SYNERGISTIC EFFECT OF MICROBIAL AND NON-MICROBIAL BIOSTIMULANTS ON GROWTH, YIELD AND NUTRITIONAL QUALITY OF ORGANIC TOMATO

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Dedicated to

My

Beloved Parents

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CERTIFICATE

*This is to certify that the thesis entitled "**SYNERGISTIC EFFECT OF MICROBIAL AND NON-MICROBIAL BIOSTIMULANTS ON GROWTH, YIELD AND NUTRITIONAL QUALITY OF ORGANIC TOMATO**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS)** in **HORTICULTURE,** embodies the results of a piece of bona fide research work carried out by **MD. NASIR HOSSAIN SANI,** Registration. No.**12-04810** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information as has been availed of during the course of this investigation has duly been acknowledged.

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ABSTRACT

The present pot experiment was conducted at the Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka from October 2017 to April 2018 to evaluate the effects of three biostimulants *Trichoderma* based biostimulants (TB), Seaweed Extract (SWE), Humic Substance (HS) and their combinations on growth, yield, nutritional quality and antioxidant properties of tomato. The experiment was laid out in Completely Randomized Design with four replications. The study was comprised of 13 treatments : T_0 = Control (No biostimulants), T_1= 25g/l TB, T_2 = 50g/l TB, T_3 = 75g/l TB, T_4 = 2 g/l SWE, T_5 = 10 g/l HS, T_6 = 25 g/l TB + 2 g/l SWE, T_7 = 25 g/l TB + 10 g/l HS, T_8= 50 g/l TB + 2 g/l SWE, T_9 = 50 g/l TB + 10 g/l HS, T_{10} = 75 g/l TB+ 2 g/l SWE, T_{11} = 75g/l TB + 10 g/L HS, T_{12} = 2 g/l SWE + 10g/l HS, respectively. The results revealed that, T_8 positively increased growth attributes resulting from the synergistic impact with better nutrient uptake and increased the yield (179.61%) over the control. The treatment T_8 also elicited an increase in total soluble solids as well as bioactive molecules such as lycopene and ascorbic acid, thereby increasing the nutritional and functional quality of the tomato fruits. Therefore, soil drenching of *Trichoderma* with seaweed extract may be considered as a noble strategy for sustainable tomato production with higher yield and superior quality.

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LIST OF ACRONYMS

BARI	=	Bangladesh Agricultural Research Institute
TB	=	*Trichoderma* based Biostimulants
SWE	=	Seaweed Extract
HS	=	Humic Substances
CRD	=	Completely Randomized Design
cm	=	Centimeter
^{0}C	=	Degree Centigrade
DAT	=	Days After Transplanting
et al.	=	and others *(at elli)*
FRG	=	Fertilizer Recommendation Guide
kg	=	Kilogram
g/l	=	Gram/liter
g	=	gram (s)
LSD	=	Least Significant Difference
pH	=	Hydrogen ion conc.
TSS	=	Total Soluble Solid
t ha^{-1}	=	ton/hectare
%	=	Percent
mg kg^{-1}	=	Milligram/kilogram

CHAPTER I

INTRODUCTION

Tomato (*Lycopersicum esculentum* L.) is a widely grown and versatile vegetable throughout the world for taste, color, high nutritive value and diversified use. It occupies a pre-eminent position among vegetables for its high nutrient content. Tomatoes are the major dietary source of the antioxidant lycopene which has been linked to many health benefits, including reduced risk of heart disease and cancer. Due to its sublime nutritional quality and health benefits, tomato has attained the uppermost position among the most consumed fresh vegetables in the world (Perveen *et al*., 2015). However, extensive use of chemical fertilizers for the production of tomato has become a major hurdle to maintain nutritional quality and food safety. In fact, non-judicious use of inorganic fertilizer may lead to environmental pollution including contamination of groundwater. Besides these, the environmental concerns and residuals on edible product is a matter of awareness and emerging demand for quality products have increased. Therefore, organic farming has generated significant interest among consumers and scientists owing to their healthier and safer characteristics to human health.

However, the major drawback of organic vegetable production is the lower yield compared to conventional agriculture (Dorais and Alsanius, 2015). Meanwhile, availability of nutrient (N and P) has been identified to be a major yield-limiting factor in organic production systems. The release of plant available mineral N and P from organic fertilizers is often not synchronized with crop demand leading to mismatch between nutrients bioavailability and plant uptake during the peak growing period (Lester and Saftner 2011). Therefore, there is an urgent need to bring new tools to increase nutrient availability, plant uptake and assimilation in order to close the gap between organic and conventional yields. A promising tool and sustainable approach would be the use of naturally derived biostimulants.

Biostimulants, sometimes referred to as agricultural biostimulants, are a diverse classification of substances that can be added to the environment around a plant and have positive effects on plant growth and nutrition, but also on abiotic and biotic stress tolerance (Patrick du Jardin, 2015).

Microbial biostimulants contain microorganism(s) whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality (Rouphael *et al*., 2017). Several studies conducted on a wide range of horticultural crops reported that foliar or substrate drench application of plant humic substances and seaweed extracts and beneficial microorganisms (*Trichoderma* sp.) can stimulate the primary and secondary metabolism in plants. They improve nutrient uptake and assimilation by promoting the synthesis and accumulation of phytochemicals as well as enhancing the tolerance to abiotic stressors thus boosting crop yields (Rouphael *et. al*., 2017).

In addition to this, due to their effects on the activation and accumulation of the bioactive compounds into edible yields, biostimulants are also a useful tool to promote production of fresh functional food with improved properties (Oancea *et al*., 2016). However, information about the potential benefits derived from applications of microbial and non-microbial biostimulants in organic farming systems is completely missing (De Pascale *et al*., 2017). Meanwhile, little or no experiment was conducted in Bangladesh to find out the impact of biostimulants on tomato in organic production systems. Therefore, more research is needed to elucidate the mode of action of biostimulants to overcome nutrient limitation throughout the improvement of nutrient availability and uptake, thus reducing the gap between organic and conventional yields.

Furthermore, none of the microbial and non-microbial biostimulants has the potential to fulfill the requirements of providing a viable alternative to mineral fertilizers. In recent literature, it has been recognized that the purposeful

combination of microbial biostimulants with other non-microbial substances can result in interactive and synergistic effects that are not achievable with single application. Therefore, this study aims to obtain maximum growth, yield and quality of tomato in a sustainable and environment friendly way with the following specific objectives-

Objectives:

- To investigate the synergistic impact of different biostimulants on growth and yield contributing attributes of organic tomato
- To determine the impact of biostimulants on quality enhancement of organic tomato

CHAPTER II

REVIEW OF LITERATURE

Over the past two decades, interest in organically grown vegetables has been on the rise worldwide, as a result of growing interest of the consumers and scientists in healthy and safer products. In fact, organic horticulture has increased worldwide by almost twofold since 2008, accounting for 3.5 million ha of cultivated organic land in 2014, with more than 87 countries practicing organic agriculture (Willer and Lernoud, 2016). Furthermore, organic horticulture has been often reported as an environmentally-friendly production system able to produce food with minimal harm to ecosystems as well as minimal use of off-farm inputs (Dorais, 2007). However, Plants grown in organic farming are often exposed to nutrient deficiency resulting from low amounts of nutrients in the soil or to the poor solubility of nutrients in soil solution which leads to the lower yield compared to the conventional production. The yield reduction observed in organic horticultural farming was mainly associated to a major biotic pressure (both seed-borne and shoot fungal and bacterial diseases; Van Bueren *et al.*, 2011; Ponisio *et al.*, 2015; Orsini *et al.* 2016) as well as nutrient limitation (Ponti *et al.*, 2012).Therefore, more land is needed to produce the same amount of food as conventional farming, leading to more deforestation, and consequently reducing the environmental benefits of organic practices (Trewavas, 2001). Organic fertilizers, such as compost, have the advantage of recycling nutrients that are already available in the agro-ecosystem, enriching soil with organic matter that converts nutrients to a stable form less susceptible to leaching, but, at the same time, less accessible due to their low solubility, thus requiring more input of energy to be easily processed by plant roots (Chen, 2004; King and Torbert, 2007). Therefore, there is an urgent need to bring new tools to increase nutrient availability, plant uptake and assimilation in order to close the gap between organic and conventional yields. One method by which it is possible overcome this problem is to grow crops with more robust root systems and higher nutrient-uptake efficiency, to

ensure that they receive the nutrients when they need them despite their lower immediate availability when they are introduced in organic form. Alternatively, nutrients can be made more available by promoting certain types of organisms within the soil microbial community (Vessey, 2003). Both of these approaches can be achieved by introducing biostimulants (PBs), to crop leaves, seeds, or soil as a means of stimulating root growth (Canellas *et al.* 2015; Khan *et al.* 2009; Zandonadi *et al.* 2007), efficient root uptake and beneficial microbial populations (Chen, 2004; Vessey, 2003) which is gaining interest globally (Colla and Rouphael, 2015; Rouphael *et al.* 2017a). However, none of the known PGPM per se has the potential to fulfill the requested requirements of providing a viable alternative to mineral fertilizers. In recent literature, it has been recognized that the purposeful combination of plant growth promoting microorganisms can result in interactive and synergistic effects that are not achievable with single applications. Besides synergistic interactions between different microbial bio-effectors, also combinations including natural compounds can provide additional benefits for plant growth.

2.1 PLANT BIOSTIMULANTS

Plant biostimulants have been defined as: substances and materials, with the exception of nutrients and pesticides, which, when applied to plants, seeds or growing substrates in specific formulations have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development, and/or stress response. (Du Jardin, 2012).

2.2 CATEGORIES OF PLANT BIOSTIMULANTS

2.2.1 Humic substances

Humic substances (HS) are natural substances belonging to the soil organic matter and resulting from the decomposition of dead cell materials and from the metabolic activity of soil microbes using these substrates. Humic substances have a high potential for increasing plant production, even in unfavorable environmental conditions.

HS are collections of heterogeneous compounds, originally classified according to their molecular weights and solubility into humins, humic acids and fulvic acids, but with loosely defined boundaries and complex molecular constituents. (Du Jardin, 2012).

Humic substances have been recognized for long as essential contributors to soil fertility, acting on physical, physico-chemical, chemical and biological properties of the soil. Most biostimulant effects of HS refer to the amelioration of root nutrition, via different mechanisms. One of them is the increased uptake of macro- and micronutrients, due to the increased cation exchange capacity of the soil containing the polyanionic HS, and to the increased availability of phosphorus by HS interfering with calcium phosphate precipitation. Another important contribution of HS to root nutrition is the stimulation of plasma membrane H+-ATPases, which convert the free energy released by ATP hydrolysis into a transmembrane electrochemical potential used for the import of nitrate and other nutrients. Besides nutrients uptake, proton pumping by plasma membrane ATPases also contributes to cell wall loosening, cell enlargement and organ growth.

2.2.2 Algal Extracts

The use of fresh seaweeds as source of organic matter and as fertiliser is ancient in agriculture, but biostimulant effects have been recorded only recently. This prompts the commercial use of seaweed extracts and of purified compounds, which include the polysaccharides laminarin, alginates and carrageenans and their breakdown products. Other constituents contributing to the plant growth promotion include micro- and macronutrients, sterols, N-containing compounds like betaines, and hormones (Khan *et al.*, 2009).

Seaweeds act on soils and on plants (Khan *et al.*, 2009). They can be applied on soils, in hydroponic solutions or as foliar treatments. In soils, their polysaccharides contribute to gel formation, water retention and soil aeration.

The polyanionic compounds contribute to the fixation and exchange of cations, which is also of interest for the fixation of heavy metals and for soil remediation. Positive effects via the soil microflora are also described, with the promotion of plant growth-promoting bacteria and pathogen antagonists in suppressive soils. In plants, nutritional effects via the provision and micro- and macronutrients indicate that they act as fertilisers, beside their other roles. Impacts on seed germination, plant establishment and on further growth and development is associated with hormonal effects, which is viewed as major causes of biostimulation activity on crop plants. There is evidence that the hormonal effects of extracts of the brown seaweed *Ascophyllum nodosum* are explained to a large extent by the down- and up regulation of hormone biosynthetic genes in plant tissues, and to a lesser extent to the hormonal contents of the seaweed extracts themselves (Wally *et al.,* 2013).

2.2.3 Protein hydrolysates and other N-containing compounds

Amino-acids and peptides mixtures are obtained by chemical and enzymatic protein hydrolysis from agro-industrial by-products, from both plant sources (crop residues) and animal wastes (Du Jardin, 2012; Calvo *et al.,* 2014; Halpern *et al.,* 2015). Chemical synthesis can also be used for single or mixed compounds. Other nitrogenous molecules include betaines, polyamines and 'non-protein amino acids', which are diversified in higher plants but poorly characterized with regard to their physiological and ecological roles (Vranova *et al.,* 2011). Glycine betaine is a special case of amino acid derivative with well-known anti-stress properties (Chen 2004).

Direct effects on plants include modulation of N uptake and assimilation, by the regulation of enzymes involved in N assimilation and of their structural genes, and by acting on the signalling pathway of N acquisition in roots. By regulating enzymes of the TCA cycle, they also contribute to the cross talk between C and N metabolisms.

Hormonal activities are also reported in complex protein and tissue hydrolysates (Colla *et al.*, 2014). Chelating effects are reported for some amino acids (like proline) which may protect plants against heavy metals but also contribute to micronutrients mobility and acquisition. Antioxidant activity is conferred by the scavenging of free radicals by some of the nitrogeous compounds, including glycine betaine and proline, which contributes to the mitigation of environmental stress.

Indirect effects on plant nutrition and growth are also important in the agricultural practice when protein hydrolysates are applied to plants and soils. Protein hydrolysates are known to increase microbial biomass and activity, soil respiration and, overall, soil fertility. Chelating and complexing activities of specific amino acids and peptides are deemed to contribute to nutrients availability and acquisition by roots.

2.2.4 Beneficial fungi

Fungi interact with plant roots in different ways, from mutualistic symbioses (i.e. when both organisms live in direct contact with each other and establish mutually beneficial relationships) to parasitim (Behie and Bidochka, 2014). Mycorrhizal fungi are a heterogeneous group of taxa which establish symbioses with over 90 % of all plant species. Among the different forms of physical interactions and taxa involved, the Arbuscule-Forming Mycorrhiza (AMF) are a widespread type of endomycorrhiza associated with crop and horticultural plants (Bonfante and Genre, 2010; Behie and Bidochka, 2014). There is an increasing interest for the use of mycorrhiza to promote sustainable agriculture, considering the widely accepted benefits of the symbioses to nutrition efficiency (for both macronutrients, especially P, and micronutrients), water balance, biotic and abiotic stress protection of plants (Gianinazzi *et al.*, 2010; Hamel and Plenchette, 2007; Harrier and Watson, 2004; Siddiqui *et al.*, 2008; Van der Heijden *et al.*, 2004). Fungal-based products applied to plants to promote nutrition efficiency, tolerance to stress, crop yield and product quality should fall under the concept of biostimulants.

Major limitations on their use are the technical difficulty to propagate AMF on a large scale, due to their biotrophic character (Dalpé and Monreal, 2004), and, more fundamentally, the lack of understanding of the determinants of the host specificities and population dynamics of mycorrhizal communities in agro-ecosystems. Nevertheless, other fungal endophytes, like *Trichoderma* spp. (Ascomycota) distinct from the mycorrhizal species, are able to live at least part of their life cycle away from the plant, to colonize roots and, as shown recently, to transfer nutrients to their hosts, using poorly understood mechanisms (Behie and Bidochka, 2014). They are receiving increasing attention, both as plant inoculants easier to multiply in vitro and as model organisms for dissecting the mechanisms of nutrient transfer between fungal endosymbionts and their hosts. There is convincing evidence that many plant responses are also induced, including increased tolerance to abiotic stress, nutrient use efficiency and organ growth and morphogenesis (Colla *et al.* 2015; Shoresh *et al.* 2010). On the basis of these effects, these fungal endophytes may be regarded as biostimulants, though their agricultural uses are currently supported by claims as bio-pesticides.

2.2.5 Beneficial bacteria

Bacteria interact with plants in all possible ways (Ahmad *et al.* 2008): (i) as for fungi there is a continuum between mutualism and parasitism; (ii) bacterial niches extend from the soil to the interior of cells, with intermediate locations called the rhizosphere and the rhizoplane; (iii) associations may be transient or permanent, some bacteria being even vertically transmitted via the seed; (iv) functions influencing plant life cover participation to the biogeochemical cycles, supply of nutrients, increase in nutrient use efficiency, induction of disease resistance, enhancement of abiotic stress tolerance, modulation of morphogenesis by plant growth regulators.

With regard to the agricultural uses of biostimulants, two main types should be considered within this taxonomic, functional and ecological diversity: (i) mutualistic endosymbionts of the type Rhizobium and (ii) mutualistic, rhizospheric PGPRs ('plant growth-promoting rhizobacteria'). Rhizobium and related taxa are commercialized as bio-fertilizers, i.e. microbial inoculants facilitating nutrients acquisition by plants. PGPRs are multifunctional and influence all aspects of plant life: nutrition and growth, morphogenesis and development, response to biotic and abiotic stress, interactions with other organisms in the agro ecosystems (Ahmad *et al.* 2008; Berg *et al.* 2014; Bhattacharyya and Jha, 2012; Gaiero *et al.* 2013; Philippot *et al.* 2013; Vacheron *et al.* 2013). Several of these functions are generally fulfilled by the same organisms, some are strain-specific, and others are dependent on synergisms within bacterial consortia. Agricultural uses of PGPRs are constrained by this complexity, by the variable responses of the plant cultivars and the receiving environments. Also the technical difficulties associated with the formulation of the inoculants give rise to inconsistent results in practice (Arora *et al.* 2011; Brahmaprakash and Sahu, 2012). Despite this, the world market of bacterial biostimulants is growing and PGPR inoculants are now regarded as some kind of plant 'probiotics', i.e. efficient contributors to plant nutrition and immunity (Berendsen *et al.* 2012).

2.2.6 Inorganic compounds

Chemical elements that promote plant growth and may be essential to particular taxa but are not required by all plants are called beneficial elements (Pilon-Smits *et al.* 2009). The five main beneficial elements are Al, Co, Na, Se and Si, present in soils and in plants as different inorganic salts and as insoluble forms like amorphous silica ($SiO_2.nH_2O$) in Graminaceaous species. These beneficial functions can be constitutive, like the strengthening of cell walls by silica deposits, or expressed in defined environmental conditions, like pathogen attack for selenium and osmotic stress for sodium.

Many effects of beneficial elements are reported by the scientific literature, which promote plant growth, the quality of plant products and tolerance to abiotic stress. This includes cell wall rigidification, osmoregulation, reduced transpiration by crystal deposits, thermal regulation via radiation reflection, enzyme activity by co-factors, plant nutrition via interactions with other elements during uptake and mobility, antioxidant protection, interactions with symbionts, pathogen and herbivore response, protection against heavy metals toxicity, plant hormone synthesis and signaling (Pilon-Smits *et al.,* 2009).

2.3 PHYSIOLOGICAL FUNCTIONS AND MECHANISMS INVOLVED INTO PLANT BIOSTIMULATION BY *Trichoderma* spp.

Plant biostimulants *Trichoderma* strains produce several bioactive compounds, which modify the pattern of plant growth and development and induce modifications into plant metabolism, down and up-regulating gene expression. The effects of such bioactive compounds could be summarized in relation to the main recognized activities of plant biostimulants – improved nutrients uptake and use, increased crop stress tolerance and enhanced quality of the edible yield.

2.3.1 Effect of *Trichoderma* spp. on Plant Growth

Dorais, (2007) reported that *Trichoderma* spp. may induce plant growth promotion by improving the availability of nutrients such as N, P and Fe .Several reports have shown the beneficial effects of *Trichoderma* spp. on horticultural crops such as cucumber, periwinkle, chrysanthemum and lettuce on seed germination, vegetative growth and flowering (Hermosa *et al.,* 2012; Studholme *et al.,* 2013).

Azarmi *et al.* (2011) reported that, Seed germination rate was not affected by *Trichoderma* application, whereas shoot height, shoot diameter, shoot fresh and dry weights, number of leaves, leaf area and chlorophyll content were increased by application of *Trichoderma* spp.

Brotman *et al.* (2012) reported that *Trichoderma* live in close relationship with plants and colonize the plant roots that lead to significant changes in plant metabolism and alteration in the content of hormones, soluble sugars, phenolic compounds and amino acids, photosynthetic rate, transpiration and water content and enhance plants growth and development.

Meanwhile, Kotasthane *et al.* (2015) and Zeilinger *et al.* (2016) reported that *Trichoderma* spp. and their secondary metabolites released in the rhizosphere show promotive effect on plant growth and nutrition.

Several studies demonstrated that *Trichoderma* spp. increase root development and crop yield, the proliferation of secondary roots, seedling fresh weight and leaf area (Harman 2000; Janarthanam 2013).

Benitez *et al.* (2004) reported that crop productivity in the fields increased up to 30% after the addition of *T. hamatum or T. koningii.*

Chacon *et al.* (2007) showed that *T. harzianum* is able to promote tomato plant growth by colonizing the roots, increasing the foliar area and secondary roots, as well as changing the root system architecture.

Molla *et al.* (2012) evaluated the ability of *Trichoderma* spp. to enhance growth of tomato plants when supplied together with fertilizer. It was found that *Trichoderma*-enriched biofertilizer such as BioF/compost (household/kitchen wastes composted with *T. harzianum* T22) and BioF/liquid (broth culture containing spores and mycelia of *T. harzianum* T22) alone or in combination with chemical (N: P: K) fertilizer enhanced plant production by 50% compared with a standard dose of N, P, and K macronutrients, minimizing the use of fertilizer and their potential negative effects in the environment.

Azarmi *et al.* (2011) stated that plants grown on soil amended with *Trichoderma* sp. And *T. harzianum* T-969 marked increase in leaf number, leaf area and chlorophyll content.

2.3.2 Effect of *Trichoderma* spp. on Solubility of Micronutrients and P

Accumulating information has shown the efficacy of *Trichoderma* spp. as biostimulants, since their application to soil, seeds or plant surfaces increases the solubility of nutrients as well as the nutrient uptake capacity of the root and/or their distribution within plant parts.

Samolski *et al.* (2012) and Zhao *et al.* (2014) stated that beneficial properties are explained via modulation of root architecture or through the exudation of substances that increase nutrient availability such as siderophores and organic acids.

Altomare *et al.* (1999) reported that *T. harzianum* Rifai 1295-22 showed the ability to solubilize insoluble minerals via various mechanisms including redox activity and chelating metabolites.

Rudresh *et al.* (2005); Anil and Lakshmi (2010) and Saravanakumar *et al.* 2013 reported that the role of *Trichoderma* spp. in solubilization tricalcium phosphate and other phosphorus has been well investigated and results indicated the enhanced availability of P to the plants.

Further, Khan *et al.* (2016) mentioned that *Trichoderma* spp. enriched biofertilizer enhanced tomato growth, leaf greenness, mineral contents (P, K, Ca, Mg, Cu, Fe, Mn and Zn) in tomato roots and produced 12.9% higher yield compared to the recommended doses of NPK.

Furthermore, Lugtenberg and Kamilova, (2009) and Colla *et al.* (2015) reported that through producing siderophores *Trichoderma* atroviride can enhance iron solubility and hence uptake and translocation by plant.

2.3.3 Effect of *Trichoderma* spp. on Nutrient Uptake

Trichoderma spp. plays a vital role in soil nutrient cycling through mobilization and uptake. Several studies indicated that *T. harzianum* can solubilize a number of plant nutrients (Khan *et al.* 2016; Saravanakumar *et al.* 2013; Rudresh *et al.* 2005; Altomare *et al.* 1999).

Yedidia *et al.* (2001) reported that the colonization of cucumber roots by T. asperellum has been shown to enhance the availability of P and Fe with signifcant enhancement in plant biomass.

Singh *et al.* (2010) reported that application of T. *harzianum* (Th 37) formulation (@20 kg ha−1) in sugarcane enhanced the availability of primary nutrient N, P and K by 27, 65 and 44%, respectively.

In another study, Azarmi *et al.* (2011) showed *T. harzianum* isolate T-969, increased the concentrations of Ca, Mg, P and K compared with the control, with positive effects on shoot height, shoot diameter, and shoot fresh and dry weights in tomato seedlings.

Molla *et al.* (2012) tested the ability of *Trichoderma* spp. to increase growth of tomato plants when supplied together with fertilizer. It was found that supplementation of fertilizer with *Trichoderma* enhanced plant production by 50% compared with a standard dose of N, P, and K macronutrients, minimizing the use of fertilizer and their potential negative effects in the environment

2.3.4 Effect of *Trichoderma* spp. on Stress Tolerance

López-Bucio *et al.* (2015) reported that *Trichoderma* spp. help plants to resist stresses via reinforcing plant growth, activation of the antioxidant machinery and defense signalizing pathways in roots and shoots.

Mastouri *et al.* (2010) reported that the treatment of tomato seeds with T. harzianum accelerates seed germination, increases seedling vigour and ameliorates water, osmotic, salinity, chilling and heat stresses by inducing physiological protection in plants against oxidative damage.

Contreras-Cornejo *et al.* (2009) reported that *Trichoderma* spp. may enhance drought tolerance to plants via improved root development activating antioxidant protection against damage by dehydration and delaying drought induced changes in stomatal opening, photosynthesis and chlorophyll leaf content.

Lewis *et al.* (2011) stated that *Trichoderma* colonized plants display an in increased endogenous level of auxins, ethylene and gibberellins, plant enzymes, antioxidants and compatible solutes and compounds like phytoalexins and phenols that provide tolerance to environmental stress. Since ethylene is considered a plant growth regulating compound that inhibits root development and plant growth, its production by fungi or in plants in response to fungal root colonization may be important to fine-tune induced biotic or abiotic stress responses.

The beneficial effects of *Trichoderma* species on alleviating the adverse effects of salt stress have been recently documented in Arabidopsis and crop plants (Mastouri *et al.* 2010, 2012; Rawat *et al.* 2013; Contreras-Cornejo *et al.* 2014; Hashem *et al.* 2014).

Zörb *et al.* (2013) reported that Auxin signaling is a major target of salinity stress in plants and Auxin production by *T. virens* (Tv29.8) and *T. atroviride* promoted plant growth in both normal and saline conditions, which was related to altered root architecture and biochemical changes.

2.4 PHYSIOLOGICAL FUNCTIONS AND MECHANISMS INVOLVED INTO PLANT BIOSTIMULATION BY HUMIC SUBSTANCES (HS)

Nardi *et al.* (2007) reported that Humic substances (HSs) have considerable effects on soil fertility and crop productivity owing to their unique physiochemical and biochemical properties, and play a vital role in establishing biotic and abiotic interactions within the plant rhizosphere. HS are heterogeneous organic molecules that form in the soil as byproducts of microbial metabolism of dead organic matter.

2.4.1 Effect of Humic Substances on Plant Growth

Ayuso *et al.* (1996) reported that HS have a number of positive effects on plant growth, including increased biomass

Arancon *et al.* (2006) reported an increased number of fruits and flowers due to the application of HS.

Yildirim, (2007) stated that HS has positive effect on the nutritional quality improvement of tomato fruits.

Ramos *et al.* (2015) reported the most convincing demonstration of the effect of HS has been the report of their role in lateral root development and root hair formation.

Rose *et al.* (2014) stated that a recent random-effect meta-analysis of HS applied to plants concluded on an overall dry weight increase of 22 ± 4% for shoots and of by 21 ± 6% for roots.

Siddiqui *et al.* (2008) and Olivares *et al.* (2015) reported HS were combined with beneficial microorganisms as plant growth promoter or biological control agents. Since HS are considered recalcitrant to microbial activity, it is possible to use them as a carrier to introduce beneficial microorganisms in the field.

Canellas *et al.* (2010) found significant positive effects on maize crop yield due the combined application of microorganisms with HS.

2.4.2 Effect of Humic Substances on Nutrient Availabiltiy

du Jardin, (2012) stated that the most biostimulant effects of HS refer to stimulation of root growth and improvement of plant nutrition resulting from the increase of soil nutrient availability.

Canellas *et al.* (2015) and du Jardin, (2015) reported that HS act on soil nutrient availability by increasing cation exchange capacity and buffering (neutralize) soil pH. They also stated another important positive effect of HS on soil nutrient availability for plant uptake is the formation of soluble HS complexes with micronutrients (i.e., iron).
Chen *et al.* (2004) reported that the trace element-humic complex has been often considered as a strategy to improve plant nutrition of micronutrients by preventing leaching and making micronutrients more available to plants.

Canellas *et al.* (2015) stated that it is also well established that application of HS stimulates plasma membrane H+-ATPase activity, thereby increasing H+ extrusion from roots and lowering root surface pH, and thus triggering soil nutrient availability for a better uptake and translocation.

Moreover, García *et al.* (2004) reported that HS mainly affect nutrient bioavailability via their ability to form complexes with metallic ions, which enhances the availability of micronutrients (zinc, manganese, copper, and iron); and macronutrients (phosphorus), and particularly when these nutrients are scarce in the soil.

Chen *et al.* (2004) further reported that under some circumstances, micronutrients and P are highly insoluble. HS added to the nutrient solution enhance Fe and Zn solubility by forming metal–humic complexes.

Cesco *et al.* (2000) reported that application of the water-soluble fraction of HS increased the solubility of Fe-hydroxides, as well as their mobility in the soil .

Schmidt *et al.* (2007) showed that water-soluble HS derived from peat cause an increase in root-hair density in Arabidopsis

2.4.3 Effect of Humic Substances on Nutrient Uptake

Plant nutrient uptake depends on a number of factors, including plant species, environmental conditions, and microorganisms associated with plant roots. Root growth and function play a fundamental role in nutrient uptake especially in organic farming where nutrients are often available in soil solution at relatively low concentrations. Several studies have shown that plant biostimulants like HS, PHs and seaweed extracts (SWE) have the potential to boost the root growth and development allowing a better soil exploration and resource acquisition (nutrients and water) by plant roots.

Ayuso *et al.* (1996) showed that HS from a number of different parent materials can improve the uptake of total N as well as other nutrients, such as P, Mn, Cu, Zn, and Fe in barley over the course of an entire growing season.

Paksoy *et al.* (2010) stated that HS improve plant nutrition by affecting soil processes and by directly affecting the plant's physiology. The mechanisms that affect the soil processes include: (1) improvement of the soil structure, (2) improvement of micronutrient solubility in the soil. Direct effects on the plant's physiology include: (3) changes in root morphology, (4) an increase in root activity and (5) an increase in the activity of NO_3-assimilation enzymes.

2.4.4 Effect of Humic Substances on Stress Tolerance

García *et al.* (2014) reported that optimization of plant growth conditions by HSand provision of water, nutrients, and plant growth regulators can help in preventing abiotic stresses.
Türkmen *et al.* (2004) stated that the effectiveness of HS to improve salinity tolerance was also reported by on tomato and okra, respectively. The former authors showed that HS application can induce salt tolerance by increasing root growth, decreasing membrane damage as well as improving the chemical, physical and microbiological properties of soil.

Zhang *et al.* (2014) reported that integrated application of HS and seaweed extract increases drought tolerance, as well as endogenous levels of cytokinins.

Furthermore, Aydin *et al.* (2012) observed that HS application under saline conditions increased proline content, and reduced membrane leakage and reactive oxygen species (ROS) generation in the common bean (*Phaseolus vulgaris* L.), reflecting better adaptability to saline conditions.

2.5 PHYSIOLOGICAL FUNCTIONS AND MECHANISMS INVOLVED INTO PLANT BIOSTIMULATION BY SEAWEED EXTRACT (SWE)

In recent years, use of seaweed extracts have gained in popularity due to their potential use in organic and sustainable agriculture especially in rainfed crops, as a means to avoid excessive fertilizer applications and to improve mineral absorption. Unlike, chemical fertilizers, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Dhargalkar and Pereira, 2005)

2.5.1 Effect of Seaweed Extract on Plant Growth

Battacharyya *et al.* (2015) reported that Seaweed extract (SWE) are a complex mixture of bioactive compounds like polysaccharide, fatty acids, vitamins, phytohormones and mineral nutrients

Pacholczak *et al.* (2016) reported a stimulation of rhizogenesis and root growth after SWE application on cuttings or plants.

Recently, Hernández-Herrera *et al.* (2016) reported that polysaccharide-enriched extracts have a strong root growth-promoting activity suggesting that oligosaccharides can act as signaling molecules inducing changes in endogenous phytohormone metabolism of treated plants by a selective regulation of phytohormone metabolic genes.

Rouphael *et al.* (2017) further reported that polysaccharide-enriched extracts promoted the formation of longer roots in Mung bean hypocotyl cuttings compared with the control and synthetic rooting hormone (Indole-3-butyric acid). Contradictory results have been also reported with no significant effects of SWE application on root growth.

Fan *et al.* (2013) and Jannin *et al.* (2013) conducted several researches and stated that one of the characteristic responses of seaweed extract treatment is an increase in chlorophyll content in the treated plants; this effect has been observed in a wide range of crops including grapevine and strawberry.

Calvo *et al.* (2014) reported that Seaweed extracts have shown in different studies that foliar application leads to enhanced root development and biomass accumulation in different plant varieties including tomato.

2.5.2 Effect of Seaweed Extract on Nutrient Uptake

Seaweed extracts alter physical, biochemical and biological properties of the soil and may also affect the architecture of plant roots facilitating efficient uptake of nutrients.

Lattner *et al.* (2003) reported that the presence of highly cross-linked polymeric network in SWE improved water retention capacity of the soil and as a consequence better growth and development of the plants.

Craigie *et al.* (2011) reported that *Ascophyllum nodosum* extract up regulated the expression of a nitrate transporter gene NRT1.1 which improved nitrogen sensing and Auxin transport resulting in enhanced growth of lateral roots and improved nitrogen assimilation.

Kuwada *et al.* (2006) reported that chemical components of brown seaweed extract are known to induce growth and root colonization of beneficial soil fungi.

Ishi *et al.* (2000) reported that Alginic acid, a major component of brown seaweed extracts, promoted hyphal growth and elongation of arbuscular myocorrhizal fungi and such proliferation of mycorrhizal fungi lead to an improvement in phosphorus nutrition of plants.

Jannin *et al.* (2012) found an increased root and shoot growth in rapeseed treated with extract was associated with enhanced uptake and accumulation of nitrogen and sulphur.

Dobromilska *et al.* (2008) reported that application of a commercial product made with brown seaweed increased mineral nutrient (N, P, K, Ca, Zn and Fe) content of tomato.

2.5.3 Effect of Sea Weed Extract on Stress Tolerance

Seaweed extracts (SWE) as biostimulants are emerging as commercial formulations for use as plant growth promoting factors and a method to improve tolerance to salinity, heat, and drought. Bioactive compounds present in the seaweed extracts enhance the performance of plants under abiotic stresses.

Mancuso *et al.* (2006) reported that foliar applications of extracts have been shown to improve plant tolerance to freezing temperature stress.

Xu *et al.* (2015) reported that *A. nodosum* SWE increased RWC, Fresh Weight, and Dry Weight in spinach (Spinacia oleracea L.) plants under drought stress with some adverse effects on the nutritional value through reduced ferrous ion chelating ability.

Neily *et al.* (2010) found in greenhouse studies that the treatment of vegetables, bedding plants and turf crops with a commercial extract of *A. nodosum* significantly delayed wilting; decreased water use increased leaf water content and improved the recovery of drought- wilted plants, as compared to controls.

Biostimulant treatments of agricultural crops have the potential to improve plant resilience to environmental perturbations. In order to fine-tune application rates, biostimulant-plant specificities and techniques is identified that may yield highest impact on production, quality improvement and stress protection; high priority should be given to better understanding of the causal/functional mechanism of biostimulants. Only once a good understanding of these mechanisms has been reached; we will be able to move to the next generation of biostimulants where synergies and complementary mechanisms can be functionally designed.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from November, 2017 to April, 2018 with the intention to investigate the synergistic impact of *Trichoderma* based microbial biostimulant, humic substance and Sea weed extract powder (*Ascophyllym nodosum*) on growth, yield and nutritional quality of organically grown tomato. The materials and methods that were used for conducting the experiment have been presented in this chapter. It includes a short delineation of the location of experimental site, soil and climatic condition of the experimental area, materials used for the experiment, design of the experiment, data collection and data analysis procedure.

3.1 Location of the experimental site

The experiment was conducted at the Horticulture Research Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka. The GPS (Global Positioning System) of experimental was at 24.09^0N latitude and 90.26^0E longitude with an elevation of 8.4 m above the mean sea level.

3.2 Characteristics of soil that used in pot

Soil of the experimental site belongs to the Salna series representing the Shallow Red Brown Terrace soil which falls under the agro-ecological zone (AEZ) of Modhupur Tract under AEZ No. 28 (UNDP, 1988). The physiochemical properties of the experimental soil including initial status of N: P: K was analyzed in the Soil Research and Development Institute Dhaka, and result has been presented in Appendix I.

3.3 Climatic condition of the experimental site

Experimental area is situated in the sub-tropical climate zone, which is characterized by heavy rainfall during the months of April to September and scanty rainfall during the rest period of the year. Details of the meteorological

data during the period of the experiment was collected from the Bangladesh Meteorological Department, Agargoan, Dhaka and presented in Appendix II.

3.4 Planting materials

Seeds of tomato (*cv.* BARI tomato 14) were collected from the Horticulture Research Centre of Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Seedlings of 30 days of BARI Tomato-14 were used as planting material. The seedlings of tomato were grown at the nursery of Horticulture Farm in of Sher-e-Bangla Agricultural University. BARI Tomato-14, a high yielding variety of Tomato was developed by the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. Its total growing duration is about 110-120 days.

3.5 Treatment of the experiment

T_0= Control (No biostimulant)

T_1= 25 g/l *Trichoderma*

T_2= 50 g/l *Trichoderma*

T_3= 75 g/l *Trichoderma*

T_4= 2 g/l SWE (Seaweed Extract)

T_5 = 10 g/l HS (Humic Substances)

T_6= 25 g/l *Trichoderm*a + 2 g/l SWE

T_7= 25 g/l *Trichoderma* + 10 g/l HS

T_8= 50 g/l *Trichoderma* + 2 g/l SWE

T_9 = 50 g/l *Trichoderma* + 10 g/l HS

T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE

T_{11}= 75 g/l *Trichoderma* + 10 g/l HS

T_{12}= 2 g/l SWE + 10 g/l HS

3.6 Source of Biostimulants: Different biostimulants have been collected from the following sources-

***Trichoderma* Powder:** GME Agro Limited, Savar, Dahaka, Bangladesh.

Seaweed Extract powder: Global Laboratory, Vadodara, Gujrat, India.

Humic Substance: Laksam Scientific Company, 32/1, Shahid Nazrul Islam Sarak, Hatkhola road , Dhaka-1203, Bangladesh.

3.7 Design and layout of the experiment

The pot culture based experiment was laid out in Completely Randomized Design (CRD) with four replications where 13 treatments were allotted at random. Two plants were placed under each treatment. There were 104 unit pot altogether in the experiment. Each pot was 35 cm (14 inches) in diameter and 30 cm (12 inches) in height.

3.8 Raising of seedlings

Seedlings were raised in the seed bed with optimum care and management. Tomato Seedlings were raised in one seedbed on a relatively high land at Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka. The size of the seedbed was 3m× 1 m. The soil was well prepared with spade and made into loose friable and dried mass to obtain fine tilth. All weeds and stubbles were removed by spade and khurpi and 5 kg well rotten cow dung was applied during seedbed preparation. The seeds were sown in the seedbed at 16 October; 2017. Germination was visible 3 days after sowing of seeds. After sowing, seeds were covered with light soil to a depth of about 0.6 cm. The emergence of the seedlings took place within 5 to 6 days after sowing. Necessary shading by white polythene was provided over the seedbed to protect the young seedlings from scorching sun or heavy rain. Weeding, mulching and irrigation were done from time to time as and when required and no chemical fertilizer was used in this seedbed.

3.9 Preparation of Pot

Air dried soil was used for pot preparation. A ratio of 1:3 well decomposed cow dung and silt loam soil was mixed thoroughly and pots were filled 15 days before transplanting. All 117 pots were filled on October, 2017. Each pot was filled with 10 kg of soil mixed with cowdung. Weeds and stubbles were completely removed from the soil before mixing the soil with decomposed cowdung.

3.10 Uprooting and Transplanting of Seedlings

Healthy and uniform 30 days old seedlings were uprooted separately from the seedbed and were transplanted in the experimental pots in the afternoon of 16 November, 2017 maintaining two seedlings in each pot. Each pot was placed 45 cm apart from each other. The seedbed was watered before uprooting the seedlings from the seedbed so as to minimize damage to roots with ensuring maximum retention of roots. The seedlings were watered after transplanting.

3.11 Application of manure

As the tomato was grown organically, no inorganic fertilizers were applied. As per supplementation of the nutrient required for BARI Tomato-14 recommended in FRG (2012) by BARC (Bangladesh Agricultural Research Council), well decomposed cowdung was used. For higher yield of tomato, the required amount of N: P: K was calculated based on the following equation of Bangladesh Agricultural Research Council (BARC), 2012.

$$\mathrm{Fr} = \mathrm{Uf} - \frac{\mathrm{Ci}}{Cs} \times (\mathrm{St} - \mathrm{Ls})$$

Where,

Fr is the fertilizer nutrient required for given soil test value

Uf is the upper limit of the recommended fertilizer nutrient for the respective soil test value interpretation (STVI) class (Appendix III).

Ci is the units of class intervals used for the STVI class (Appendix III),

St is the test value of experimental soil (Appendix I),

Ls is the lower limit of the soil test value within the STVI class

Using the data given in Appendix I (soil test value) and Appendix III (STVI class and nutrient recommendation) a calculation was done to find out the required amount of N: P: K . The required amount of N: P: K per meter^2 was 13.45 g, 2.42 g and 1.13 g, respectively. To supplement this well decomposed cowdung 20 ton/ha was applied during pot preparation.

3.12 Application of the treatments

3.12.1 Preparation of solution

The solutions of plant biostimulants were prepared by dissolving them directly in water.

3.12.2 Method of application

All the treatments were applied as soil drenching. As per treatment, all the *Trichoderma* solution was applied immediately after transplanting of seedling through soil drenching. All doses of Humic Substance and Sea Weed Extract (*Ascophyllum nodosum)* were applied in three split application at 15 DAT, 30 DAT and 45 DAT as soil drench.

3.13 Intercultural operations

3.13.1Gap filling

Only a very few seedlings were damaged after transplanting and these were replaced by the healthy new seedlings from the same stock.

3.13.2 Weeding

Weeding was done whenever it was necessary, mostly in vegetative stage by hand pulling.

3.13.3 Staking

When the plants were well established, staking was provided to each plant by bamboo sticks tied with rope for support to keep them erect.

3.13.4 Plant Protection Measures

Tomato fruit borer, white fly, leaf miner are the major insect pest of tomato in Banlgadesh. To reduce their intensity of infestation clean cultivation was done along with the whole experimental area was covered with nylon net. However, to control these major insect pest neem seed extract was sprayed (1 kg broken neem seed soaked in 20 L of water) three times at 10 days interval after flowering starts. BARI tomato 14 is resistant to bacterial wilt. To control blight of tomato seeds were treated with BAU Bio-fungicide (3%) solution before sowing. In case of leaf curl virus, infected leaves and plants were collected and destructed.

3.14 Harvesting

Fruits were harvested at 3-5 days interval during early ripe stage when they developed slightly red color. Harvesting was started from last week of February and was continued up to first week of April, 2018.

3.15 Data collection

Data were collected from plant of each unit pot.

A. Morphological characters

3.15.1 Plant height (cm)

Plant height was measured from plant of each unit pot from the ground level to the tip of the longest stem and mean value was calculated. Plant height was recorded at 20 days interval starting from 30 days of planting up to 70 days to observe the growth rate of plants.

3.15.2 Number of branches per plant

The total number of branches per plant was counted from plant of each unit pot. Data was recorded at 20 days interval starting from 30 days of planting up to 70 days.

3.15.3 Number of leaves per plant

The total number of leaves per plant was counted from plant of each unit pot. Data was recorded at 20 days interval starting from 30 days of planting up to 70 days.

B. Physiological characters

3.15.4 SPAD value

SPAD value was determined from plant samples by using an automatic SPAD meter. A Minolta SPAD-502 Meter (Minolta, Japan) was used to determine the chlorophyll content of leaves. SPAD reading were taken from three apical leaflets of the young fully expanded leaf at flowering stage. Three readings were taken in each leaf. The results were expressed as SPAD units.

3.15.5 Root dry weight (g)

Two plants were uprooted at harvest from each treatment, the fruits were separated and the roots were washed thoroughly with tap water and the excess water adhering to the roots was removed with the help of blotting paper and dried at 70 ºC for 72 hours in hot air oven till two consecutive weights remained unchanged and expressed in grams per plant.

3.15.6 Shoot dry weight (g)

Two plants were uprooted at harvest from each treatment, the fruits and roots were separated, cleaned and dried at 70 ºC for 72 hours in hot air oven till two consecutive weights remained unchanged and expressed in grams per plant.

C. Yield contributing and yield characters

3.15.7 Number of flower cluster plant^{-1}

The number of flower cluster was counted from plant of each unit pot and the numbers of flower clusters produced per plant were recorded.

3.15.8 Number of flowers cluster^{-1}

The number of flower was counted from plant of each unit pot and number of flower produced per cluster was recorded on the basis of flower cluster per plant.

1.15.9 Number of fruits cluster^{-1}

The number of fruits per cluster was counted from plant of each unit pot and the number of fruits per clusters was recorded.

3.15.10 Number of fruits plant^{-1}

The number of fruit per plant was counted from plant of each unit pot and the number of fruits per plant was recorded.

3.15.11 Length of fruit (cm)

The length of fruit was measured with a slide calipers from the neck of the fruit to the bottom of 5 selected marketable fruits from each pot and there average was taken and expressed in cm.

3.15.12 Diameter of fruit (cm)

Diameter of fruit was measured at the middle portion of 5 selected marketable fruit from each pot with a slide calipers and the average was taken and expressed in cm.

3.15.13 Weight of individual fruit (g)

Individual fruit weight was measured by the following formula:

$$\text{Weight of individual fruit} = \frac{\text{Total weight of fruits per plant}}{\text{Total number of fruits per plant}}$$

3.15.14 Yield plant^{-1} (kg)

Yield of tomato per plant was recorded as the whole fruit per plant harvested in different time and was expressed in kilogram.

3.15.15 Total soluble solid (TSS)

Total soluble solid (TSS) was determined using hand refractometer immediately after harvesting. The refractometer was calibrated with distilled water before use. The readings were recorded for each sample by putting a drop of juice on the prism and value was recorded. A temperature correction was applied when it was above or below 20^0C and the readings were expressed in degree brix (^{0}B).

3.15.16 Fruit pH

The pH of fruit juice was measured using an automatic pH meter (Labmeter PHS-3B) as recommended by AOAC.

3.15.17 Total Sugar

For estimation of sugars, 25 ml of juice extract was taken and filtered through Whatman No.4 filter paper and the final volume was made to 75 ml with distilled water. This was then neutralized with 1N NaOH and then 2 ml of lead acetate (45%) was added to it and kept for 10 minutes to remove the impurities. Excess of lead acetate was removed from the sample by using a sufficient quantity of potassium oxalate (22%) in a 250 ml volumetric flask.

Again the volume was made to 250 ml with distilled water and filtered through Whatman No.4 filter paper. Clear filtrate was used for estimation of total sugars as below-

For estimation of total sugar 50 ml of filterate was taken in a beaker and 5g of citric acid was added to it and boiled on a hot plate for complete inversion of sugars. It was then neutralized with 1N NaOH and the final volume was made to 250 ml with distilled water (aliquate). The total sugars were estimated by titrating a boiling solution of 5 ml each Fehling A and B against aliquate using methylene blue as an indicator. The end point was noted by obtaining brick red colour and total sugars were expressed as percentage of fresh weight of fruit aril and calculated as given below (Ranganna, 1995).

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{*Factor x Dilution x Dilution}}{\text{Titre value x Weight or Volume of sample taken}} \times 100$$

*Factor = 0.05

3.15.18 Ascorbic acid (mg/100g)

10 g of blended fruit pulp from five randomly selected red ripe fruits served as a sample for estimation of ascorbic acid by using 2, 6-dichlorophenol indophenol titration method. Four per cent oxalic acid was added to sample. The volume made up to 50 ml in a volumetric flask and filtered using Whatman No.4 paper and 25 ml of this filtrate was taken and titrated against 2, 6-Dichlorophenol-indophenol dye. The titration was carried out up to a light pink colour to appear. The dye was prepared using 50 mg of sodium salt of 2, 6-Dichlorophenol-indophenol dye in approximately 200 ml of double distilled water containing 4.2 mg of sodium bicarbonate. It was used for titration and standardizing ascorbic acid by Ranganna (1986).

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titratable value} \times \text{Dye factor} \times \text{vol. made up}}{\text{Aliquot of extraction} \times \text{vol. of sample taken}} \times 100$$

3.15.19 Lycopene (mg/100g)

The lycopene content of tomato was analyzed by using the following procedure- One gram of blended fruit sample was taken into a mortar and pulp was extracted repeatedly with acetone until the residue turned colorless. The acetone extract was transferred to separating funnel containing 10 to 15 ml of hexane layer by diluting the acetone with water. Hexane containing pigments were transferred to 25 ml volumetric flask and diluted to the mark with hexane. Then one ml of aliquot was further diluted to 4 ml with hexane and the absorbance (Ab.) was read in a spectrophotometer at 503 nm. The lycopene content was calculated by using the formula.

$$\text{Lycopene (mg/100g)} = \frac{\text{Ab. of sample} \times \text{Vol. made up} \times \text{dilution factor}}{1 \times \text{weight of sample (g)} \times 100} \times 100$$

3.15.20 Determination of mineral contents in shoots of tomato plants

To determine the concentration of minerals (N, P, K, Fe and Zn) in shoots two plants from each replication were uprooted carefully at final harvest and then washed with clean water. Harvested plants were air dried in the laboratory at room temperature. After 72 hours, shoots were oven dried at 70^0 C until constant weight was obtained. Dry matter was pooled (shoots), ground and digested with concentrated HNO_3-H_2O_2 using the methods described by Yedidia et al. (2001). The digest were used to determine the mineral contents as the methods described by Piper (1966) using atomic adsorption spectrophotometer.

3.16 Statistical analysis

The data obtained for different characters were statistically analyzed by using SPSS computer package program to find out the significance of the difference for different microbial and non-microbial biostimulants on growth, yield contributing characters, mineral contents and nutritional quality of tomato. The significance of the difference among the treatment combinations of means was estimated by Tukey's Test at 0.05% level of significance.

Chapter IV

RESULTS AND DISCUSSION

This chapter represents the results and discussion of the present study. The experiment was conducted to investigate the synergistic impact of application of *Trichodrma* based microbial biostimulants and non-microbial organic plant biostimulants on growth, yield and national quality of organically grown tomato. Data on growth, yield and quality parameter were recorded in both field and laboratory. The analyses of variance (ANOVA) of the data on different growth, yield and quality parameters are presented in Appendix V-XVI. The results have been presented and discussed with the help of Tables and graphs and possible interpretations are given under the following headings:

4.1 Plant Height (cm)

The data pertaining to the effect of microbial and non-microbial plant biostimulants on plant height of tomato at 30, 50 and 70 DAT are presented in Figure 1, 2, 3 & Appendix V. The application of biostimulants significantly increased the plant height at 30, 50 and 70 DAT. At 30 DAT the maximum plant height (46.42 cm) was recorded in treatment T_8 (50g/l *Trichoderma*a + 2g/l SWE) which was statistically similar to T_{12} (2g/l SWE + 10 g/l HS) but was significantly superior to all other treatments. This was followed by treatment T_2 (40.50 cm) which also exerted a significant increase in plant height and was statistically at par with T_3 (37.76 cm), T_4 (38.96cm), T_6 (39.46 cm), T_9 (39.82 cm), T_{10} (38.42 cm) and T_{11} (38.33 cm). The minimum plant height (29.75 cm) was observed in treatment T_0 (control) where no biostimulants were applied.

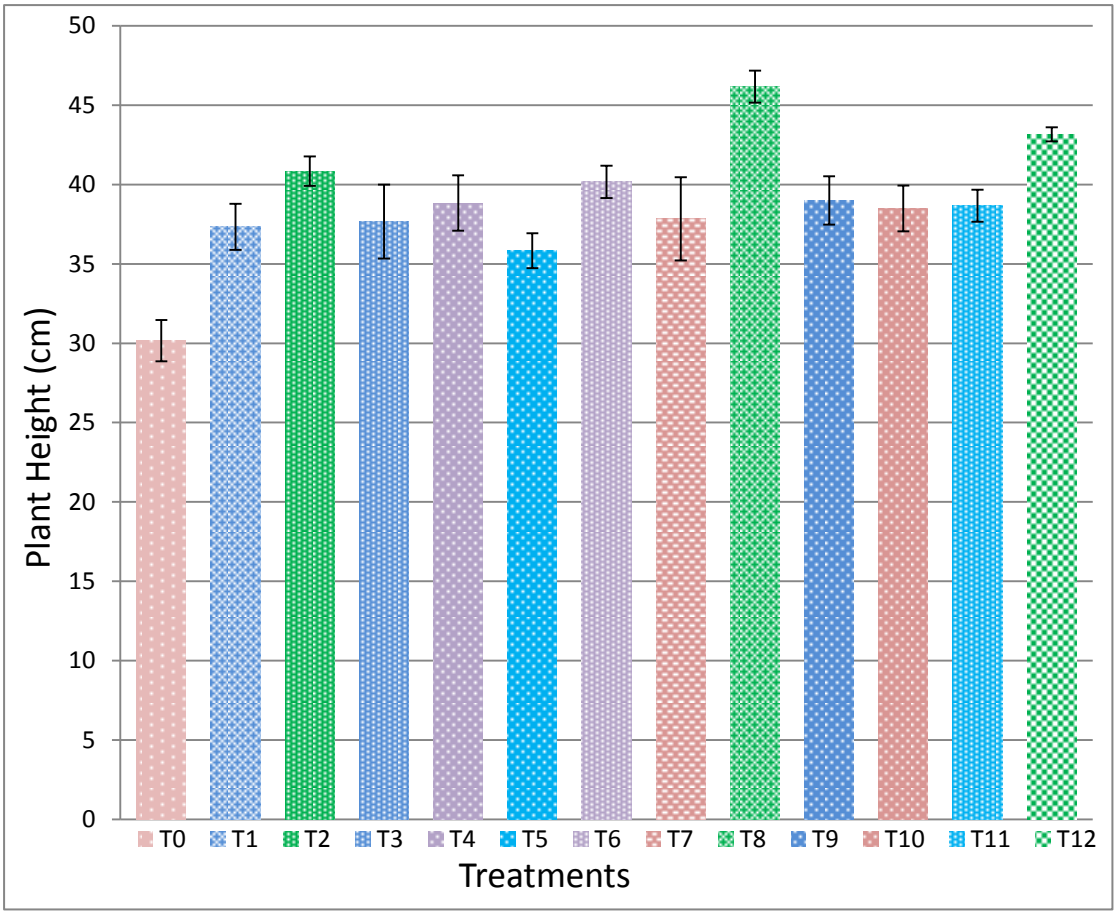

Figure 1. Effect of microbial and non-microbial biostimulants on plant height of organic tomato at 30 days after transplanting (DAT)

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

Vertical bar represent the standard error of the means

At 50 DAT, the longest plant (88.31 cm) was found from T_8 (50g/l *Trichoderma*a + 2g/l SWE) which was statistically similar to T_6, T_7, T_9 and T_{12}. On the other hand, the shortest plant (65.37 cm) was found from the treatment T_0 (control) at 50 DAT. The results further showed that all other treatments also exerted a significant increase in plant height and was statistically similar for the character plant height at 50 DAT.

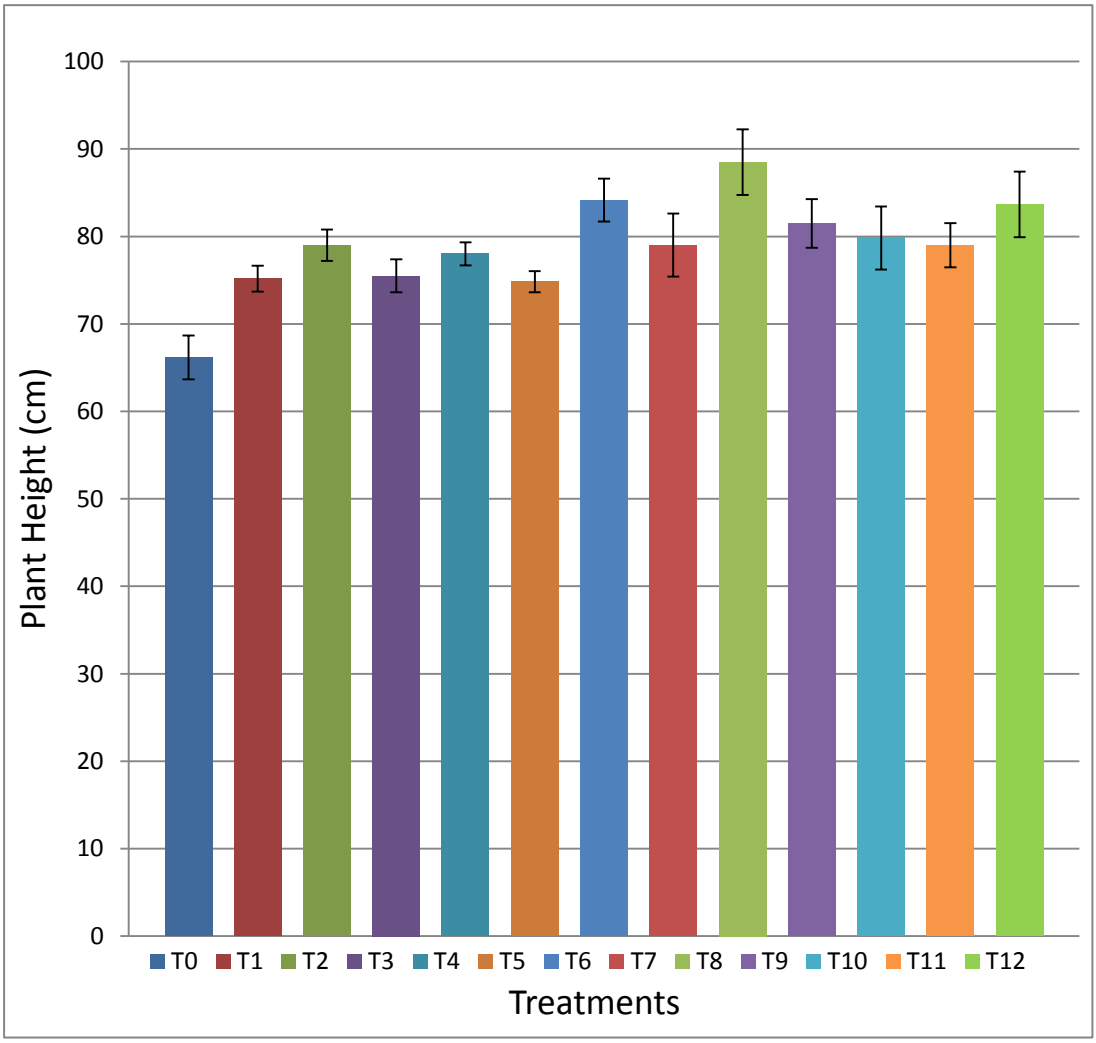

Figure 2. Effect of microbial and non-microbial biostimulants on plant height of organic tomato at 50 days after transplanting (DAT)

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderma* + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

Vertical bar represent the standard error of the means

At 70 DAT, the longest plant (111.81 cm) was obtained from T_8,which was statistically at per with T_9 and T_{12} while the shortest plant (85.62 cm) was found from the treatment T_0 (control) where no biostimulants were applied. All other treatments showed intermediate range of this parameter plant height of tomato.

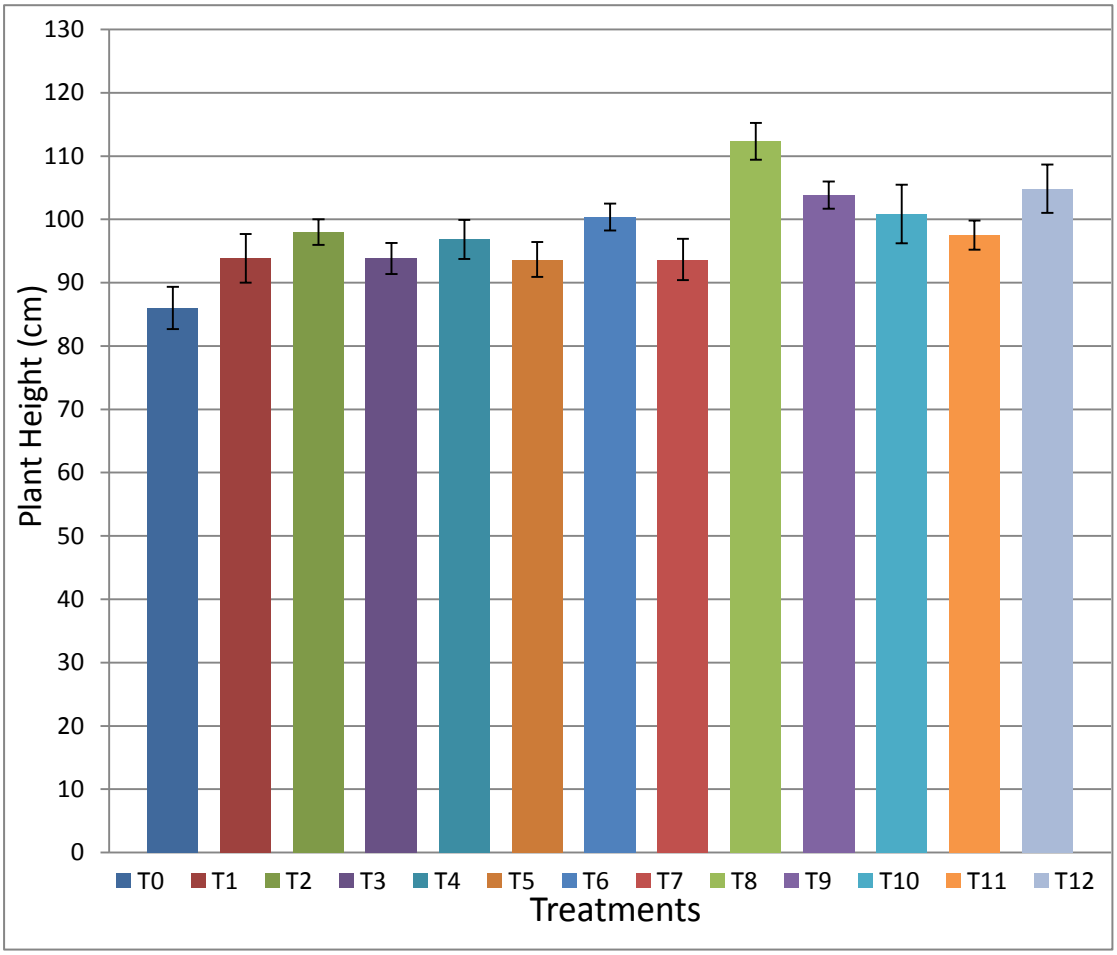

Figure 3. Effect of microbial and non-microbial biostimulants on plant height of organic tomato at 70 days after transplanting (DAT)

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

Vertical bar represent the standard error of the means

Looking at the result in the graphs in more detail it is clearly evident that application of biostimulants had significant positive impact in increasing plant height of tomato over the control at all dates of data recording. Meanwhile combined application of microbial and non-microbial biostimulants showed better performance in increasing plant height of tomato than sole treatments. Amongst the treatments, the tallest plant was recorded in T_8 (50g/l *Trichoderma*a + 2g/l SWE) at all three dates of data recording. This might be due to the synergistic impact of *Trichodema* along with Sea Weed Extract (*Ascophyllum nodosum*) which exerted a significant growth promotion over all other treatments. The result is in agreement with the Dorais, 2007 and Chacon *et al.* 2007 who reported that *Trichoderma* spp. may induce plant growth promotion by colonizing the roots, increasing the foliar area and secondary roots, as well as changing the root system architecture. Meanwhile, Kotasthane *et al.* 2015 and Zeilinger *et al.* 2016 reported that *Trichoderma* spp. and their secondary metabolites released in the rhizosphere show promotive effect on plant growth and nutrition. Application of product made with brown seaweed (*Ascophyllum nodosum)* are reported to induce growth and root colonization of beneficial soil fungi (Kuwada *et al.* 2006, Dobromilska *et al.* 2008). Alginic acid, a major component of brown seaweed extracts, promoted hyphal growth and elongation of beneficial fungi (Ishi *et al.* 2000) and such proliferation lead to an improvement in mineral nutrition uptake and growth promotion of plants and our present findings is in strong agreement with their findings.

4.2 Number of branches plant^{-1}

The data with respect to number of branches per plant recorded at 30 DAT, 50 DAT and 70 DAT are presented in Table 1 & Appendix VI. The number of branches per plant was found to deviate significantly due the application of different plant biostimulants treatments either alone or in combination at every stage of observations. Among the treatments, T_8 having 50 g/l *Trichodrma* + 2 g/l SWE resulted in significantly higher number of branches per plant at every stage as compared to the other treatments.

At 30 DAT, the maximum number of branches (5.22) was found from T_8 which was statistically similar to T_6, T_9, T_{10} and T_{12}. Meanwhile, the minimum number of branches (2.39) per plant was obtained from the treatment T_0 (control) where no biostimulants were applied.

Table 1. Effect of microbial and non-microbial biostimulants on number of branches per plant of tomato at different days after transplanting (DAT)

Treatments	Number of branches plant^{-1}		
	30 DAT	50 DAT	70 DAT
T_o	2.39 c	5.59 d	5.67 e
T_1	3.07 bc	6.33 cd	5.74 de
T_2	3.25 bc	7.52 ac	8.48 be
T_3	2.91 bc	7.24 bd	7.42 ce
T_4	2.84 bc	6.88 bd	7.06 ce
T_5	3.36 bc	6.58 bd	6.77 de
T_6	3.87 abc	8.20 ab	8.97 bd
T_7	3.12 bc	7.16 bd	8.24 be
T_8	5.22 a	9.21 a	12.70 a
T_9	3.52 abc	7.96 ac	9.72 ac
T_{10}	3.56 abc	7.52 ac	8.24 be
T_{11}	3.33 bc	7.05 bd	7.72 ce
T_{12}	4.20 ab	7.56 ac	11.71 ab
SE(±)	0.486	0.482	1.001
CV	20.00	9.35	17.32

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

AT 50 DAT, the maximum number of branches (9.21) was recorded in T_8 which was significantly superior to all other treatments. On the other hand, lowest number of branches (5.59) was found from the T_0 (control) treatment.

Accordingly, at 70 DAT stage, the maximum number of branches up to 12.70 was recorded in treatment T_8 followed by 11.71 in case of T_{12} and 9.72 in case of T_9. In contrast, the minimum number of branch (5. 67) was found from the control treatment. The results further showed that treatment T_6, T_8, T_9, T_{10} and T_{12} which are the combined treatment of *Trichodemra* along with other biostimulants were significantly superior compared to the control. The findings indicate that the *Trichodemra* along with HS and SWE had synergistic effect on the growth resulting from the better nutrient uptake and assimilation which might have facilitated the increased vegetative growth and the number of branches per plant. Our findings are in agreement with the findings of Calvo *et al.* 2014 and Saa *et al.* 2015.

4.3 Number of leaves plant^{-1}

The data with respect to the number of leaves per plant was recorded at 30, 50 and 70 DAT are presented in Table 2 & Appendix VII. Significant difference was observed in terms of number of leaves per plant at different DAT due to the application of plant biostimulants. At 30 DAT, the highest number of leaves (16.12) per plant was recorded from the treatment T_8 which was superior to all other treatments. This was followed by treatment T_{12} (12.96), T_9 (11.56) and T_6 (11.37) which were superior compared to other treatments. On the contrary, the minimum number of leaves (6.40) per plant was recorded in the control (T_0).

At 50 DAT, the highest number of leaves (35.46) per plant was found from T_8 which was statistically similar to T_3, T_4, T_6, T_7, T_9, T_{11} and T_{12}. On the other hand, the minimum number of leaves (22.10) per plant was found from the treatment T_0 (control).

The results further showed that all other treatments also exerted a significant increase in the number of leaves per plant and was statistically similar for the character plant height at 50 DAT.

Table 2. Effect of microbial and non-microbial biostimulants on number of leaves per plant of tomato at different days after transplanting (DAT)

Treatments	Number of leaves plant^{-1}		
	30 DAT	50 DAT	70 DAT
T_0	6.40 c	22.10 c	51.22 d
T_1	8.70 bc	26.56 bc	55.23 cd
T_2	10.46 bc	27.71 bc	62.54 abc
T_3	7.81 bc	30.24 ab	55.92 cd
T_4	10.12 bc	31.45 ab	55.34 cd
T_5	10.69 bc	26.12 bc	58.41bcd
T_6	11.37 abc	31.61 ab	63.96 abc
T_7	9.51 bc	27.81 bc	55.22 cd
T_8	16.12 a	35.46 a	71.56 a
T_9	11.56 abc	29.17 ab	62.80 abc
T_{10}	10.20 bc	28.46 bc	58.27 bcd
T_{11}	8.70 bc	29.51 ab	55.94 cd
T_{12}	12.96 ab	30.71 ab	67.47 ab
SE(±)	1.49	1.83	2.68
CV	20.36	8.92	6.37

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

As regard number of leaves per plant T_8 was found significantly superior to all other treatments. The maximum number of leaves (71.56) was found from T_8 followed by T_{12} (67.47), T_9 (62.80) and T_2 (62.54). All other treatments also showed significant increase in of number of leaves per plant compared to control. The result further showed that T_8 was found superior to all other treatments in every stage of observations. This might be due the synergistic effect of *Trichoderma* and SWE (*Ascophyllum nodosum*) which promoted the significant increase in number of leaves per plant at all stages of observations. Several studies reported that the plant growth promotion resulting from better nutrient uptake induced by microbial based biostimulants and SWE have been associated with making soil nutrients more available to plant uptake (Hayat *et. al.* 2010; Calvo *et. al.,* 2014; Colla *et. al.*, 2015; Rouphael *et. al.,* 2015). This was also supported by Thirumaran *et. al.,* 2009 and Sashikumar *et. al.,* 2011.

4.4 Chlorophyll (%)

The effect of various plant biostimulants on the chlorophyll content is detailed in Fig 4 and Appendix VIII. Significant variation was observed for SPAD values due to the application of different plant biostimulants at flowering stage. At flowering stage highest SPAD value (50.40) was recorded from T_{12} (2g/l SWE + 10g/l HS) which was statistically similar to T_8 (50g/l *Trichoderma* +2g/l SWE) and T_9 (50 g/l *Trichoderma* + 10g/l HS). Meanwhile, the lowest SPAD value (40.25) was recorded from T_0 (control) which was statistically identical that of T_4. This might be due to the synergistic effect of *Trichoderma*, HS and SWE which resulted in significant increment in chlorophyll content of tomato leaves over control and single treatments. It has been reported that seaweed extract (*Ascophyllum nodosum*) contains betanines which increased chlorophyll content in the treated plants and this effect has been observed in a wide range of crops (Fan *et al.,* 2014; Jannin *et al.,* 2013; Mancuso *et al.* 2006; Sivasankari *et al.,* 2006; Spinelli *et al.,* 2010). Our finding is in complete agreement with their findings.

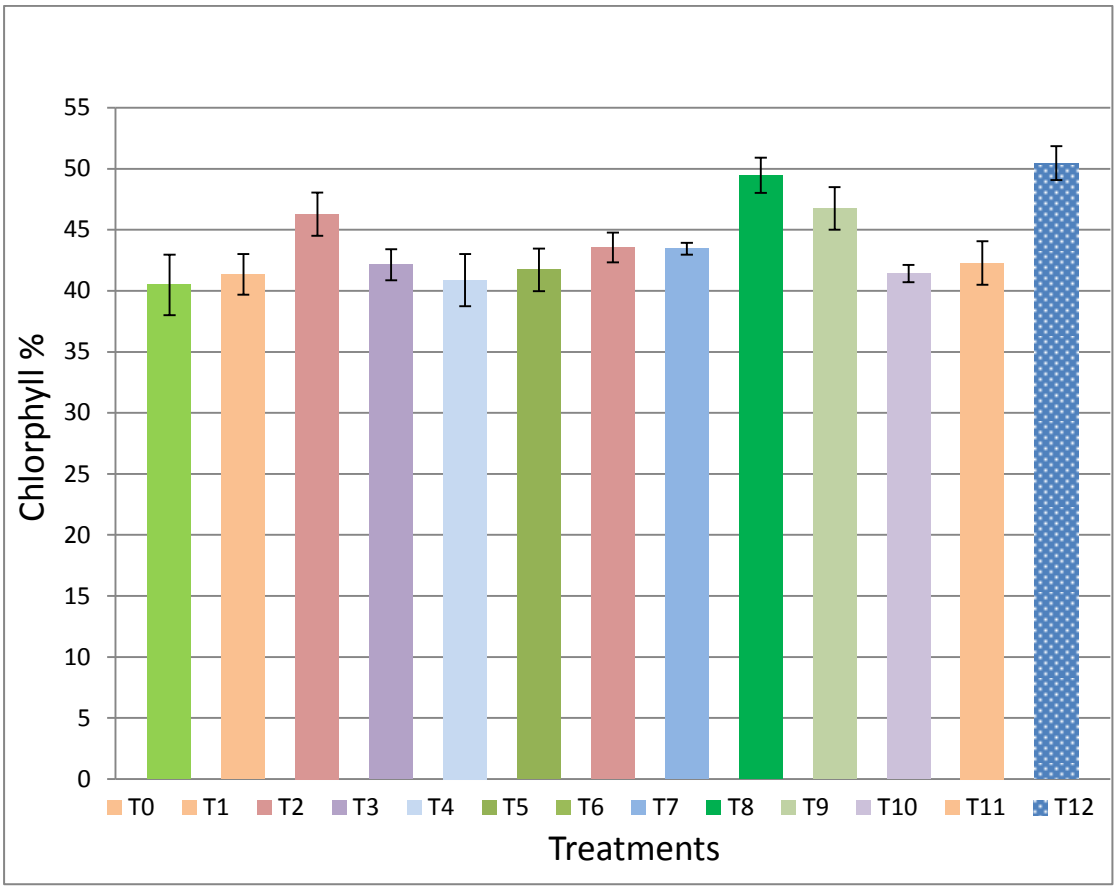

Figure 4. Effect of microbial and non-microbial biostimulants on chlorophyll percentage (SPAD value) of tomato

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderma* + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

Vertical bar represent the standard error of the means

4.5 Root Dry Weight (g)

The data pertaining to the effect of different biostimulants on root dry matter accumulation of tomato are presented in Table 3 & Appendix IX. The root dry weight was found to differ significantly amongst the different treatments of biostimulants.

Amongst the treatments, T_8 resulted in significantly higher root dry weight (5.56 g) compared to the reaming treatments. This was closely followed by the treatments T_{12} (5.12 g), T_2 (4.72g) and T_9 (4.44 g) and were also statistically similar to that of T_8. The significantly lowest root dry weight (3.49 g) was found from the T_0 (control) where no biostimulants were applied. All other treatments also exerted a significant increase in root dry weight over the control and were statically similar to each other. Seaweed extract has been reported to improve root development (Khan *et al.,* 2009; Xu *et al.,* 2015; Rose *et al.,* (2014) and Hernández-Herrera *et al.* 2016. Meanwhile, Rouphael *et al.,* 2015 reported that Microbial-based biostimulants can also stimulate root growth which was also supported by Rubin *et al.,* 2017 who reported an increase of root mass by 35% and 43% under well-watered and drought conditions, respectively due to the application of microbial biostimulants.

4.6 Shoot Dry Weight (g)

The shoot dry weight of tomato as influenced by the biostimulant treatments are presented in Table 3 & Appendix IX. The application of biostimulants significantly increased the shoot dry weight of tomato. The observations on shoot dry weight varied from 61.16 g to 82.65 g. The maximum shoot dry weight (82.65 g) was obtained from the T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which is superior to all other treatments. On the other hand, minimum shoot dry weight (61.16g) was obtained from the T_0 (control). All other treatments brought about the intermediate range of this parameter of shoot dry weight. Similar kind of result was also reported by Rayorath *et al.,* 2008 and Battacharyya *et al.,* 2015 and our findings are in accordance with their findings.

Table 3. Effect of microbial and non-microbial biostimulants on root and shoot biomass of tomato

Treatments	Root Dry Weight (g)	Shoot Dry Weight (g)
T_o	3.49 c	61.16 d
T_1	3.95 bc	64.71 cd
T_2	4.72 ac	68.57 bd
T_3	3.91 bc	66.99 bd
T_4	4.15 bc	65.19 cd
T_5	3.92 bc	65.26 cd
T_6	4.15 bc	72.06 bc
T_7	4.03 bc	65.85 cd
T_8	5.56 a	82.65 a
T_9	4.44 ac	66.55 bd
T_{10}	4.22 bc	66.89 bd
T_{11}	4.17 bc	67.45 bd
T_{12}	5.12 ab	73.91b
SE(±)	0.38	2.19
CV	12.40	4.54

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

4.7 Number of Flower Cluster Plant^{-1}

The observations on number of cluster per plant in tomato as influenced by the application of biostimulants are given in Table 4 and Appendix X. The number of flower cluster varied from a low of 3.34 to as high as of 6.91 per plant. The maximum number of flower cluster (6.91) per plant was recorded in treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all

other treatments and was closely followed by treatment T_{12} (5.84) and T_6 (5.28). The minimum number of flower cluster (3.34) was observed in treatment T_0 (control) which was significantly lower than all other treatments. On comparing all the treatments, it is clear that all the treatments of biostimulants either single or combined increased the number of flower cluster per plant of tomato compared to the control. Similar kind of result was reported by Koyama *et al.,* 2012; Briceno-Dominguez *et al.*, 2014 and Lucini *et al.*, 2015.

4.8 Number of Flower Cluster^{-1}

Application of plant biostimulants exhibited a significant influence on the number of flower per cluster of tomato Table 4 & Appendix X. The number of flower varied from a low of 5.59 to as high as 9.00 per cluster of tomato. The maximum number of flower (9.00) per cluster was obtained from the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all other treatments. This was closely followed by treatment T_6 (8.32), T_4 (7.97) and T_{12} (7.97) which had exhibited a significant increase in the number of flower per cluster and were statistically similar to that of T_8. Meanwhile the minimum number of flower (5.59) was found from the treatment T_0 (control) where no biostimulants were applied and was significantly lower than all other treatments. All other treatments brought about the intermediate range of this parameter of number of flower per cluster of tomato and were statistically similar to each other. Seaweed extract has been reported to trigger flowering and fruit set in several crop plants, including tomato (Crouch and van Staden, 1992; Khan *et al.*, 2009) which might have the reason for higher number of flower cluster in tomato plants under the present investigation and our findings is in agreement with their findings.

Table 4. Effect of microbial and non-microbial biostimulants on number of cluster and number of flower per cluster of tomato

Treatments	Number of Flower Cluster Plant^{-1}	Number of Flower Cluster^{-1}
T_o	3.34 d	5.59 b
T_1	3.82 cd	6.98 ab
T_2	4.86 bd	7.60 ab
T_3	4.36 bd	6.80 ab
T_4	4.81 bd	7.97 a
T_5	3.59 cd	6.70 ab
T_6	5.28 ac	8.32 a
T_7	4.53 bd	7.07 ab
T_8	6.91 a	9.00 a
T_9	4.44 bd	7.06 ab
T_{10}	4.80 bd	7.51 ab
T_{11}	4.37 bd	6.87 ab
T_{12}	5.84 ab	7.97 a
SE(±)	0.495	0.672
CV	14.93	12.96

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

4.9 Number of Fruit Cluster^{-1}

Number of fruits per cluster differed significantly due to the application of different plant biostimulants under the present investigation (Table 5 & Appendix XI). The number of fruit varied from a low of 3.78 to as high as 6.01 per cluster of tomato.

The highest number of fruit per cluster was recorded from the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was statistically superior to all other treatments. This was closely followed by the treatment T_{12} (5.02) which were statistically similar to that of T_{10} (4.81) and T_8 (6.01). Significantly lower number of fruit per cluster (3.78) was obtained from the treatment T_0 (control) which was closely followed by the treatment T_4 (3.96) with the lower values. The remaining treatments attained intermediate values for the parameter of number of fruit per cluster of tomato and were statistically similar to each other. The result further showed that combined application of Trichoderma based microbial biostimulants along with Sea weed Extract facilitated higher number of fruit per cluster as it has been reported in several crops that seaweed extract can trigger flowering and fruit set Khan *et al.,* 2009 which was also supported by Danesh *et al.,* 2012; Bozorgi 2012.

4.10 Number of Fruit Plant^{-1}

The data pertaining to the effect of plant biostimulants on number of fruits per plant are presented in Table 5 and Appendix XI. It is obvious from the table that application of plant biostimulants produced significantly higher number of fruits per plant compared to control. The maximum number of fruits (31.94) per plant was obtained from the treatment T_8 (50 g/l *Trichoderma*+ 2 g/l SWE) which was superior to all other treatments. This was closely followed by the treatment T_{12} (27.31) which were also statistically similar to that of T_8. The second best treatments were T_{10} (22.55) and T_6 (22.30). On the other hand, significantly lowest number of fruits (12.34) per plant was obtained from the treatment T_0 (control) where no biostimulants were applied. All other treatments of biostimulants also exerted a significant increase in the number of fruits per plant over the control and were statistically more or less similar to each other.

Table 5. Effect of microbial and non-microbial biostimulants on number of fruit per cluster and fruit per plant of tomato

Treatments	Number of Fruit Cluster^{-1}	Number of fruit plant^{-1}
T_o	3.78 b	12.34 e
T_1	4.15 b	16.82 ce
T_2	4.53 b	21.17 bd
T_3	4.53 b	18.04 ce
T_4	3.96 b	17.95 ce
T_5	4.02 b	14.06 de
T_6	4.27 b	22.30 bc
T_7	4.24 b	19.25 ce
T_8	6.01 a	31.94 a
T_9	4.71 b	18.97 ce
T_{10}	4.81 ab	22.55 bc
T_{11}	4.34 b	18.04 ce
T_{12}	5.02 ab	27.31 ab
SE(±)	0.359	2.142
CV	11.33	15.11

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

The results further showed that combined application of *Trichoderma* based biostimulants (50 g/l) along with SWE (2g/l) produced almost 3 times higher number of fruits compared to the control. Looking at the Table in more detail it is evident that all the combined treatments of *Trichoderma* along with SWE (*Ascophyllum nodosum*) performed better in terms of producing higher number of fruits per plant compared to other treatments.

This might be due to the synergistic effect of *Trichoderma* and SWE which have prompted the number of fruits per plant. Similar kind of result was reported Khan *et al.,* 2009; Briceno-Dominguez *et al.,* 2014 and our findings is in accordance with their findings.

However, combined application of 2g/l SWE (*Ascophyllum nodosum*) and HS (10 g/l) also produced the second highest number of fruits per plant. Therefore, there also might have synergistic effect which has facilitated the production of higher number of fruits per plant. Similar result was reported by Satish *et al.,* 2015.

4.11 Fruit Length (cm)

The data with respect of length of fruit as influenced by the application of different plant biostimulatns are presented in Table 6 & Appendix XII. It is evident from the table that treatments with biostimulants resulted in fruits of significantly higher length in comparison to control. The maximum fruit length (5.56 cm) was observed in treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE), which was significantly superior to all other treatments, followed by treatment T_{12} (5.31 cm) and T_9 (5.12).Whereas, the treatments T_{12} (5.31 cm), T_9 (5.12) and T_{11} (4.91 cm) were statistically similar with each other. The minimum fruit length (4.16) was observed in treatment T_0 (control) which was statistically similar with T_1 (4.47) and T_4 (4.55 cm) treatments. All other treatments brought about the intermediate range of this parameter of fruit length of tomato due to the application of different plant biostimulants. It has been reported that SWE and microbial biostimulants not only improve fruit yield but also large size fruits with superior quality (Crouch and van Staden, 1992; Khan *et al.,* 2009).

4.12 Fruit diameter (cm)

The fruit diameter of tomato as influenced by the application of *Trichoderma* based biostimulant, Seaweed Extract Powder (*Ascophyllum nodosum*) and Humic Substance (HS) are presented in Table 6 and Appendix XII. It is obvious from Table 5 that biostimulants produced fruits of markedly bigger diameter in comparison to control. The maximum fruit diameter (6.01 cm) was recorded in treatment T_8 (75g/l *Trichoderma*+ 2g/l SWE) which was statistically similar to treatments T_{12} (5.64 cm), T_9 (5.21 cm) and T_{11} (5.05 cm). The minimum fruit diameter (4.57 cm) was observed in treatment T_0 (Control) which was significantly lower than all other treatments. On the basis of the mean value of fruit diameter obtained from all the concentrations of different biostimulants, synergistic effect was found mostly from the combined application of *Trichoderma* based biostimulant along with other biostimulants. However combined application of 2g/l SWE + 10 g/l HS also showed the second best result for the parameter of fruit diameter. Crouch and van Staden, 1992; Khan *et al.*, 2009 reported the larger fruit with superior quality due to the application of different biostumulants and our findings of large sized fruit is in complete agreement with their findings.

4.13 Individual Fruit weight (g)

The data pertaining to individual fruit weight of tomato as influenced by the different plant biostimulants are presented in Table 6 and Appendix XII. The individual fruit weight varied from 68.14 g to 85.21 g. The maximum individual fruit weight (85.21 g) was recorded in treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all other treatments, followed by treatments T_6 (79.73 g) and T_{12} (78.28 g). The minimum individual fruit weight (68.14 g) was observed in treatment T_0 (control). All other treatments exerted a significant increase in the individual fruit weight over the control and were statistically more or less similar to each other.

On the basis of mean value of fruit weight obtained from the different concentrations of each biostimulant, it was highest in T_8 (75g/l *Trichoderma*+ 2g/l SWE) and was followed by T_6 (25 g/l *Trichoderma*+ 2g/l SWE). This might be due to the better nutrient uptake and accumulation of carbohydrate by microbial biostumulants and SWE which produced larger fruit with higher weight. Similar kind or results were reported by Ming *et al.*, 2013 and Kowalczyk and Zielony, 2008.

Table 6. Effect of microbial and non-microbial biostimulants on fruit length, fruit diameter and individual fruit weight of tomato

Treatments	Fruit length (cm)	Fruit diameter (cm)	Individual Fruit Weight (g)
T_o	4.16 d	4.57 d	68.14 e
T_1	4.47 cd	4.95 cd	70.16 de
T_2	4.64 bd	5.23 bd	75.93 bd
T_3	4.79 bd	4.88 bd	72.57 be
T_4	4.55 cd	4.98 cd	74.40 be
T_5	4.89 bc	4.91 bc	70.79 ce
T_6	4.76 bd	5.63 bd	79.73 ab
T_7	4.51 cd	4.98 cd	73.62 be
T_8	5.56 a	6.01 a	85.21 a
T_9	5.12 ac	5.21 ac	75.51 be
T_{10}	4.66 bd	5.16 bd	75.04 be
T_{11}	4.91 ac	5.05 ac	71.12 ce
T_{12}	5.31 ab	5.64 ab	78.28 ac
SE(±)	0.188	0.153	2.168
CV	5.54	4.20	4.11

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract r), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

4.14 Yield plant^{-1} (kg)

Application of plant biostimulants exhibited a significant influence on the yield per plant of tomato (Fig 8 and Appendix XIII). The perusal of data in the (Fig 4. And Appendix XIII) indicated that the treatments were varied significantly with respect to yield per plant of tomato.

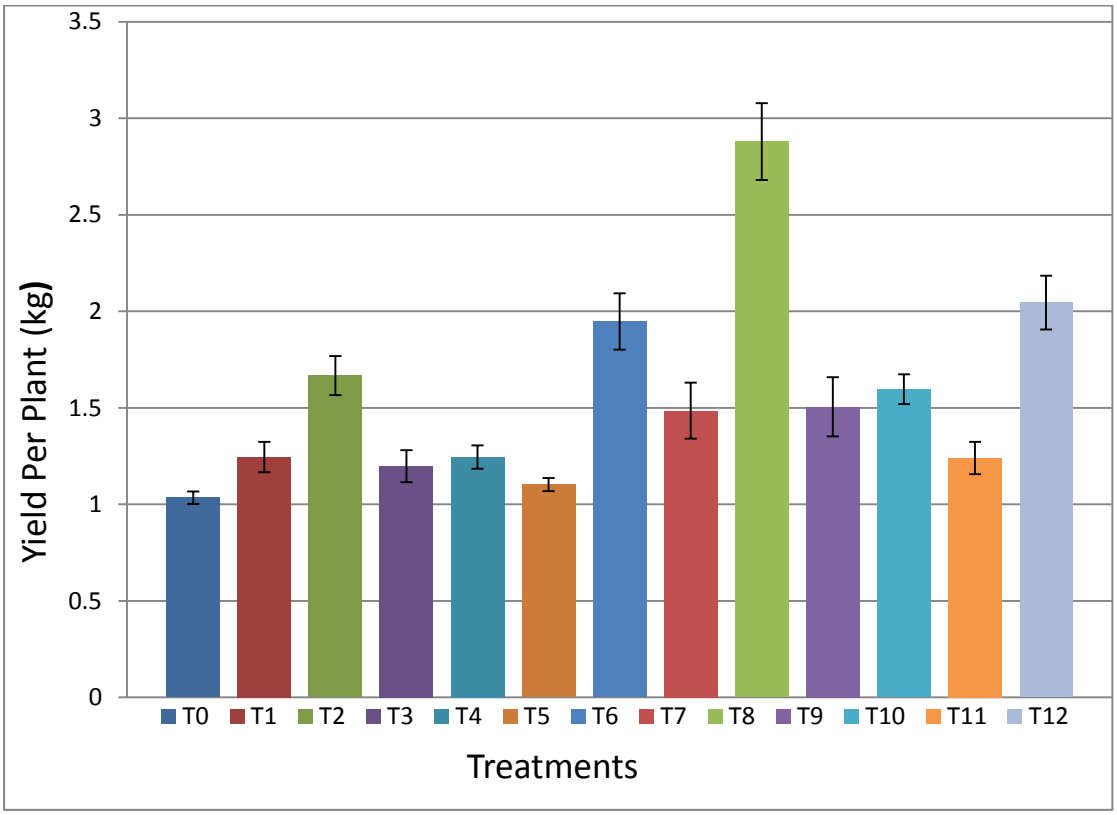

Figure 5. Effect of microbial and non-microbial biostimulants on yield per plant of tomato

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract Powder), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderma* + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

The highest yield (2.88 kg) per plant was obtained from the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all other treatments. This was followed by the treatment T_{12} (2.04 kg) and T_6 (1.94 kg) and were statistically at per with each other.

Meanwhile, significantly lowest yield per plant of tomato (1.03kg) was obtained from the treatment T_0 (control) where no biostimulants were applied. All other treatments also exhibited significantly higher yield per plant compared to control and brought about the intermediate range of this parameter of yield per plant of tomato. Our results are consistent with the findings of Ali *et al.*, 2016 who observed that the foliar application of *Ascophyllum nodosum* SWE (0.5%) enhanced the fruit yield of potted tomato (+54%) compared with the control whereas Molla et al 2012 reported 50% increase in yield of tomato due to the application of *Trichoderma* along with standard doses of NPK. Therefore the highest yield in T_8 might be due to the synergistic action of *Trichoderam* along with SWE.

4.15 Total soluble solids

The data with respect of total soluble solids (TSS) content of tomato fruit as influenced by the application of different plant biostimulatns are presented in Table 7 and Appendix XIV. The data revealed that the total soluble solids content values varied from 4.90 to 6.24. The maximum total soluble solids (6.24) were recorded in treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all other treatments which was statistically at par with treatment T_6 (6.01) and T_{12} (6.00) respectively. On the contrary, the minimum soluble solids (4.90) were observed in treatment T_0 (control) which was significantly lower than all other treatments. All other treatments of biostimulants also exerted a significant increase in the soluble solids content compared to the control. The results further showed that the mean value of total soluble solids from all the concentrations was highest in 50 g/l *Trichoderma*+ 2g/l SWE followed by the mean value of 25 g/l *Trichoderma*+ 2g/l SWE respectively. This might be due to the synergistic effect of *Trichoderma* along with SWE which might have enhanced total soluble solid content in tomato fruits. This result is in accordance with earlier results reported by Zodape *et al.*, 2011 in tomato.

This result was also supported by the result of Sharma *et al.,* 2009 who stated that plants treated SWE resulted in higher total soluble solids, total sugars and longer shelf life.

4.16 Fruit pH

The data with respect of fruit pH of ripe tomato fruit as influenced by the application of different plant biostimulants either sole or in combination are presented in the Table 7 and Appendix XIV. The fruit pH was found to differ significantly amongst the different treatments of biostimulants. The fruit pH varied from a low of 4.02 to as high as of 4.75. The highest fruit pH (4.75) was recorded from the treatment T_6 (25 g/l Trichoderma +2 g/l SWE) which was superior to all other treatments. This was closely followed by treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) and was statistically similar to that of T_6 (25 g/l Trichoderma +2 g/l SWE). On the contrary, lowest fruit pH (4.02) was recorded from the treatment T_0 (control) which was statistically similar to T_3, T_4, T_7, T_9 and T_{11} with the lower values. Similar result was reported by Khan *et al.* 2009.

4.17 Total sugar

The data pertaining to the influence of microbial and non-microbial biostimulants on suagar content of tomato are presented in Table 7 and Appendix XIV. The sugar content of tomato were found to vary significantly due to the application of biostimulants either sole or combined treatment. Amongst the treatment, the maximum sugar content (4.54 mg) was found from the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all other treatments. This was closely followed by the treatment T_6 (4.06 mg) and T_{12} (3.99 mg) and were statistically similar to that T_8 as well as with each other. On the other hand, the lowest sugar content was obtained from the treatment T_0 (control) where no biostimulants were applied and was significantly lower than all other treatments.

All other treatments also exerted a significant increase in sugar content of tomato fruit and brought about the intermediate range of this parameter of sugar content of tomato fruits.

Table 7. Effect of microbial and non-microbial biostimulants on Total Soluble Solid (TSS), total sugar content and fruit pH of tomato

Treatments	TSS	Total Sugar	pH
T_o	4.90 d	3.08 f	4.02 c
T_1	5.03 d	3.19 ef	4.26 bc
T_2	5.37 cd	3.62 bf	4.25 bc
T_3	5.02 d	3.41 cf	4.11 c
T_4	5.17 d	3.28 df	4.11 c
T_5	5.19 d	3.16 ef	4.19 bc
T_6	6.01 ab	4.06 ab	4.75 a
T_7	5.53 bd	3.67 bf	4.11 c
T_8	6.24 a	4.54 a	4.47 ab
T_9	5.05 d	3.80 bd	4.14 c
T_{10}	4.97 d	3.68 be	4.27 bc
T_{11}	5.15 d	3.67 bf	4.13 c
T_{12}	6.00 ac	3.99 ac	4.33 bc
SE(±)	0.178	0.166	0.088
CV	4.72	6.50	2.94

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

The results further showed that, application of *Trichoderma* along with sea weed extract (*Ascophyllum nodosum*) might have synergistic impact on the increment of sugar content of tomato fruit which might have facilitated higher

sugar content (4.54 mg mg) in the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE), (4.06 mg) in treatment T_6 (25 g/l Trichoderma+ 2g/l SWE) and (3.68 mg) in T_{10} (75 g/l *Trichoderma*+ 2 g/l SWE). Similar kind of result was also observed in T_{12} (2 g/l SWE + 10 g/l HS) which also showed higher sugar content of tomato fruit. Kossak and Dyki 2008; Kowalczyk and Zielony, 2008 reported a higher total sugar to titratable acid ratios as an effect of Sea weed extract application in tomato and our result is in consistent with their result.

4.18 Ascorbic Acid content of tomato fruit (mg/100g fruit)

The data pertaining to ascorbic acid content of tomato as influenced by the different plant biostimulants are presented in Table 8 and Appendix XV. The ascorbic acid content of tomato were found to vary significantly due to the application of biostimulants either sole or combined treatment. Amongst the treatment, the maximum ascorbic acid content (13.14 mg) was found from the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all other treatments. This was closely followed by the treatment T_6 (12.17mg) and T_{12} (11.80 mg) and were statistically similar with each other. On the other hand, the lowest vitamin C content was obtained from the treatment T_0 (control) where no biostimulants were applied. All other treatments brought about the intermediate range of this parameter of ascorbic acid content of tomato fruits. There result elicited an increase in total ascorbic acid content of tomato due to the application of biostimulants. It is evident from the results that application of *Trichoderma* along with sea weed extract (*Ascophyllum nodosum*) might have synergistic impact on the increment of ascorbic acid content which might have facilitated higher ascorbic acid content (13.14 mg) in the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) and (12.17 mg) in treatment T_6 (25 g/l *Trichoderma*+ 2g/l SWE). Dobromilska and Gubarewicz 2008 showed positive effects of biostimulants based on *A. nodosum* on vitamin C content and dry weight of cherry tomato fruits.

Kossak and Dyki (2008) and Kowalczyk and Zielony 2008 also noted an increase in both dry weight of greenhouse tomato fruits and vitamin C content after the application of Seaweed Extract.

4.19 Lycopene content of tomato fruit (mg/100g fruit)

The lycopene content of tomato as influenced by the application of *Trichoderma* based biostimulant, Seaweed Extract Powder (*Ascophyllum nodosum*) and Humic Substance (HS) are presented in Table 8 and Appendix XV. The lycopene content was found to differ significantly amongst the different treatments of biostimulants. Amongst the treatments, the heighest lycopene content (0.015) was found from the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE) which was significantly superior to all other treatments. That was closely followed by the treatment T_{12} (0.75), T_4 (0.94), T_6 (0.94) and T_{10} (0.91) and were statically similar to each other. On the other hand, the lowest lycopene content was recorded in treatment T_0 (control) where no biostimulants were applied. All other treatments brought about the intermediate range of this parameter of lycopene content of tomato fruits. The result depicts an increase in lycopene content of tomato due to the application of plant biostimulants compared to control. The results further showed that, application of *Trichoderma* along with sea weed extract (*Ascophyllum nodosum*) might have synergistic impact on the increment of lycopene content of tomato fruit which might have facilitated higher lycopene content (0.105 mg) in the treatment T_8 (50 g/l *Trichoderma*+ 2g/l SWE), (0.094 mg) in treatment T_6 (25 g/l *Trichoderma*+ 2g/l SWE) and (0.091 mg) in T_{10} (75 g/l *Trichoderma*+ 2 g/l SWE). Similar kind of result was also observed in T_{12} (2 g/l SWE + 10 g/l HS) which also showed higher lycopene content of tomato fruit. Similar result was reported by Binoy *et al.* 2004. Ertani *et al.* 2013; Guinan *et al.* 2013 stated that biostimulants improve stress tolerance in plants due to the higher production of antioxidant which might be the reason for higher lycopene content in ripe tomato fruits.

Table 8. Effect of microbial and non-microbial biostimulants on antioxidant properties of tomato

Treatments	Ascorbic acid (mg/100g fruit)	Amount of lycopene (mg/100g fruit)
T_o	8.14 g	0.038 f
T_1	8.83 fg	0.063 e
T_2	10.03 df	0.081 c
T_3	8.97 eg	0.063 de
T_4	9.06 eg	0.094 ac
T_5	9.02 eg	0.078 ce
T_6	12.17 ab	0.094 ac
T_7	9.92 df	0.080 cd
T_8	13.14 a	0.105 a
T_9	10.60 be	0.085 bc
T_{10}	11.15 bd	0.091 ac
T_{11}	10.32 cf	0.083 c
T_{12}	11.80 ac	0.75 ab
SE(±)	0.478	4.675
CV	6.61	8.11

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

4.20 Mineral Concentration in shoots

Mineral concentration (macro nutrient) in shoots of tomato plants were determined at harvest and are presented in Table 10 and Appendix XVI. N, P, K contents were found significantly higher in shoots in all the biostimulants treated plants compared to the control plants. The highest N content (12.85%) was found from the treatment T_8 (50g/l *Trichoderma* + 2g/l SWE) which was

statistically similar to the treatments T_6, T_7, T_9, T_{10}, T_{11}, T_{12}. On the other hand the minimum N content (9.24%) was obtained from the treatment T_0 (control). In case of P content, the highest P content (0.415 %) recorded from the treatment T_8 (50g/l *Trichoderma* + 2g/l SWE) which was superior to all other treatments. That was followed by the treatments $T_{9,}$ T_{10} and was statistically similar to $T_{8.}$ All other treatments of biostimulants also exerted a significant increase in P content in shoots over the control. Meanwhile the lowest P content (0.192%) was obtained from the control plants. In case of K content in tomato shoots, the highest K content (4.95%) was obtained from the treatments T_7 (25 g/l *Trichoderma* + 10 g/l HS) and T_8 (50g/l *Trichoderma* + 2g/l SWE). That was followed by the treatments T_9, T_{10}, T_{11}, T_{12} and were statistically more or less similar to that T_7 and T_8. On the other hand, the lowest K content (3.89%) was obtained from the control treatment T_0. Looking into the information in Table 10 it is clearly evident that all the treatment of biostimulats had significant positive impact in nutrient uptake by tomato plants over the control plants. Meanwhile, it is more obvious that combined application of microbial and non-microbial biostimulants showed better nutrient uptake than single application. It might be due to the synergistic effect of microbial and non-microbial biostimulants in increasing nutrient solubility, nutrient uptake capacity, mobilization and assimilation which might have facilitated higher N, P, K contents in T_8 as well as in all other combined application treatments. Our findings is in complete agreement with the findings of Samolski *et al.* 2012 and Zhao *et al.* 2014 who reported that *Trichoderma* spp , HS and SWE enhance nutrient uptake via modulation of root architecture or through the exudation of substances that increase nutrient availability such as siderophores and organic acids. Saravanakumar *et al.* 2013 reported higher P uptake while Colla *et al.* 2015 reported that *Trichoderma* spp can enhance iron solubility and hence uptake and translocation by plant. Dobromilska *et al.*, 2008 and Jannin *et al.* 2013 reported enhanced uptake and accumulation of N, P, K, S, Fe and Zn due to the application of SWE.

Table 9. Effect of microbial and non-microbial biostimulants on macro nutrient concentrations in tomato shoots

Treatments	N (%)	P (%)	K (%)
T_o	9.24 e	0.1925 d	3.89 d
T_1	9.88 de	0.2225 cd	4.08 cd
T_2	10.84 b-e	0.2475 cd	4.29 b-d
T_3	10.22 c-e	0.275 d	4.25 bd
T_4	10.62 b-e	0.2075 d	4.45 ad
T_5	10.54 b-e	0.2125 d	4.09 cd
T_6	11.95 a-c	0.3400 b	4.79 ab
T_7	11.72 a-d	0.2950 bc	4.95 a
T_8	12.85 a	0.4150 a	4.95 a
T_9	11.20 a-d	0.3475 ab	4.59 a-c
T_{10}	12.16 ab	0.3650 ab	4.44 a-d
T_{11}	11.47a-d	0.3225 b	4.51 a-d
T_{12}	12.31 ab	0.2925 bc	4.69 a-c
SE(±)	0.531	0.021	0.179
CV	6.73	10.38	5.69

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

The data pertaining to the mineral concentration (micro nutrient) in shoots of tomato plants were determined at harvest and are presented in Table 10 and Appendix XVI. Fe and Zn contents were found significantly higher in shoots in all the biostimulants treated plants compared to the control plants.

Table 10. Effect of microbial and non-microbial biostimulants on micro nutrient concentrations in tomato shoots

Treatments	Fe ($mgkg^{-1}$)	Zn ($mgkg^{-1}$)
T_o	171.02 c	43.93 e
T_1	207.46 ab	47.55 de
T_2	209.48 ab	48.30 c-e
T_3	215.17 ab	47.74 c-e
T_4	210.83 ab	47.52 de
T_5	206.35 b	48.99 c-e
T_6	235.10 ab	56.84 ab
T_7	223.87 ab	51.37 bd
T_8	237.94 a	62.00 a
T_9	216.39 ab	48.70 c-e
T_{10}	220.16 ab	53.69 bc
T_{11}	219.19 ab	52.24 bd
T_{12}	229.79 ab	51.88 bd
SE(±)	2.646	1.692
CV	5.67	4.71

T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS

The highest Fe content (237.94 mg Kg^{-1}) was found from the treatment T_8 (50g/l *Trichoderma* + 2g/l SWE) which was statistically similar to the treatments most of the treatments except T_5 and T_0. The minimum Fe content (171.02 mg kg^{-1}) was recorded from the control treatment. In case of Zn, the maximum Zn content (62.00 mg kg^{-1}) was recorded from the T_8 (50g/l *Trichoderma* + 2g/l SWE) which was statistically similar to T_6.

On the other hand minimum Zn content (43.93 mg kg^{-1}) was obtained from the control treatment. Higher Fe and Zn content in tomato shoots might be due to the synergistic effect which facilitated better nutrient uptake and accumulation in plants. It was reported in several studies that plant biostimulants increase in soil enzymatic and microbial activities, modifications in root architecture as well as an enhancement in micronutrient mobility and solubility (Ertani *et al.* 2009; Colla *et al.*, 2014; Lucini *et al.*, 2015). Dobromilska *et al.*, 2008 reported that application of a commercial product made with brown seaweed increased mineral nutrient (N, P, K, Ca, Zn and Fe) content of tomato and our finding is in agreement with their finding.

CHAPTER V

SUMMERY AND CONCLUSION

The experiment was conducted at the Horticulture Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka during the period from October 2017 to April 2018 to study the synergistic impact of microbial and non-microbial biostimulants on growth, yield and nutritional quality enhancement of organically grown tomato and their nutrient uptake potential to find a purposeful combination which can be a viable alternatives to mineral fertilizers in organic production system. The single factor pot experiment consisted of T_0= Control (No biostimulant), T_1= 25 g/l *Trichoderma*, T_2= 50 g/l *Trichoderma*, T_3= 75 g/l *Trichoderma*, T_4= 2 g/l SWE (Seaweed Extract), T_5 = 10 g/l HS (Humic Substances), T_6= 25 g/l *Trichoderm*a + 2 g/l SWE, T_7= 25 g/l *Trichoderma* + 10 g/l HS, T_8= 50 g/l *Trichoderma* + 2 g/l SWE, T_9 = 50 g/l *Trichoderma* + 10 g/l HS, T_{10}= 75 g/l *Trichoderma* + 2 g/l SWE, T_{11}= 75 g/l *Trichoderma* + 10 g/l HS, T_{12}= 2 g/l SWE + 10 g/l HS. The experiment was laid out in Completely Randomized Design (CRD) with four replications. Data on growth, yield and quality parameter were recorded in both field and laboratory.

At 30 DAT, 50 DAT, and 70 DAT, the tallest plant (46.42, 88.31, 111.81cm respectively), maximum number of leaves plant^{-1} (16.12, 35.46, 71.56 respectively) and maximum number of branches plant^{-1} (5.22, 9.21, 12.70 respectively) was recorded from T_8, whereas the shortest plant (29.75, 65.37, 85.62 cm respectively), minimum number of leaves plant^{-1} (6.40, 22.10, 51.50 respectively) and minimum number of branches plant^{-1} (2.39, 5.59, 5.21 respectively) was observed from T_0. As regards with the treatments effect T_8= 50 g/l *Trichoderma* + 2 g/l SWE performed the best in encouraging all the growth parameters up to significant extent at every stage of observations.

The tremendous variations in physiological parameters viz. SPAD value, root dry weight and shoot dry weight occurred among the thirteen biostimulants treatments. At flowering stage highest SPAD value (50.40) was recorded from T_{12} which was closely followed by the treatment T_8 (48.80) whereas the minimum SPAD value was recorded from the treatment T_0. At harvest, the highest root dry weight (5.56 g), highest shoot dry weight (82.65 g) was recorded from the treatment T_8= 50 g/l *Trichoderma* + 2 g/l SWE. On the other hand the lowest dry root dry weight (3.49 g), lowest shoot dry weight (61.16 g) was recorded from the treatment T_0 (control) where no biostimulants were applied.

Significant variation was found in yield contributing attributes of tomato grown organically due to the application of biostimulants. Amongst the treatments, highest number of flower cluster plant^{-1} (6.91), highest number of flowers cluster^{-1} (9.00), highest number of fruits cluster^{-1} (6.01), highest number of fruits plant^{-1} (31.94), highest length of fruit (5.561 cm), highest diameter of fruit (6.01cm), highest weight of individual fruit (85.21 g), maximum yield plant^{-1} (2.88 kg) was found from T_8; lowest number of flower cluster plant^{-1} (3.34), lowest number of flowers cluster^{-1} (5.59), minimum number of fruits cluster^{-1} (3.78), lowest number of fruits plant^{-1} (12.34), lowest length of fruit (4.16 cm), lowest diameter of fruit (4.57 cm), lowest weight of individual fruit (68.14 g), lowest yield plant^{-1} (1.03 kg) was observed from T_0.

In case of quality parameters, enhanced nutritional quality was observed due to the application of plant biostimulants whereas the quality in control plant was probably suboptimal. Amongst the treatments, T_8= 50 g/l *Trichoderma* + 2 g/l SWE performed better and was superior to all other treatments in improving fruit quality. The highest Ascorbic acid content (13.14 mg), highest lycopene content (0.105 mg) per 75g tomato fruit was recorded from the treatment T_8= 50 g/l *Trichoderma* + 2 g/l SWE whereas minimum Ascorbic acid content (8.14 mg), minimum lycopene content (0.038 mg) per 75 g tomato fruit was

recorded from the treatment $T_{0.}$ The highest fruit pH (4.75) was recorded from the treatment T_6 (25 g/l *Trichoderma* + 2 g/l SWE) which was closely followed by the treatment T_8= 50 g/l *Trichoderma* + 2 g/l SWE. On the contrary, minimum fruit pH was recorded from the $T_{0.}$ Meanwhile the highest sugar content (4.54 mg) was recorded from the treatment T_8= 50 g/l *Trichoderma* + 2 g/l SWE whereas minimum sugar content (3.08 mg) was recorded from the control treatment T_0.

Mineral concentration (macro nutrient) in shoots of tomato plants were determined at harvest and N, P, K contents were found significantly higher in shoots in all the biostimulants treated plants compared to the control plants. The highest N content (12.85%), the highest P content (0.415 %) was recorded from the treatment T_8= 50 g/l *Trichoderma* + 2 g/l SWE. Besides this, the highest K content (4.95%) was obtained from the treatments T_7 (25 g/l *Trichoderma* + 10 g/l HS) and T_8 (50g/l *Trichoderma* + 2g/l SWE) with the similar value. On the other hand, minimum N content (9.24%), the lowest P content (0.192%) and the the lowest K content (3.89%) was obtained from the control treatment $T_{0.}$ In case of micronutrient (Fe and Zn) content in shoots, the highest Fe content (237.94 mg Kg^{-1}), the highest Zn content (62.00 mg Kg^{-1}) was recorded from the treatment T_8= 50 g/l *Trichoderma* + 2 g/l SWE whereas the The minimum Fe content (171.02 mg Kg^{-1}) and the minimum Zn content (43.93 mg Kg^{-1}) was obtained from the control treatment T_0 where no biostimulants were applied.

CONCLUSION

The present results revealed that treatment T_8= 50 g/l *Trichoderma* + 2 g/l SWE has emerged as the best treatment with respect to growth, yield and nutritional quality enhancement of tomato grown organically. Therefore, on the basis of the results it can be concluded that combined application of 50 g/l *Trichoderma* + 2 g/l SWE (Seaweed Extract) can be considered as a noble strategy for sustainable tomato production with higher yield and superior quality.

SUGGESTIONS

- More varieties may be included in the further program before final recommendation.
- Further studies are needed to clarify mode of action involved and metabolites dynamics after the combine microbial and non-microbial biostimulants application

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APPENDICES

Appendix I. Physio-chemical characteristics of the experimental soil

Physical characteristics	
Textural class	Silty clay loam to clay loam
Bulk density (g cm^{-3})	1.33
Particle density (g cm^{-3})	2.61
Porosity (%)	46.9
Chemical characteristics	
pH	6.2
Organic carbon (%)	0.75
Organic matter (%)	1.12
Total N (%)	0.092
Available P (µg/g)	18
Available K (meq/75g)	0.32

Source: Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka

Appendix II. Soil test interpretation (STVI) class and the respective recommended fertilizer nutrients for tomato (BARC 2012)

STVI class	Limit of the soil test value within the STVI class			Nutrient recommendation of the respective STVI class (kg $ha^{-1)}$		
	Total N (%)	Available P(mg kg^{-1})	Exchangeable K (c-mol kg^{-1})	N	P	K
Very low	0-0.09	<7.5	<0.09	136-180	40-60	76-75
Low	0.091-0.18	7.51-15.0	0.091	91-135	31-45	51-75
Medium	0.181-0.27	15.1-22.5	0.181-0.27	46-90	16-30	26-50
Optimum	0.271-0.36	22.51-30	0.271-0.36	0-45	0-15	0-25
High	0.361-0.45	30.1-37.5	0.361-0.45	-	-	-
Very high	>0.45	>37.5	>0.45	-	-	-

Appendix III. Monthly meteorological information during the period from October, 2017 to April, 2018

Year	Month	Air temperature (^{0}C)		Relative humidity (%)	Total rainfall (mm)	Sunshine (hr.)
		Maximum	Minimum			
2017	October	29.50	19.40	81.10	22	6.9
	November	28.50	17.90	78.50	00	6.8
	December	27.60	15.20	74.60	00	6.3
2018	January	24.60	13.50	68.50	00	5.7
	February	28.90	18.00	67.00	30	6.7
	March	33.60	29.50	54.70	11	8.2
	April	33.50	25.90	64.50	119	8.2

Source: Meteorological centre, Agargaon, Dhaka (Climate Division)

Appendix IV. Physio-chemical composition of SWE (*Ascophyllum nodosum*), Humic Substance and *Trichoderma* (Technical information)

Component	**Amount (% from dry powder)**
SWE powder (*Ascophyllum nodosum*)	
Organic matter	45-55 %
Alginic acid	10-12%
Manitol	4-6%
Amino acids	4-6%
Other organic compounds	10-12%
Macronutrients in Ash (N:P:K)	0.8-1.5%, 0.5-1.0%, 14-18%
Micronutrients in Ash (Fe, Mn, Zn)	75-250ppm, 8-12ppm, 15-25 ppm
Humic Substance (HS)	
Humic acid	75%
Fulvic acid	15%
Other organic substances	10-15%
Water solubility	100%
***Trichoderma* Powder**	
Fungi spores (CFUg-^{1} powder)	1×10^8

Appendix V: Effect of microbial and non-microbial biostimulants on plant height of tomato at different DAT

Source of Variation	Degrees of freedom	Mean Square		
		Plant height (cm) at		
		30 DAT	50 DAT	70 DAT
Replication	3	8.31	31.33	87.05
Treatment	12	58.54*	122.70**	179.00**
Error	36	4.77	14.07	15.86
Total	51			

Appendix VI: Effect of microbial and non-microbial biostimulants on number of branches plant^{-1} of tomato at different DAT

Source of Variation	Degrees of freedom	Mean Square		
		Number of branch plant^{-1}		
		30 DAT	50 DAT	70 DAT
Replication	3	0.45	2.62	3.51
Treatment	12	1.99*	3.23**	19.25**
Error	36	0.47	0.46	2.03
Total	51			

Appendix VII: Effect of microbial and non-microbial biostimulants on number of leaves plant^{-1} of tomato at different DAT

Source of Variation	Degrees of freedom	Mean Square		
		Number of leaves plant^{-1}		
		30 DAT	50 DAT	70 DAT
Replication	3	1.95	8.01	10.18
Treatment	12	23.69*	41.48**	133.20**
Error	36	4.44	6.69	14.39
Total	51			

Appendix VIII: Effect of microbial and non-microbial biostimulants on chlorophyll content (SPAD) value of tomato

Source of Variation	Degrees of freedom	Mean Square
		SPAD value
Replication	3	5.79
Treatment	12	44.22*
Error	36	6.67
Total	51	

Appendix IX : Effect of microbial and non-microbial biostimulants on root and shoot biomass of tomato

Source of Variation	Degrees of freedom	Mean Square	
		Root Dry Weight (g)	Shoot Dry Weight (g)
Replication	3	0.07	44.95
Treatment	12	1.22**	115.94**
Error	36	0.28	9.59
Total	51		

Appendix X: Effect of microbial and non-microbial biostimulants on number of cluster and number of flower per cluster of tomato

Source of Variation	Degrees of freedom	Mean Square	
		Number of Flower Cluster Plant^{-1}	Number of Flower Cluster^{-1}
Replication	3	0.72	1.17
Treatment	12	3.57**	2.95*
Error	36	0.49	0.90
Total	51		

Appendix XI: Effect of microbial and non-microbial biostimulants on number of fruit per cluster and fruit per plant of tomato

Source of Variation	Degrees of freedom	Mean Square	
		Number of Fruit Cluster^{-1}	Number of fruit plant^{-1}
Replication	3	0.53	9.54
Treatment	12	1.33**	108.88**
Error	36	0.25	9.18
Total	51		

Appendix XII: Effect of microbial and non-microbial biostimulants on fruit length, fruit diameter and individual fruit weight of tomato

Source of Variation	Degrees of freedom	Mean Square		
		Fruit length (cm)	Fruit diameter (cm)	Individual Fruit Weight (g)
Replication	3	0.02	0.02	4.96
Treatment	12	0.55*	0.59**	82.81**
Error	36	0.07	0.04	9.40
Total	51			

Appendix XIII: Effect of microbial and non-microbial biostimulants on yield per plant of tomato

Source of Variation	Degrees of freedom	Mean Square
		Yield per plant (kg)
Replication	3	0.05
Treatment	12	1.02 **
Error	36	0.05
Total	51	

Appendix XIV: Effect of microbial and non-microbial biostimulants on Total Soluble Solid (TSS), total sugar content and fruit pH of tomato

Source of Variation	Degrees of freedom	Mean Square		
		TSS	Total Sugar	pH
Replication	3	0.01	0.09	0.01
Treatment	12	0.81*	0.69**	0.14**
Error	36	0.06	0.05	0.01
Total	51			

Appendix XV: Effect of microbial and non-microbial biostimulants on antioxidant properties of tomato

Source of Variation	Degrees of freedom	Mean Square	
		Ascorbic acid (mg/75g fruit)	Amount of lycopene (mg/75g fruit)
Replication	3	1.66	9.14
Treatment	12	8.84 **	1.30 *
Error	36	0.45	4.37
Total	51		

Appendix XVI: Effect of microbial and non-microbial biostimulants on macro nutrient concentrations in tomato shoots

Source of Variation	Degrees of freedom	Mean Square		
		N (%)	P (%)	K (%)
Replication	3	0.64	0.00126	0.11
Treatment	12	4.40**	0.02082 **	0.44 **
Error	36	0.56	0.00086	0.06
Total	51			

Appendix XVII: Effect of microbial and non-microbial biostimulants on micro nutrient concentrations in tomato shoots

Source of Variation	Degrees of freedom	Mean Square	
		Fe($mgkg^{-1}$)	Zn ($mgkg^{-1}$)
Replication	3	105.93	27.69
Treatment	12	1127.54**	88.54**
Error	36	149.53	5.73
Total	51		

*Significant at 5 % level of probability

** Significant at 1 % level probability

Plate 1.1: Field View of experimental plot

Plate 1.2: Field view of experimental plot

Plate 2: Photographs of flower, fruits from experimental plot

Plate 3: Harvested tomato fruits from the experimental plot