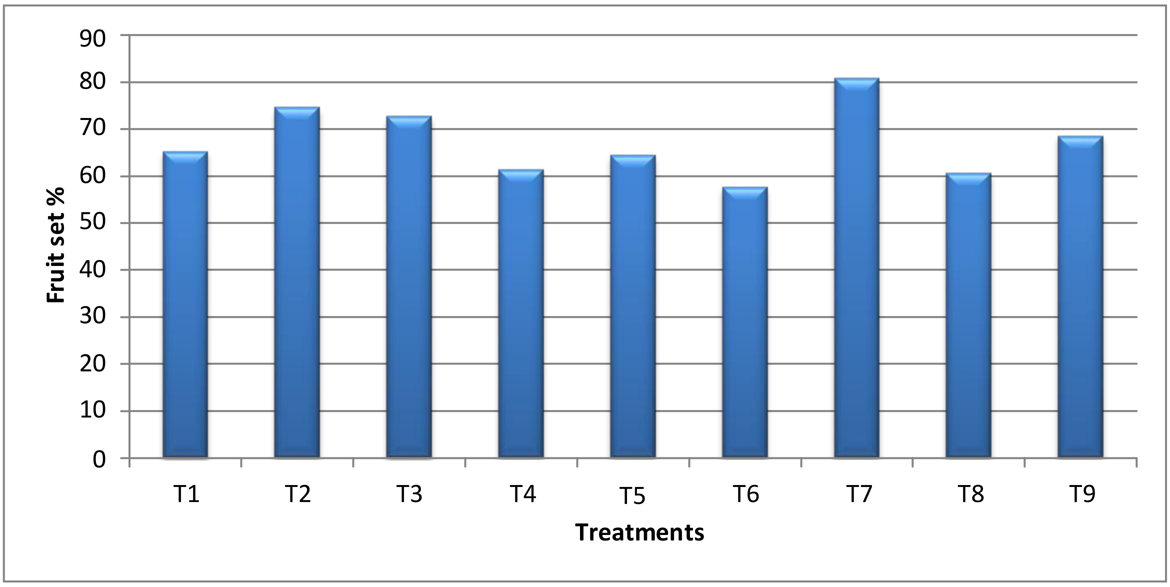


Fig 4.2.2. Effect of different bio-stimulants on fruit set percentage of strawberry cv. Festival

4.2.3. Total number of fruits/plant

The data in Table 4.2 indicated that the application of bio-stimulants had a significant influence on the quantity of fruits produced which ranged from 32.9 to 23.2. T_7 yielded the highest number of fruits per plant (32.9). Treatment T_1, on the other hand, had the minimum number of fruit production (23.2) with statistically identical number in T_8 (32.4), T_9 (30.2), T_3 (28.3), and T_6 (27.9).

Humic acid significantly increases the rate of photosynthesis, respiration and hormonal activities in plants, resulting in increased flower and fruit production (Chen *et al* 1990). Humic acid contains cytokinins; hence its application results in enhanced endogenous cytokinin and auxin levels, perhaps leading to improve plant development as well as production of more number of berries per plant (Zhang *et al* 2000).

The findings are consistent with those of Arancon *et al* (2006) who treated strawberry plants with humic acid and vermicost and discovered a substantial influence of fruit number. Arancon *et al* (2003) also investigated the influence of humic acid from various sources on the growth of green house plants and discovered a significant effect in number of fruits.

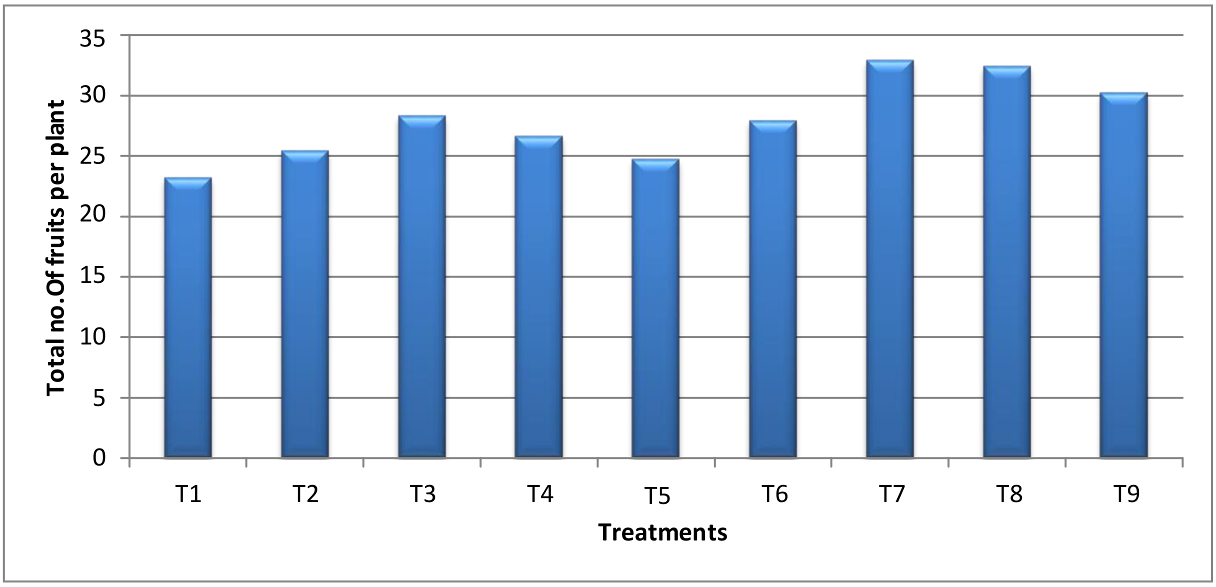


Fig 4.2.3. Effect of different bio-stimulants on total number fruits per plant of strawberry cv. Festival

4.2.4. Fruit weight (g)

The data in Table 4.2 revealed that the application of different bio-stimulants had a significant effect on the fruit weight of strawberry cv. Festival. T_6 reported to have the maximum fruit weight (20.75 g), which was statistically at par to T_8 (19.96 g). T_1 treatment, on the other hand, had the minimum fruit weight (16.36 g). T_7 and T_9 treatments had statistically equivalent effects, with corresponding values of 19.76 and 19.10 g, respectively. When compared toT_1, the best treatment of T_6 resulted in an increase of 4.39g.

The presence of plant growth regulators including cytokinin, auxins, and gibberellins in seaweed extract may be the cause of the rise in average fruit weight. The plant growth regulators especially cytokinin is associated with nutrient partitioning. The application of seaweed extract may have increased cytokinin availability, which may have led to a larger buildup of photoassimilates and an increase in fruit weight. The process of chlorophyll and photosynthesis may be another factor that contributed to the increased fruit weight by enhancing the buildup of photo-assimilates (Khan *et al* 2010).

The results in the present study are in the confirmity with the findings of Miniawy *et al* (2014).

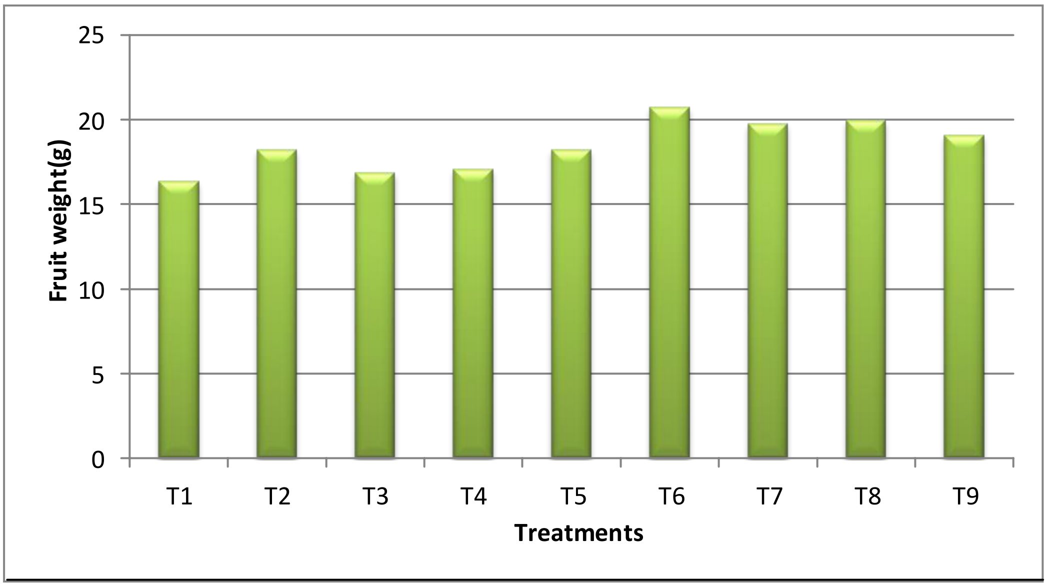


Fig 4.2.4. Effect ofd ifferent bio-stimulants on fruit weight of strawberry cv. Festival

4.2.5. Fruit diameter (cm)

The results demonstrated that several bio-stimulants had a significant influence on the diameter of strawberry fruits (Table 4.2). T_6 had the maximum fruit equatorial diameter (3.60 cm), which was statistically equal to T_8 (3.50 cm). T_1, T_3, and T_5 treatments, on the other hand, achieved a minimum (2.00 cm) fruit equatorial diameter.

The increase in fruit diameter caused by the application of seaweed extract may be attributed to the presence of cytokinins in seaweed extract, which help regulate cell division (Stephen *et al* 1985 and Whapman *et al* 1993). When applied at the first stage of cell division, seaweed extract may have boosted the cytokinin levels in fruits, leading to a rise in cell numbers and, eventually, fruit diameter (Lews *et al* 1996).

Farrag *et al* (2015) also observed increase in fruit diameter by using seaweed extract bio-stimulant which supports the present result.

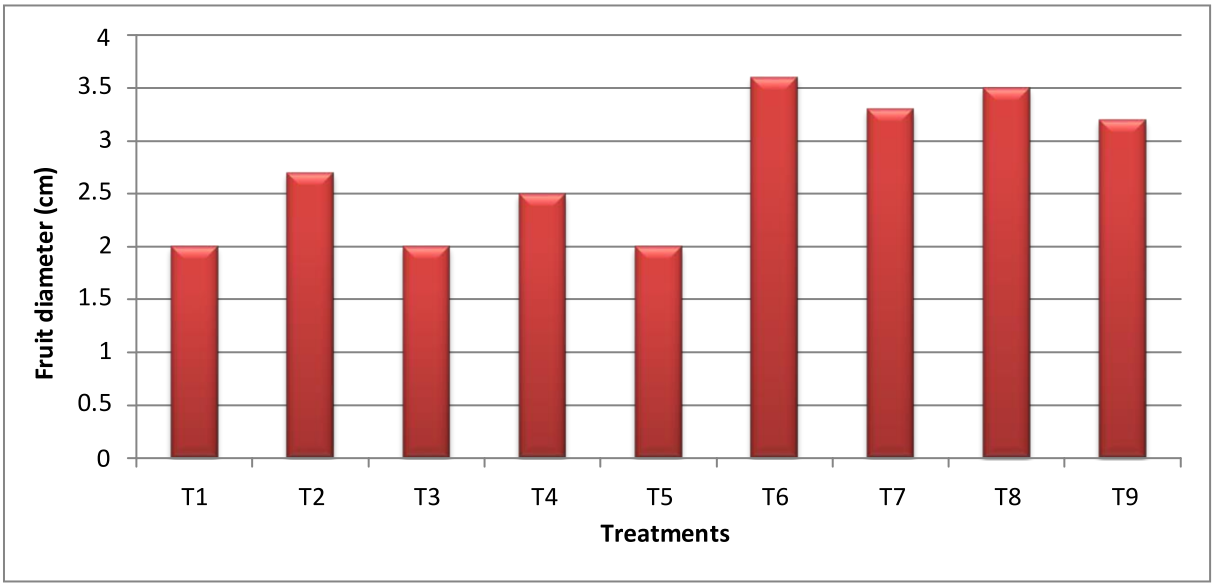


Fig 4.2.5. Effect of different bio-stimulants on fruit diameter of strawberry cv. Festival

4.2.6. Yield (g/plant)

When humic acid was applied to strawberry plants, it greatly increased fruit yield when compared to other bio-stimulants (Table 4.2). T_7 had the highest fruit production (650.10 g/plant) which was substantially higher than all other treatments, whereas T_1 had the lowest yield per plant (379.55 g/plant).

Humic acid significantly boosts plant respiration, photosynthesis, and hormonal activities which increases flower and increases plant fruit production significantly (Chen *et al* 1990). Zhang *et al* (2000) reported that humic acid contains cytokinins; hence, its application results in higher endogenous cytokinin and auxin levels, which may boost plant development and increase the quantity of berries produced per plant.

Saruhan *et al* (2011) demonstrated that humic acid treatments greatly enhanced the yield and yield components of the plants. Kaya *et al* (2005) also observed that, as compared to the control, foliar humic acid treatment increased the production of the crop.

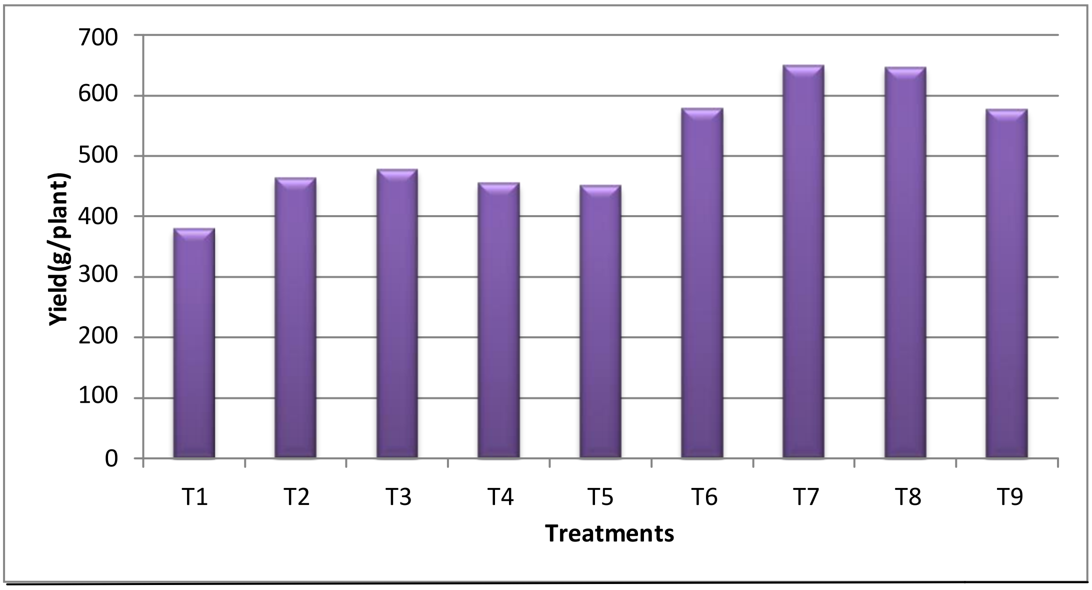


Fig 4.2.6. Effect of different bio-stimulants on yield of strawberry cv. Festival

4.3.1. Shoot fresh weight (g)

Comparing amino acid to other bio-stimulants, it significantly improved fresh weight of shoot when applied to strawberry plants (Table 4.3). T_5 had the highest fresh weight of shoot (23.45 g), whereas T_2 had the lowest fresh weight of shoot (14.66g).

All fraction of photosynthetic pigments in faba bean plants were boosted by foliar spraying with amino acid. Amino acid treatments had stimulatory effects on photosynthetic pigments. In contrast to the control plants, amino acid treatments not only reduced the inhibitory effects of salt stress but also, in the majority of cases, caused an increased in fresh weight of shoot (Sadak *et al* 2015).

Table 4.3 Effect of bio-stimulants on fresh and dry weight of shoot and root of strawberry plants

Treatments	Shoot weight (g)		Root weight (g)	
	Fresh	Dry	Fresh	Dry
T_1	18.36^c	7.21^c	4.55^d	3.04^b
T_2	14.66^{de}	6.21^d	5.15^c	2.73^c
T_3	13.67^e	6.99^c	3.84^e	2.23^d
T_4	19.79^c	9.21^b	4.84^{cd}	2.73^c
T_5	23.45^a	8.64^b	6.30^a	2.78^c
T_6	15.53^d	6.64^{cd}	4.74^{cd}	3.44^a
T_7	16.00^d	10.96^a	4.60^d	3.63^a
T_8	22.97^{ab}	8.81^b	5.68^b	3.58^a
T_9	21.67^b	9.22^b	6.48^a	2.62^c
S.Em±	0.52	0.23	0.14	0.08
LSD($P \leq 0.05$)	1.57	0.70	0.43	0.25

**Means with same letter are not significantly differs from each other*

**T_1= Control (Water spray) at 30 + 60 DAP;T_2=Seaweed extracts - 4mll^{-1} at 30 DAP;T_3= Humic acid - 4gl^{-1} at 30 DAP;T_4= Fulvic acid - 4gl^{-1} at 30 DAP;T_5= Amino acid - 4mll^{-1} at 30 DAP;T_6= Seaweed extracts - 4mll^{-1} at 30 + 60 DAP;T_7= Humic acid - 4gl^{-1} at 30 + 60 DAP;T_8= Fulvic acid - 4gl^{-1} at 30 + 60 DAP;T_9= Amino acid - 4mll^{-1} at 30 + 60 DAP

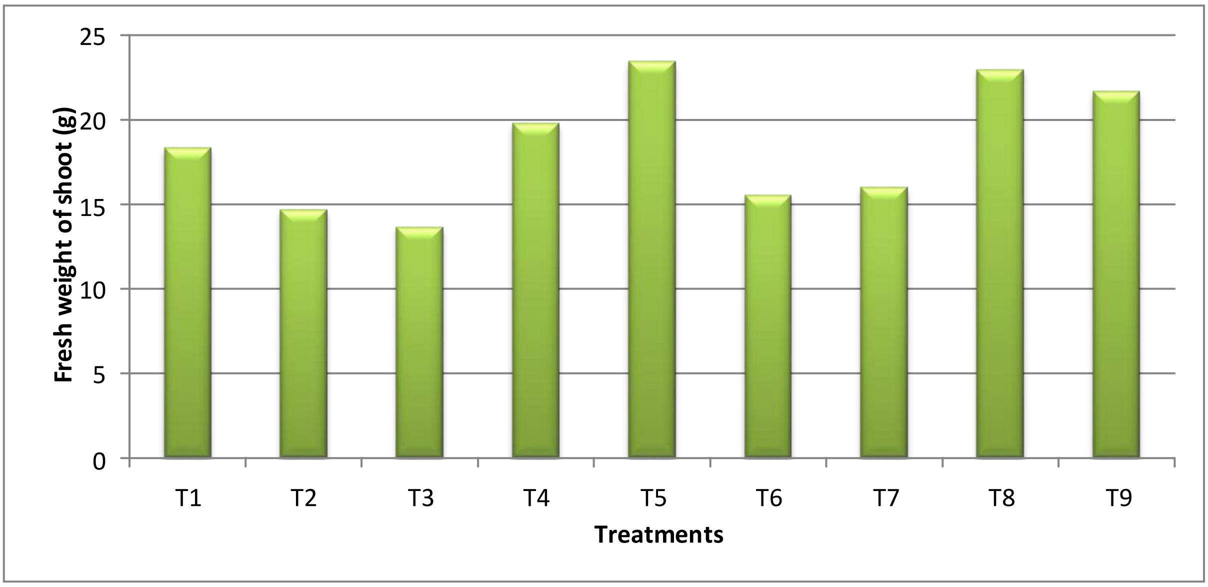


Fig 4.3.1. Effect of different bio-stimulants on fresh weight of shoot of strawberry cv. Festival

4.3.2. Root fresh weight (g)

The data in Table 4.3 showed that the fresh weight of root of strawberry cv. Festival was significantly affected by the application of several bio-stimulants. The fresh weight of root recorded maximum in T_9 (6.48g) with statistically at par in T_5 (6.30g). On the other hand, T_3 treatment had the minimum fresh weight of root (3.84g) which was statistically similar to

T_1, T_6, and T_4 with corresponding values of 4.55, 4.74, and 4.84 g. T_9 treatment led to an increase of 2.64 g in comparison to T_3 which had minimum fresh weight of root.

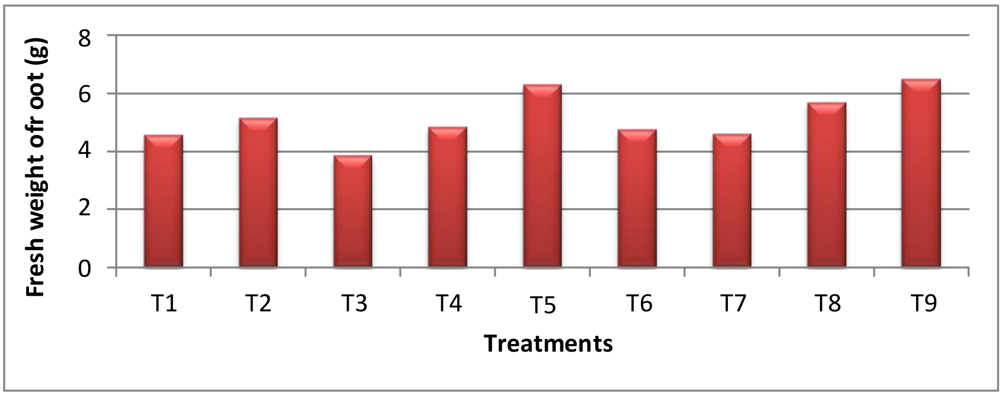


Fig 4.3.2. Effect of different bio-stimulants on fresh weight of root of strawberry cv. Festival

4.3.3. Shoot dry weight (g)

The results showed that different bio-stimulants significantly affected the shoot dry weight of strawberry (Table 4.3). The maximum dry weight of shoot was in T_7 (10.96g) which was statistically similar to T_9 (9.22 g). On the other hand, T_2, T_3, and T_6 treatments produced a minimum dry weight of shoot of 6.21 g, 6.99 g, and 6.64 g.These mean results are significantly differed among the treatments.

According to (Ehsan *et al* 2014), 110 mgl^{-1} nitrogen yields the maximum shoot fresh and dry weights, fruit production, and number of fruits, where as nitrogen levels over this amount cause these attributes to decline. It is evident that the foliar application of humic acid enhances the permeability of the cells, resulting in the quick entrance of minerals into the leaves and better nutrient absorption by the plant. As a result, vegetative growth of the plant was improved with increased fruit yield (Sarhan *et al* 2011)

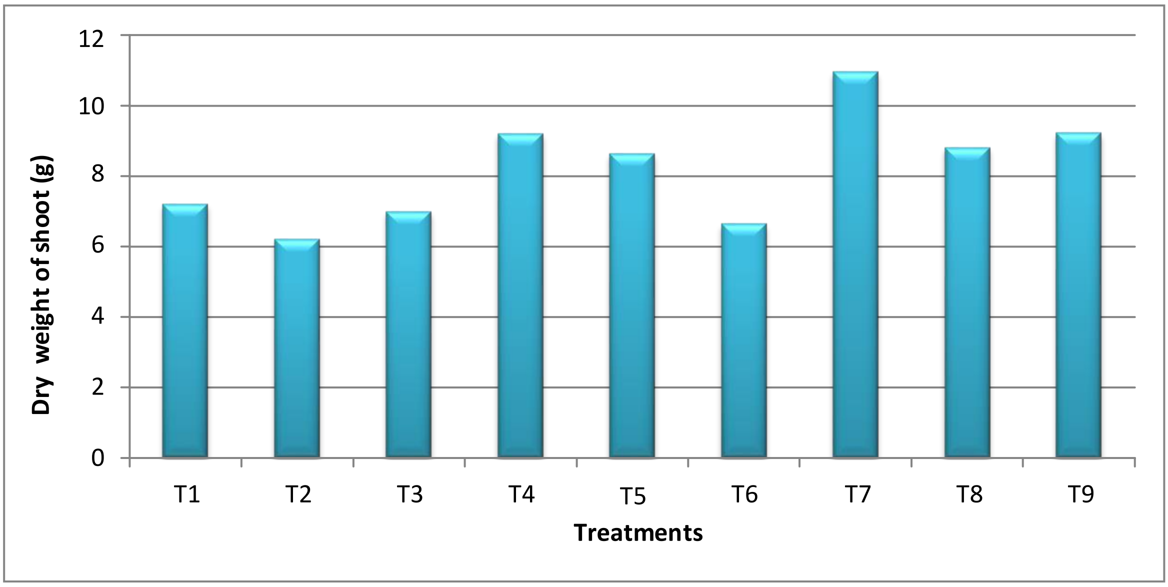


Fig 4.3.3. Effect of different bio-stimulants on dry weight of shoot of strawberry cv. Festival

4.3.4 Root dry weight (g)

The data in Table 4.3 showed that the dry weight of the root of the strawberry cv. Festival was influenced significantly by the application of several bio-stimulants. T_7 generated the maximum weight from dry roots (3.63 g). On the other hand, treatment T_3 had the minimum root dry weight (2.23 g) which is statistically at par with T_1 (3.04 g), T_6 (3.44 g), T_8 (3.58 g) and T_7 (27.9 g).

The quantity of nitrogen that produces the highest root fresh and dry weights, fruit output, and number of fruits is 110 mg l^{-1}, whereas nitrogen levels over this point result in a drop in these characteristics (Ehsan *et al* 2014). It is clear that foliar applications of humic acid increase cell permeability, which speeds up the entry of minerals into leaf cells and improves plant nutrient uptake. As a result, the plant's vegetative growth is enhanced, and number of fruits of eggplant is increased as reported by (Sarhan *et al* 2011)

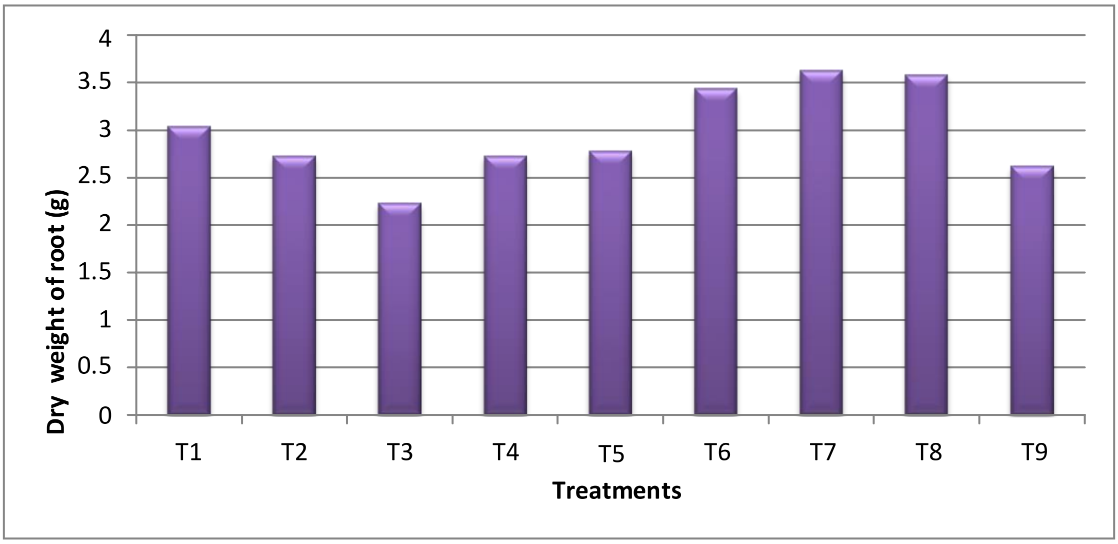


Fig 4.3.4. Effect of different bio-stimulants on dry weight of root of strawberry cv. Festival

The impact of different bio-stimulants on the biochemical characteristics of fruits

At proper ripening, 10 fruits were harvested randomly from each plot and brought to the laboratory of Pomology and Post Harvest Technology department for further analysis as per the details mentioned below.

4.4.1. Total soluble solids (°B)

The total soluble solids (TSS) content of strawberry fruits was significantly affected by foliar application of different bio-stimulants, ranging from 9.73 to 7.05 ^{o}B (Table 4.4). In comparison to the other bio-stimulants, T_7 had the highest TSS (9.73 ^{o}B) while minimum intreatment T_1 (7.05^{o}B). T_7 treatment was statistically at par to T_6, T_8, and T_9, with TSS values of 8.45, 8.97 and 8.13^{o}B, respectively.

Humic acid is known to improve photosynthesis, therefore more TSS was produced as a result (Liu *et al* 1998). Increase in TSS through application of humic acid has also been reported by Farahi *et al* (2013) in strawberry cv. Aromas.

Table 4.4. Effect of bio-stimulants on TSS, total sugar, reducing sugar, ascorbic acid content, titrable acidity of strawberry fruits.

Treatments	TSS (°Brix)	Total Sugar (%)	Reducing Sugar (%)	Titratable Acidity (%)	Ascorbic acid Content (mg/100g)
T1	7.05^{f}	3.32^{e}	2.47^{c}	0.743^{a}	36.83^{e}
T_2	7.45^{def}	3.74^{cd}	2.56^{c}	0.633^{b}	41.88^{d}
T_3	7.90^{cde}	3.87^{bcd}	2.81^{b}	0.582^{cd}	45.51^{bcd}
T_4	7.64^{def}	3.80^{cd}	2.58^{c}	0.601^{bc}	44.74^{bcd}
T_5	7.39^{ef}	3.62^{de}	2.50^{c}	0.694^{a}	43.18^{cd}
T_6	8.45^{bc}	3.99^{bc}	2.92^{ab}	0.503^{e}	46.10^{bc}
T_7	9.73^{a}	4.46^{a}	3.06^{a}	0.401^{f}	50.48^{a}
T_8	8.97^{b}	4.15^{ab}	2.90^{ab}	0.502^{e}	48.01^{ab}
T_9	8.13^{cd}	3.94^{bcd}	2.86^{ab}	0.554^{de}	47.13^{ab}
SEm±	0.23	0.11	0.08	0.023	1.27
LSD($P \leq 0.05$)	0.68	0.32	0.22	0.049	3.78

**Means with same letter are not significantly differs from each other*

**$T_{1=}$ Control (Water spray) at 30 + 60 DAP;T_2=Seaweed extracts - 4mll^{-1} at 30 DAP;T_3= Humic acid - 4gl^{-1} at 30 DAP;T_4= Fulvic acid - 4gl^{-1} at 30 DAP;T_5= Amino acid - 4mll^{-1} at 30 DAP;T_6= Seaweed extracts - 4mll^{-1} at 30 + 60 DAP;T_7= Humic acid - 4gl^{-1} at 30 + 60 DAP;T_8= Fulvic acid - 4gl^{-1} at 30 + 60 DAP;T_9= Amino acid - 4mll^{-1} at 30 + 60 DAP

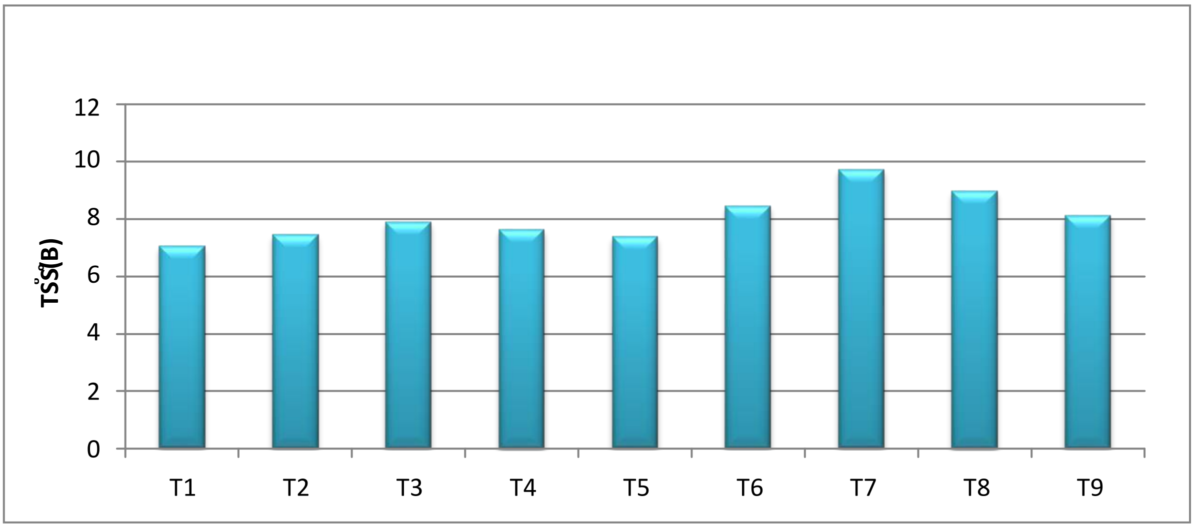


Fig 4.4.1. Effect of different bio-stimulants on TSS of strawberry cv. Festival

4.4.2. Total sugars (%)

Total sugars content of strawberry fruits was positively improved by different bio-stimulant treatment when compared to control (water spray). Treatment T_7 had a maximum total sugars level (4.46 %) which was statistically similar to T_8 (4.15 %). However, T_1 treatment had the lowest value (3.32 %) (Table 4.4)

It is possible that the greatest quantity of carbohydrates were formed in the leaf and fruit tissues in response to humic acid, and that these maximum amounts of carbohydrates were subsequently converted to the particular sugars like glucose and sucrose (Abbas *et al* 2013). The findings of Zachariakis *et al* (2001) in grape corroborates to the findings of the present experiment.

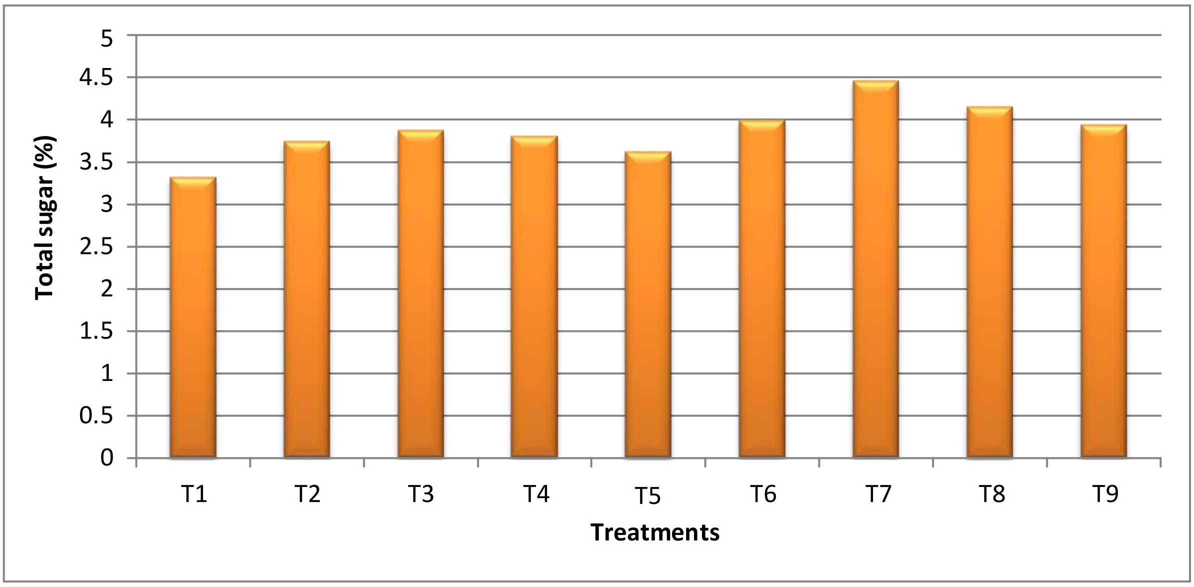


Fig 4.4.2. Effect of different bio-stimulants on Total sugar of strawberry cv. Festival

4.4.3. Reducing sugar (%)

The data showed that humic acid had a significant impact on reducing sugar content when compared to other bio-stimulants used throughout the study period. The amount of reducing sugars in each sample varied between 3.06 and 2.47 percent. The reducing sugars content was recorded maximum (3.06%) in statistically superior treatment T_7 and had at par with T_6, T_8, T_9 which has corresponding values of 2.92, 2.90, 2.86 percent. However minimum reducing sugars (2.47%) content was recorded in T_1 treatment (Table 4.4).

The enhanced translocation of additional photosynthetic assimilates to the fruit and the breakdown of starch during ripening may be the causes of the buildup of more reducing sugar by the foliar application of humic acid as reported by Abbas *et al* (2013).

Karakurt *et al* (2009) recorded that the reducing sugars in plants were greatest when humic acid was applied which is in accordance with this present experiment.

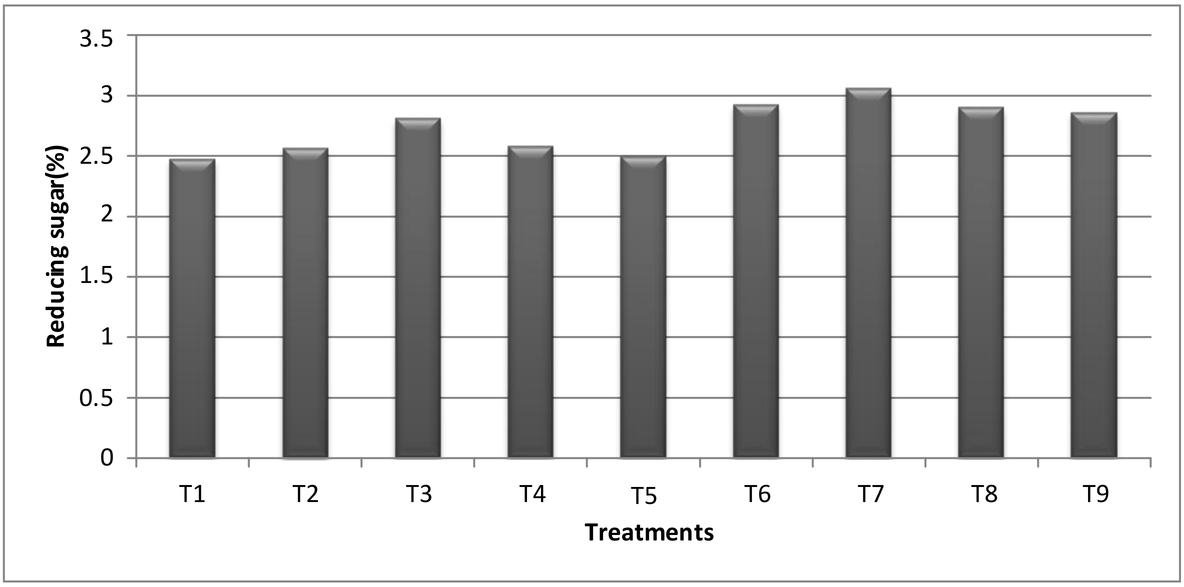


Fig 4.4.3. Effect of different bio-stimulants on reducing sugar of strawberry cv. Festival

4.4.4. Ascorbic acid content (mg/100g)

The data in Table 4.4 showed that ascorbic acid content was significantly influenced by foliar application of humic acid and other bio-stimulants.Treatment T_1 recorded the minimum quantity of ascorbic acid (36.83 mg/100g), whereas T_7 had the maximum amount (50.48 mg/100g) which was statistically equivalent to T_8 and T_9 treatment (48.01 and 47.13 mg/100g, respectively).

Humic acids have distinctive properties, such as increasing the amount of readily available vital nutrients and plant tolerance to biotic and abiotic stresses, it may increase the ascorbic acid content of fruit as reported by (Zimmer, 2004). The present experimental findings were also supported by Hameed *et al* (2018).

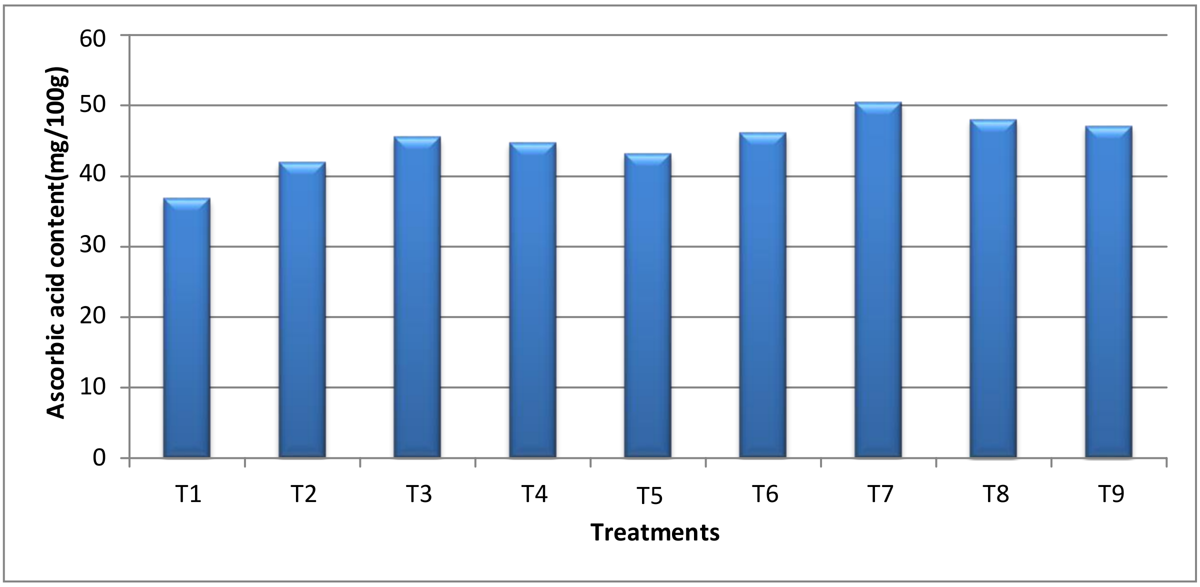


Fig 4.4.4. Effect of different bio-stimulants on ascorbic acid of strawberry cv. Festival

4.4.5. Titratable acidity (%)

When strawberry fruits were treated with various bio-stimulants, the titratable acidity was recorded minimum in T_7 (0.40%) and maximum in T_1 (0.74%). Additionally, treatment T_7 was statistically similar to treatments T_8 and T_9 (0.50 and 0.55%, respectively) (Table 4.4).

A similar decline in acidity occurs when fruit ripens properly. These findings are in line with the idea that acids can function as substrates for respiration after sugars have been consumed, or that acids can contribute to the synthesis of phenolic compounds, lipids, and volatile aromas, as well as provide a variety of metabolites that are used in various processes that reflect the predominance of sweet flavour in fruits. Increased phosphorus levels might also be associated to the decreasing trend in acidity (Reuther *et al* 1973). Similar findings were reported by Abbas *et al* (2012) in Kinnow mandarin.

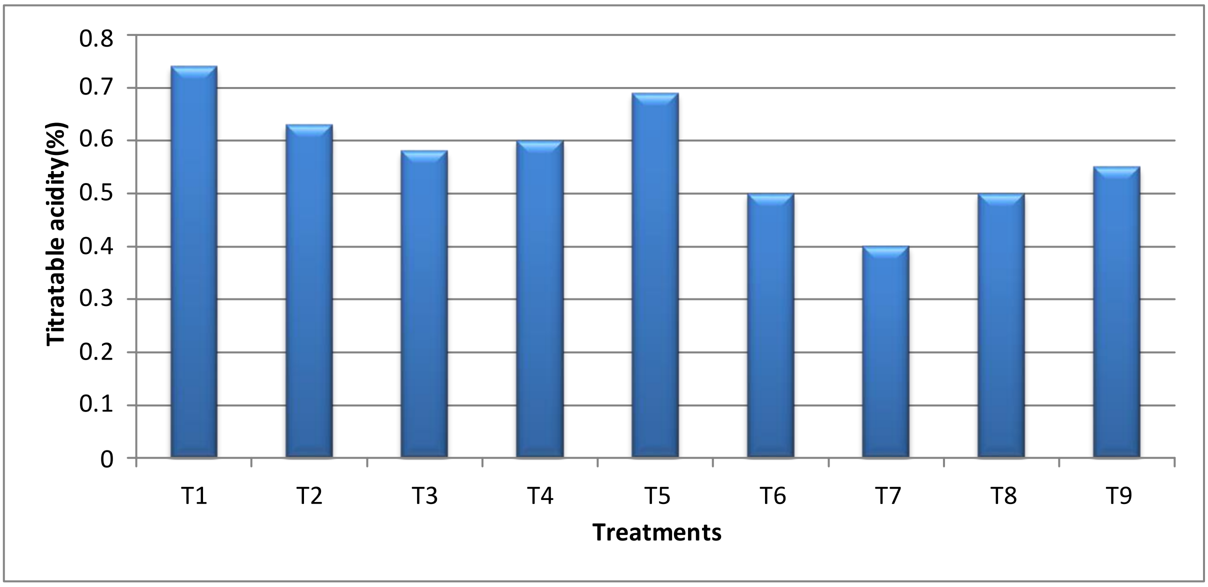


Fig 4.4.5. Effect of different bio-stimulants on titratable acidity of strawberry cv. Festival

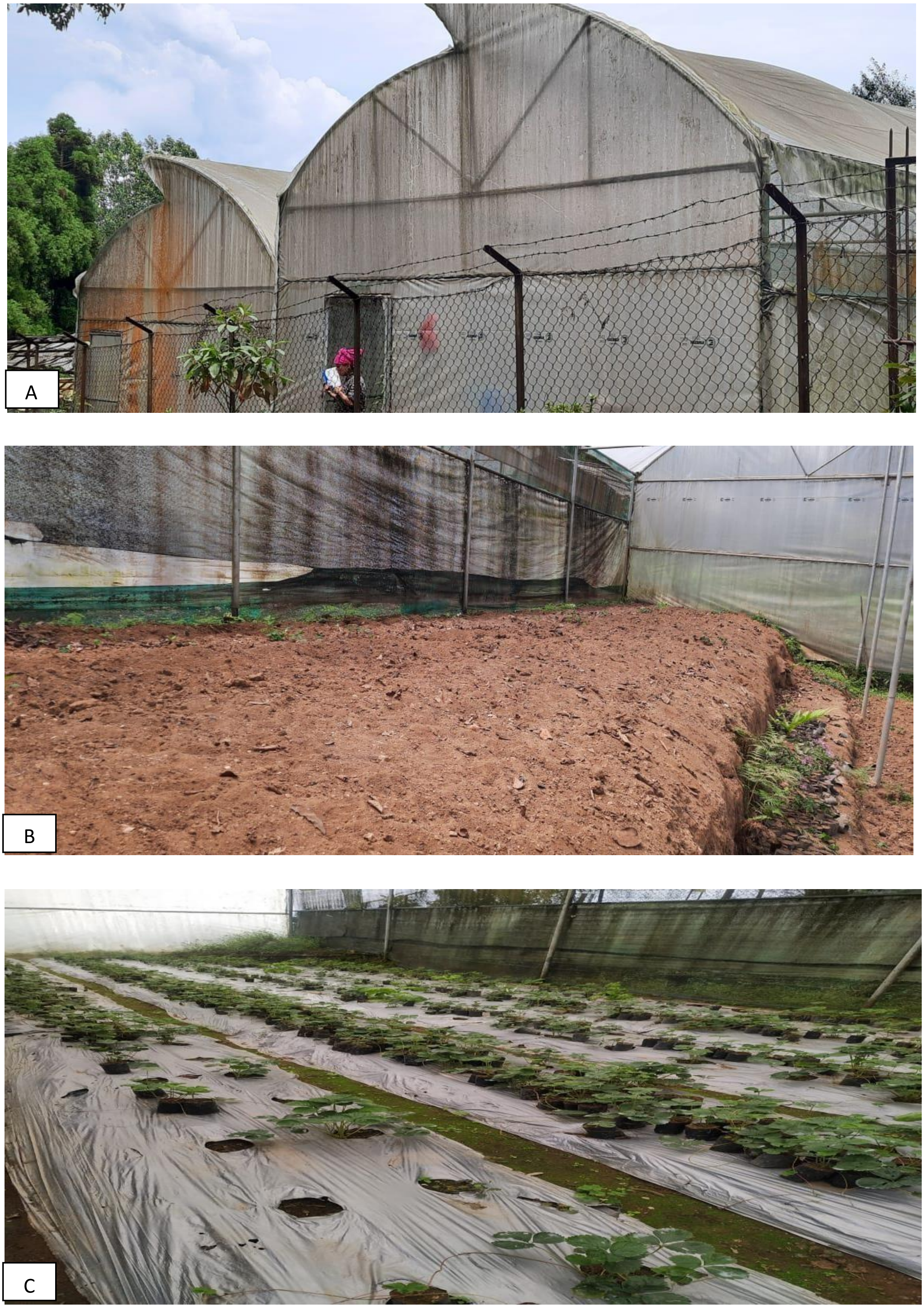


Plate-1: Experimental plot

A: Polyhouse; B: Bed ready for transplanting of strawberry runners; C: Strawberry plants after mulching

Plate -2: Cultural operations taken in field

A: Preparation of the beds; B: Application of farm yard manure; C: Transplanting of runners; D: Mulching

Plate -3: Bio-stimulents used during the experiment

A: Humic acid; B: Amino acid; C: Seaweed extract ;
D: Fulvic acid

Plate – 4: Different activities during spraying.

A: Weighing of bio-stimulants; B: Measuring of bio-stimulants; C: Spraying of bio-stimulants in the beds


A
B
C
D

Plate-5:Taking of Physical and lab observations.

A: Counting the number of flowers; B: Measuring plant height; C: Measuring fruit weight; D Measuring TSS; E Crushing the fruit sample in mortel pastel; F Measuring root length

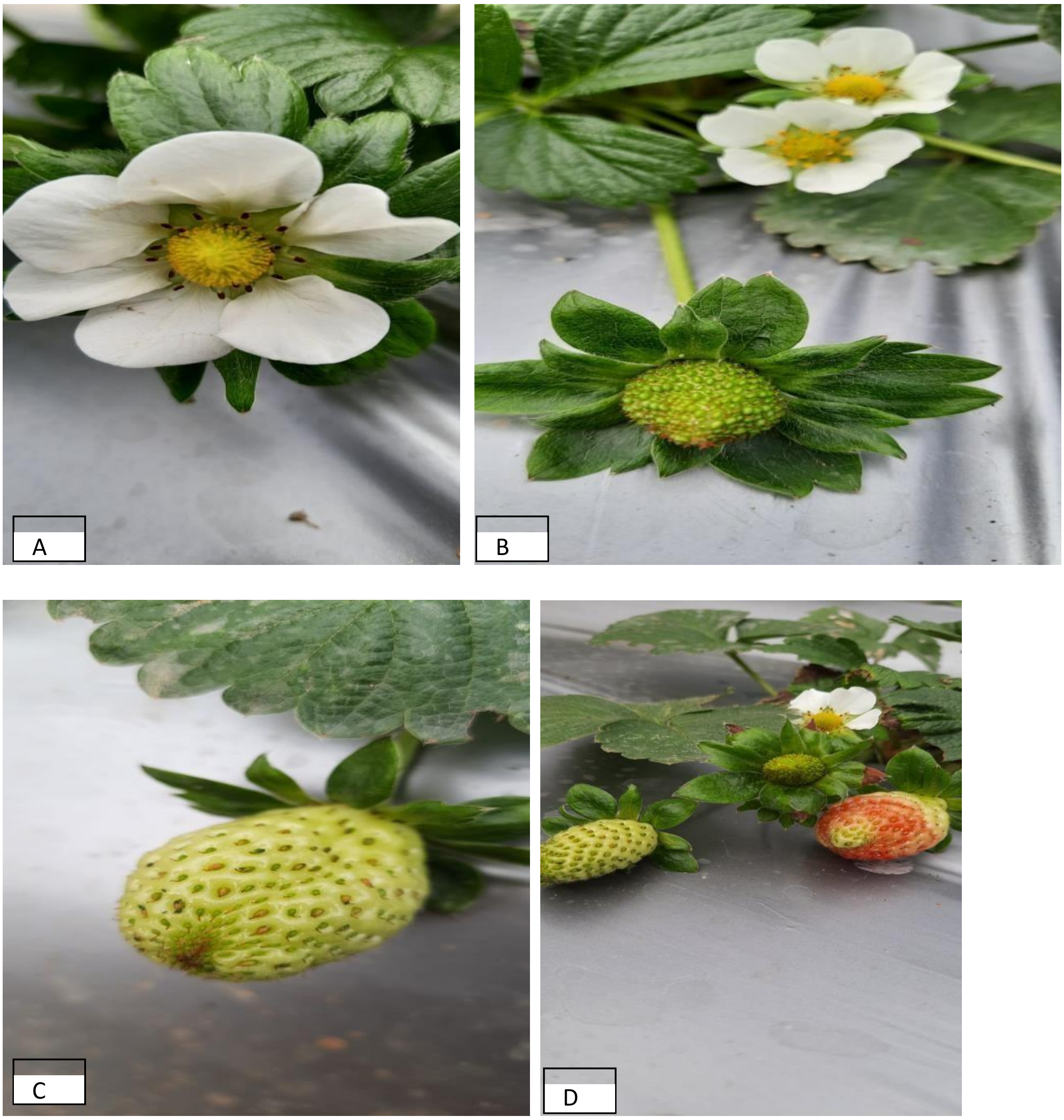

A
B
C
D

Plate-6 : Phenological stages of strawberry flower to fruit.

A Open flower; B Emergence of fruit bud; C Fruit after 18 days of fully open flower; D Appearance of red colour in fruit; E Fruits ready for harvesting; F Harvested fruits

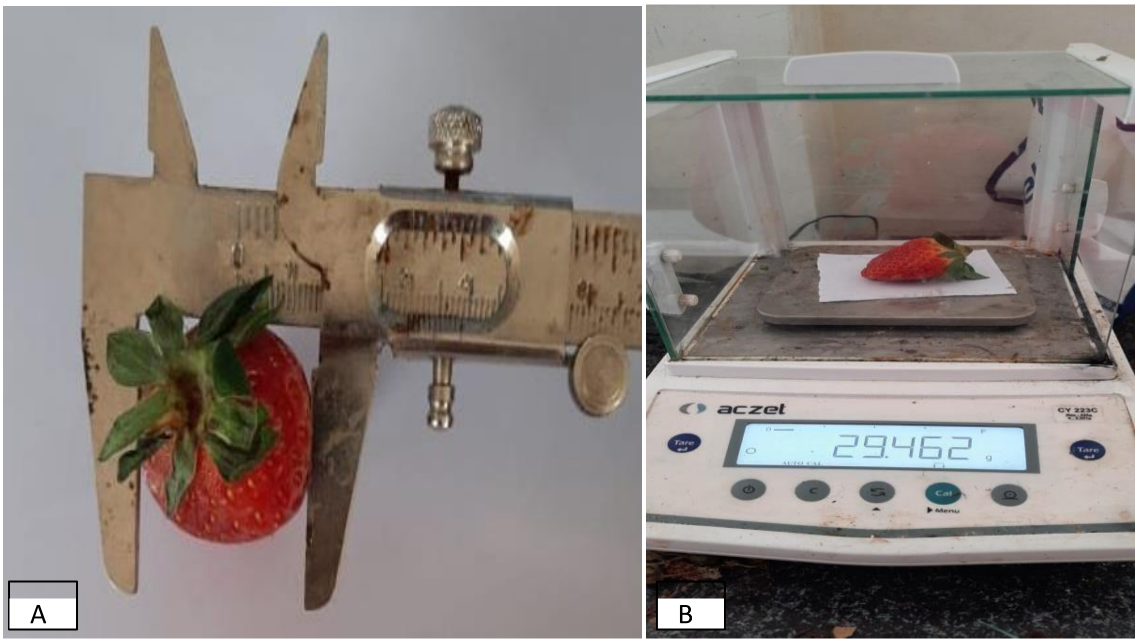


Plate-7: Measuring fruit wieght and Diameter.

A Measuring the fruit diameter; B Measuring the fruit weight

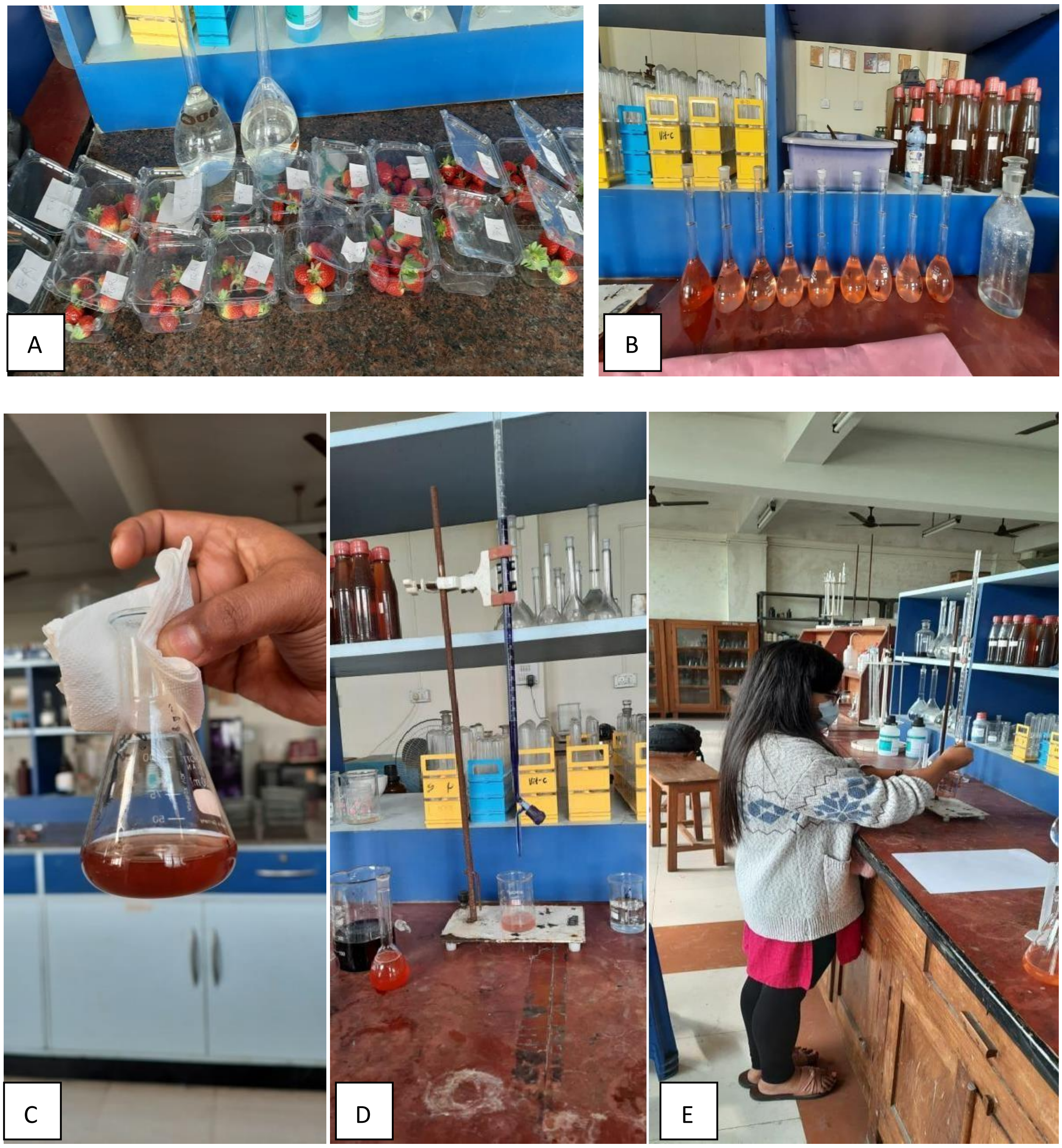


Plate-8: Different parameters lab analysis

A Sample of fruits treated with different bio-stimulants; B Preparation of fruit juice sample; C Titratable acidity estimation; D,E Ascorbic acid estimation

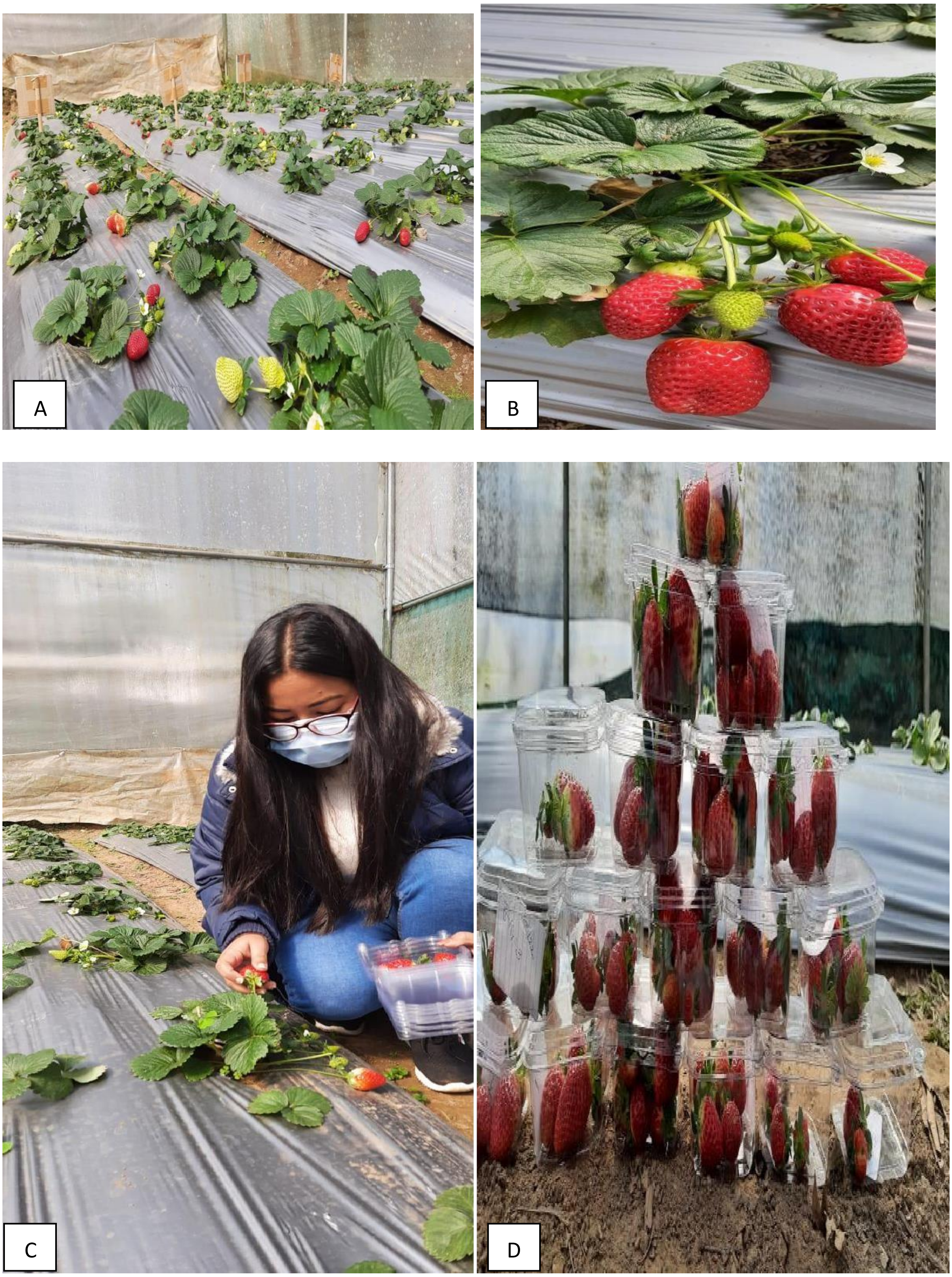


Plate 9-: Harvesting of matured fruits

A,B Matured fruits; C,D Harvested fruits

Chapter V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

An experiment was conducted in the Directorate of Cinchona and Other Medicinal Plants, Mungpoo, Darjeeling,West Bengal during September 2021 to June 2022. Results revealed that application of humic acid $4gL^{-1}$ at 30 and 60 DAP in strawberry cv. Festival is effective in improving growth, flowering and fruiting characteristics.

The physical characteristics of the plant changed as a result of foliar application with bio-stimulants at $4gL^{-1}$ during 30 and 60 DAP as compared to the control (water spray). Maximum plant height was recorded in T_7 at 30 (20.04 cm) and 60 DAP (21.31 cm). After 30 DAP and 60 DAP, T_7 attained the maximum length of the petiole 13.98cm, 15.58cm moreover, T_7 percent of increment was higher.

Maximum number of leaves were obtained by T_7 after 30 and 60 DAP (6.80 and 9.65, respectively). Further, T_7 achieved the maximum root length (22.07 cm) with, maximum number of runners per plant (14.63).

In contrast to the control (water spray), foliar treatment with bio-stimulants at $4gL^{-1}$ during the 30 and 60 DAP resulted in flowering characteristics with longer flowering duration in T_7 (98.33 days) and a minimum time for first flower bud appearance in T_3 (82.32 days) .Application of various bio-stimulants on fruiting attributes resulted in increases in the number of fruits produced per plant was maximum in T_7 (32.9) with maximum fruit set percent, (80.78%). Fruit weight and diameter was obtained maximum in T_6 (20.75 g and 3.60 cm, respectively) while fruit yield and duration of fruiting was recorded maximum in T_7(650.10 g per plant and 94.10 days respectively).

With foliar spray of bio-stimulants on strawberry plants, a significant improvement in fruit quality such as fruit diameter, weight, and fruit set% as well as the quantity of fruits produced per plant, was also seen.

Total soluble solids (TSS) in treatment T_7(9.73 ^{o}Brix), titratable acidity T_7(0.40%), and ascorbic acid concentration (50.48 mg/100g) were substantially influenced by application of bio-stimulants. Also treatment T_7 had the highest levels of reducing sugar(3.06%) and total sugar (4.46%) content in the ripe fruits.

In comparison to the control (water spray), foliar treatment with bio-stimulants at $4gL^{-1}$ during the 30 and 60 DAP produced maximum fresh weights of root by T_9 (6.48g), fresh weight of shoot by T_5 (23.45g), dry weight of root by T_7 (3.63g), and dry weight of shoot by T_7 (10.96g).

Based on the results, itcan be said that foliar applications of humic acid at a rate of $4gL^{-1}$ at two distinct times viz. 30 DAP and 60 DAP proved to be the best treatment for boosting flowering characteristics, fruit production as well as biochemical attribute of ripe strawberry fruits. The findings suggested that the use of humic acid has also demonstrated positive benefits to enhance overall strawberry crop husbandry. So it may be beneficial to apply humic acid to strawberry crops.

Effect of bio-stimulants on Strawberry (*Fragaria* × *ananassa* Duch.) cv. Festival performance under organic cultivation is the topic of the current study. The availability of nutrients already present in the soil may be increased by using humic acid on strawberry crops at the right quantity. To support the best concentration for the overall enhancement of the strawberry crop, additional research must be conducted with multiple concentrations in addition to the current study.

It is hereby concluded considering the all foregoing discussion that humic acid externally applied at a rate of 4 gL^{-1} was shown to be the most successful method for improving growth, flowering and fruiting of strawberry cv. Festival at the Directorate of Cinchona and Other Medicinal Plants ,Mungpoo, Darjeeling,West Bengal. Humic acid bio-stimulant was applied externally to improve fruit quality attributes of strawberry cv. Festival. The need for nitrogen, phosphorous, and potassium fertiliser treatments may be minimised when appropriate humic compounds are available in the soil. Among the effective assumptions put forth to describe the impact of humic acid on plant growth parameters are the formation of complexes between humic acid and mineral ions, catalysis of humic acid by plant enzymes, influence of humic acid on respiration and photosynthesis, stimulation of nucleic acid metabolism, and humic acid's hormonal activity.

Chapter VI

FUTURE SCOPE OF THERESEARCH

FUTURE SCOPE OF THE RESEARCH

The present study entitled with "Effect of bio-stimulants on performance of strawberry (*Fragaria* × *ananassa* Duch.) cv. Festival under Organic Cultivation." was conducted to examine the effect of bio-stimulants on growth, yield and quality of strawberry cv. Festival. However, there is a great deal of room for more study in this field, which may focus on the following issues:

- One can study the composition of commercial bio-stimulant products.
- It is possible to analyse the ideal time and dosage of bio-stimulants.
- It is possible to investigate the biochemical mechanisms underlying the responses of bio-stimulant to the plants.
- To gain both basic and applied knowledge about the effectiveness of bio-stimulants for a particular plant species, there is a need to conduct wide-scale research on that particular species, with various products, treatments growth stages, etc.
- The performance of additional organic manures, such as compost and biodynamic preparations as well as other bio-stimulants, may be assessed along with farm yard manure, vermicompost, and poultry manure for strawberry production.
- For organic strawberry cultivation, additional bio-stimulants such as chitosan, inorganic compounds, beneficial fungus, and bacteria may be investigated.
- Research on the impact of various strawberry bio-stimulants residual effects on the performance of future crops may be done.
- It is possible to do research on the long-term effects of bio-stimulants on the physical, chemical and biological properties of soil.
- To calculate the cost of production using different bio-stimulants.

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Fetched: 2021 10-09 06:22:29 1

Entire Document

Effect of bio-stimulants on performance of strawberry (Fragaria ×ananassa Duch.) cv. Festival under organic cultivationA thesis Submitted to the
Uttar Banga Krishi Viswavidyalaya
In partial Fulfilment of the requirements for the award of the Degree of
MASTER OF SCIENCE (HORTICULTURE) in FRUIT SCIENCE of SUDARSHANA BHUJEL (H-2020-012-M)DEPARTMENT OF POMOLOGY AND POST HARVEST TECHNOLOGY
FACULTY OF HORTICULTURE UTTAR BANGA KRISHI VISWAVIDYALAYA PUNDIBARI, COOCHBEHAR, WEST BENGAL-736165 2022
THIS THESIS IS DEDICATED TO MY BELOVED GRANDPARENTS
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CERTIFICATE I
This is to certify that the work recorded in the thesis entitled, "Effect of bio-stimulants on performance of strawberry (Fragaria ×ananassa Duch.) cv. Festival under organic cultivation" submitted by Miss Sudarshana Bhujel (H-2020-012-M) in partial fulfillment of the requirements for the degree of M.Sc. in Horticulture (Fruit Science) of the Uttar Banga KrishiViswavidyalaya, Pundibari, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the study reported in the thesis have not so far been submitted for any other degree or diploma.The assistance and help received from various sources during the course of investigation have been duly acknowledged.(Nilesh Bhowmick) Place: Pundibari Chairman, Advisory Committee Date:

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OGPA/CGPA/% MARKS : 8.43

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Award/Distinction/Fellowships/Scholarships : University merit scholarship