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# Effect of bio-stimulants on the morpho-physiological and yield traits of tomato (*Solanum lycopersicum* L.)

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**Abstract**
Bio-stimulants are the substances which contain organic and inorganic compounds that stimulate plant growth and productivity. The present study was conducted to study the influence of different microbial stimulants, humic substance and amino acid mediated bio-stimulants on the morphological, physiological and yield traits of tomato. Treatment includes T1 - Control, T2 - Hyoroplus L (1.25 L $ha^{-1}$), T3 - Heiko Root (3 capsules $plant^{-1}$) localized in root zone, T4 - Heiko Root (3 capsules $plant^{-1}$) drenched in root zone, T5 - Heiko Seed (750 g $ha^{-1}$), T6 - Hyoroplus G (20 kg $ha^{-1}$), T7 - Hyoro $My^{+}$ (20 kg $ha^{-1}$) and T8 - Viva (10 L $ha^{-1}$). Bio-stimulant treatments were given as either soil or foliar application at different growth stages. Observations were recorded at three stages *viz*., 30 Days after Transplanting (DAT), 60 DAT and 90 DAT. Better performance was observed in bio-stimulants treated plants than the untreated plants. Significant increase in shoot length, root length, total chlorophyll content, soluble protein, nitrate Reductase activity and yield traits like number of fruits per plant, fruit weight and total fruit yield were observed in bio-stimulants treated plants. Among the treatments, morpho-physiological and yield traits of T7 - Hyoro $My^{+}$ (20 kg $ha^{-1}$) and T8 - Viva (10 L $ha^{-1}$) applied tomato plants were markedly influenced. Bio-stimulants proved to be an effective tool to boost up the plant growth and productivity.

**Keywords:** bio-stimulants, microbial and humic source, morpho-physiological traits, tomato

## Introduction

Tomato (*Solanum lycopersicum* L.), a member of solanaceae family mainly cultivated for its fruits. Tomato fruits are commonly used as a vegetable and served as an ingredient for salad and pickles. It is one among the essential horticultural crops with higher export potential and foreign exchange (Farooq *et al*., 2020) [8]. Due to the presence of several nutraceutical and pharmaceutical compounds it is also called as protective food, antioxidant compounds like lycopene, carotenes in tomato possess anti-carcinogenic property thereby effectively reduce the risk of developing prostate cancer, hypertension and cardiovascular disease (Cheng *et al*., 2017) [4].

The global population is likely to expand in the coming years. To feed the global population the usage of chemical fertilizers and pesticides get increased which results in detrimental effects on soil and human health. To maintain sustainability in agriculture for the past three decades several innovations were attempted to reduce the synthetic agrochemicals, one among them is the usage of natural plant bio-stimulants (Rouphael and colla, 2020) [18]. Du Jardin (2015) [7] defined Plant bio-stimulants are the substances which are acquired from different organic, inorganic and microbial sources that helps plant by enhancing plant growth promoting activity, productivity and attenuate the adverse effects of abiotic stresses. Colla and Rouphael (2015) [5] classified plant bio-stimulants into different categories based on their microbial and non-microbial sources.

Some species of bacteria, micro algae, yeast, fungi and other organic sources are used as a bio-stimulants. Arbuscular Mycorrhizal Fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) mediated bio-stimulants helps to maintain yield stability under sub-optimal input conditions (Rouphael and colla, 2020) [18]. Calvo *et al*. (2014) [3] reported that application of bio-stimulants triggers signaling molecules in primary and secondary metabolism to enrich the plant growth and productivity. Strains belong to the genera of *Bacillus* and *Enterobacter* with PGP-activity are most promisingly used for vegetable production. Fungi belongs to the phylum Glomeromycota form symbiotic association with several agricultural and horticultural crops. *Glomus intraradices* is a Vesicular Arbuscular Mycorrhizal (VAM) fungi. This endomycorrhizal fungi have been potentially used for promoting plant growth and development. Mycorrhization of *Glomus intraradices* enhance photosynthetic activity, mineral nutrient uptake and nutrient use efficiency (Hajiboland *et al*., 2010) [9].

Commercial product from protein hydrolysates have been used for various horticultural and agricultural crops. They are rich in different amino acids and polypeptides from plant and animal fount. They assist in improvement of soil microbial activity, soil respiration, yield and quality traits of plants (Calvo *et al*., 2014) [3]. Residues of soil, plants, animals and microorganism on decomposition forms a natural constituent of organic matter called humic substances. Increased nutrient uptake and root growth may achieved by humic substances through different mechanisms like more cation exchange capacity, phosphorus availability and stimulation of $H^{+}$-ATPases (Jindo *et al*., 2012) [13]. With this background, a study was formulated to know the

effects of different bio-stimulants on morpho-physiological and yield traits of tomato.

## Material and Methods

### Experimental design and treatment details

The field experiment was conducted in field no. NA 6, Eastern Block Farm, Tamil Nadu Agricultural University, Coimbatore during July to October 2020 to study the effect of bio-stimulants on morpho-physiological and yield traits of tomato. The experiment was carried out by Randomized Block Design with eight treatments and six replications. The seeds of tomato hybrid shivam were sown in protrays and 25 days old seedlings were transplanted at the spacing of 60 x 45 cm. Package of practices under "Crop Production Techniques of Horticultural Crops" 2020 by TNAU were followed.

The treatment details are T1 - Control, T2 - Hyoroplus L (1.25 L $ha^{-1}$, 20 DAT, Pre-flowering, 2nd inflorescence), T3 - Heiko Root (3 capsules $plant^{-1}$, localized in root zone at the time of transplanting), T4 - Heiko Root (3 capsules $plant^{-1}$, drenched in root zone at the time of transplanting and 20 DAT), T5 - Heiko Seed (750 g $ha^{-1}$ at the time of transplanting and 20 DAT), T6 - Hyoroplus G (20 kg $ha^{-1}$ at the time of transplanting), T7 - Hyoro $My^{+}$ (20 kg $ha^{-1}$ at the time of transplanting), T8 - Viva (10 L $ha^{-1}$ 20 DAT, Pre-flowering, 2nd inflorescence). Hyoroplus L and Hyoroplus G are the commercial bio-stimulants which contains *Enterobacter hormaechei* similarly Heiko Root contains *Bacillus simplex*, Heiko Seed contains *Bacillus megaterium*, Hyoro $My^{+}$ contains *Glomus intraradices* and Viva is a mixture of humic acid, vitamins and amino acids.

### Data collection

Effects of bio-stimulants on morphological, physiological and growth attributes were observed at three stages viz., 30 DAT, 60 DAT and 90 DAT. Shoot length was measured from the ground soil level to the tip of the terminal leaf and denoted as centimeters (cm). Root length was calculated by measuring distance from bottom of the shoot to the longest end of the root and expressed in (cm). By following DMSO method suggested by Hiscox and Israelstam (1979) [11] total chlorophyll pigment was determined. OD at 663 and 645 nm were obtained and values were expressed as mg $g^{-1}$ of fresh weight. Soluble protein content in the leaf was estimated by Lowry *et al.* (1951) [15] procedure with folin ciocalteau reagent and expressed as mg $g^{-1}$ fresh weight. Nitrate reductase activity in physiologically active leaf was determined by adopting the procedure of Nicholas *et al.* (1976) [16] and indicated as µg $NO_2$ $g^{-1}$ $h^{-1}$. The yield parameters like individual fruit weight, No. of fruits per plant ant fruit yield per hectare were taken.

### Statistical analysis

Data were analyzed by using the software IPM SPSS Statistics 25 and a comparison of means were done at 5 % significance level with Duncan's multiple range test.

## Results

In the current study, it was observed that different microbial and humic substance mediated bio-stimulants potentially improves morpho-physiological and yield traits of tomato plants.

### Shoot length

Experimental results revealed that plants treated with bio-stimulants showed significant improvement in shoot length (Table 1). Maximum shoot length was observed in T8 (humic acid and amino acid mixture) and T7 (*Glomus intraradices*) with the length of 38.02 cm, 89.58 cm, 102.13 cm and 39.95 cm, 88.98 cm, 100.38 cm at 30, 60 and 90 DAT, respectively. Minimum shoot length was recorded in control plants (33.93cm, 70.60 cm and 87.93 cm) at different growth stages.

### Root length

Application of *Glomus intraradices* (T7) recorded maximum root length at all the three stages 19.48 cm, 38.28 cm and 45.18 cm followed by T8 (humic acid and amino acid mixture) and T5 (*Bacillus megaterium*). T7 treated plants shows maximum difference at 30 and 60 DAT. At 90 DAT there is no significant difference in root length of T5, T7 and T8, they were on par with each other (Table 1) but they were significantly differed from control plants.

**Table 1:** Effect of bio-stimulants on shoot and root length of tomato hybrid

| Treatments | Shoot length (cm) | | | Root length (cm) | | |
|---|---|---|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT | 30 DAT | 60 DAT | 90 DAT |
| T1 | 33.93 | 70.60 | 87.93 | 16.98 | 27.87 | 39.20 |
| T2 | 36.43 | 84.78 | 93.05 | 18.28 | 33.65 | 41.58 |
| T3 | 36.48 | 77.90 | 91.48 | 18.10 | 34.52 | 41.97 |
| T4 | 37.15 | 83.98 | 95.82 | 18.70 | 35.58 | 43.12 |
| T5 | 37.92 | 85.12 | 100.05 | 19.02 | 36.62 | 45.02 |
| T6 | 35.25 | 80.92 | 93.83 | 17.97 | 34.53 | 42.70 |
| T7 | 39.95 | 88.98 | 100.38 | 19.48 | 38.28 | 45.18 |
| T8 | 38.02 | 89.58 | 102.13 | 18.82 | 37.37 | 45.10 |
| Mean | 36.89 | 82.73 | 95.59 | 18.42 | 34.80 | 42.98 |
| SEd | 0.469** | 1.064** | 0.894** | 0.200** | 0.408** | 0.481** |
| CD (P=0.05) | 0.952 | 2.160 | 1.814 | 0.406 | 0.828 | 0.977 |

**- Highly significant. T2 – Foliar spray at 20 DAT, pre-flowering, 2nd inflorescence, T3 – Localized in root zone at transplanting time, T4 – Drenching in root zone at transplanting and 20 DAT, T5 – Soil application at transplanting and 20 DAT, T6 – Soil application at transplanting time, T7 – Soil application at transplanting time, T8 – Foliar spray at 20 DAT, pre-flowering, 2nd inflorescence.

### Total chlorophyll content

Total chlorophyll content was significantly improved in T8 (humic acid and amino acid mixture) treated plants with the values of 1.50, 2.39 and 1.93 mg $g^{-1}$ (Fig 1) at 30, 60 and 90 DAT respectively, followed by T7 (*Glomus intraradices*) and T5 (*Bacillus megaterium*) treated plants. Maximum difference

between the treatments were observed at 30 and 60 DAT whereas in 90 DAT, T5, T7 and T8 were on par with each other. Lesser chlorophyll content was recorded in control plants (1.30, 1.87 and 1.56 mg $g^{-1}$) at different growth stages.

**Leaf soluble protein content**

Leaf soluble protein content was significantly influenced by the tomato plants treated with T8 (humic acid and amino acid mixture) at 30, 60 and 90 DAT with values of 7.88, 10.28 and 9.33 mg $g^{-1}$ respectively, than the untreated plants (Fig 2). However, T7 (*Glomus intraradices*) inoculated plants registered maximum soluble protein content at 30 (7.71 mg $g^{-1}$), 60 (10.27 mg $g^{-1}$) and 90 (9.30 mg $g^{-1}$) DAT.

**Nitrate reductase activity**

Plants inoculated with AM fungi considerably increased the nitrate reductase activity (Fig 3). T7 (*Glomus intraradices*) plants were recorded more (144.73, 162.90 and 151.97 µg $NO_2$ $g^{-1}$ $h^{-1}$) as compared to control (121.39, 133.23 and 113.64 µg $NO_2$ $g^{-1}$ $h^{-1}$) at different growth stages. T8 (humic acid and amino acid mixture) also showed high nitrate reductase activity at 60 DAT the values obtained in T8 were on par with T7.

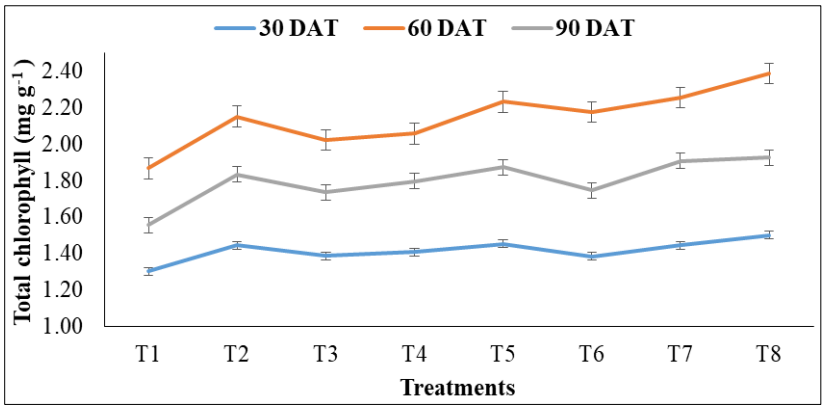


**Fig 1:** Effect of different bio-stimulants on total chlorophyll content of tomato hybrid

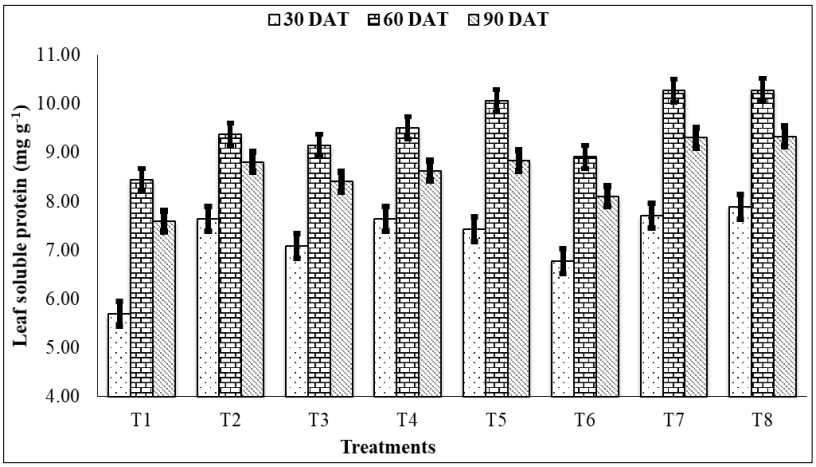


**Fig 2:** Effect of different bio-stimulants on leaf soluble protein content of tomato hybrid

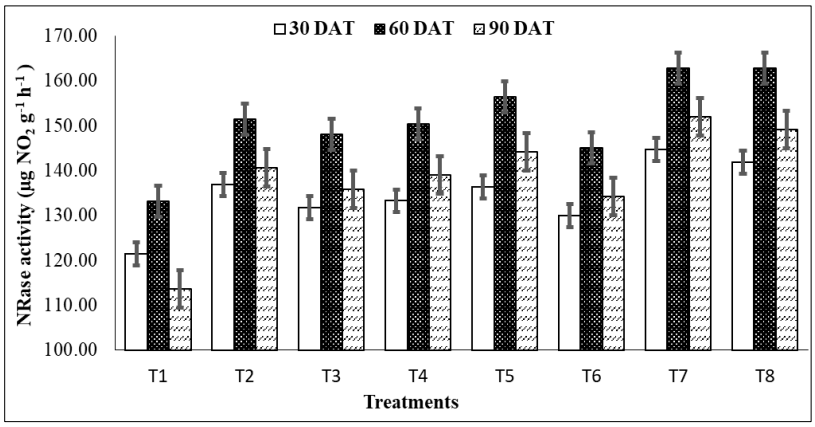


**Fig 3:** Effect of different bio-stimulants on NRase activity of tomato hybrid

**Table 2:** Effect of bio-stimulants on yield traits of tomato hybrid

| Treatments | No. of fruits per plant (no) | Single fruit weight (gm) | Yield (t ha$^{-1}$) |
|---|---|---|---|
| T1 | 20.67 | 100.53 | 70.15 |
| T2 | 24.83 | 110.33 | 97.76 |
| T3 | 24.50 | 104.15 | 78.65 |
| T4 | 25.33 | 106.61 | 91.55 |
| T5 | 26.00 | 110.63 | 98.75 |
| T6 | 24.50 | 104.23 | 81.23 |
| T7 | 26.50 | 113.23 | 102.32 |
| T8 | 28.17 | 107.29 | 102.84 |
| Mean | 25.06 | 107.12 | 90.41 |
| SEd | 0.527** | 0.956** | 1.121** |
| CD (P=0.05) | 1.070 | 1.940 | 2.277 |

**- Highly significant. T2 – Foliar spray at 20 DAT, pre-flowering, 2nd inflorescence, T3 – Localized in root zone at transplanting time, T4 – Drenching in root zone at transplanting and 20 DAT, T5 – Soil application at transplanting and 20 DAT, T6 – Soil application at transplanting time, T7 – Soil application at transplanting time, T8 – Foliar spray at 20 DAT, pre flowering, 2nd inflorescence.

**Yield traits**

The influence of different bio-stimulants on yield parameters like number of fruits per plant, fruit weight and yield per acre were observed (Table 2). Maximum number of fruits per plant (28.17 nos) were recorded in T8 (humic acid and amino acid mixture). Data on fruit weight shows that T7 (*Glomus intraradices*) inoculated plants registered higher fruit weight (113.23 grams). Highest fruit yield was observed in T8 (humic acid and amino acid mixture) and T7 (*Glomus intraradices*) treated plants with the values of about 102.32 t ha$^{-1}$ and 102.84 t ha$^{-1}$ respectively and the values are on par with each other.

**Discussion**

Different bio-stimulants used in this study showed a significant difference in plant morphological physiological and yield traits. In the current study, foliar application of (humic acid and amino acid mixture) significantly increase shoot length. Husein *et al*. (2015) [12] suggested that foliar application of humic and fulvic acid individually or in a combination of both enhance growth and yield attributes of tomato. Growth promoting substance in the bio-stimulants modify different plant growth promoting mechanisms *viz*., cell division, cell permeability and cell division to improve the vegetative growth. In this study, AMF (*Glomus intraradices*) inoculated plants also have notable effect on plant height. In line with our results, AMF infested tomato plants recorded significant difference in stem length, crop establishment, plant vigor and yield also reflected in AMF treated plants under open field condition (Vuksani *et al*., 2015) [20].

Mycorrhizal plant have considerably high root area, root length and root volume (He *et al*., 2019) [10]. Rahman *et al*. (2014) [17] reported that Arbuscular Mycorrhizal Fungi (AMF) inoculated amaranthus plants have significantly higher root length than non-inoculated plants. Our results also in agreement with the above findings that the (*Glomus intraradices*) treated plants have longer roots than the control plants.

Estimation of total chlorophyll content is necessary to understand the photosynthetic process and assimilate production. Lotfi *et al*. (2015) [14] reported that rapeseed plants treated with humic acid as a foliar spray have increased chlorophyll content under both normal and limited water conditions. Abdel-Fattah and mohamedin (2000) [1] reported that *Glomus intraradices* inoculated sorghum plants showed increased photosynthetic pigments. Total chlorophyll content in Mycorrhizal infested plant was significantly higher than control plants. Our results of increased total chlorophll content in T8 (humic acid and amino acid mixture) and T7 (*Glomus intraradices*) were supported by the above findings.

Effect of different bio-stimulants on leaf soluble protein content was observed in this study. Results indicate that T8 (humic acid and amino acid mixture) and T7 (*Glomus intraradices*) applied plants have significantly high protein content in all the growth stages. In line with our results, Rapeseed plants treated with 3 and 6 mg/L of humic acid have higher protein content than untreated plants (Lotfi *et al*., 2015) [14]. AMF colonization induce soluble protein content and plant growth in *Ephedra foliate* (Al-Arjani *et al*., 2020) [2].

Nitrate reductase is the nitrogen cycle enzyme which is responsible for nitrogen absorption in plants. In the present study, T7 (*Glomus intraradices*) inoculated plants showed high Nitrate reductase activity in different growth stages. Meanwhile T8 (humic acid and amino acid mixture) treated plants also recorded maximum NRase activity. Sulistiono *et al*. (2020) [19] showed that AMF application maximize the Nitrate reductase activity and improve plant growth. It positively regulate nitrogen metabolism which maximize amino acid production in plants. Tomato plants treated with humic acid exhibits high nitrate reductase activity (Zandonadi D *et al*., 2014) [22].

Number of fruits per plant and total yield were highly influenced by T8 (humic acid and amino acid mixture). Our results were in harmony with Yildirim (2007) [21] who concluded that foliar application of humic acid resulted in maximum fruit number and total fruit yield. T7 (*Glomus intraradices*) inoculated plants registered higher fruit weight. Conversa *et al*. (2013) [6] suggested that AMF inoculation enhance the fresh weight of tomato fruits.

**Conclusion**

Results obtained in the present study concluded that, compare to control plants bio-stimulants treated plants significantly improves morphological, physiological and yield traits of tomato (*Solanum lycopersicum* L.). Among the different bio-stimulants studied, markedly T7 - Hyoro My$^{+}$ (*Glomus intraradices* 20 kg ha$^{-1}$) at the time of transplanting and T8 - Viva (humic acid and amino acid mixture) at 20 DAT, Pre-flowering, 2nd inflorescence have improved shoot length, root length, total chlorophyll content, leaf soluble protein, NRase activity, fruit number, fruit weight and total fruit yield. Hence these bio-stimulants are the useful tool to promote plant growth and productivity in a sustainable and ecofriendly way.

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