

**BIO EFFICACY OF *Trichoderma harzianum* SPORE CONCENTRATIONS  
ON GROWTH AND YIELD OF TOMATO**

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ON GROWTH AND YIELD OF TOMATO**

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*This is to certify that the thesis entitled “**BIO EFFICACY OF *Trichoderma harzianum* SPORE CONCENTRATIONS ON GROWTH AND YIELD OF TOMATO**” submitted to the Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE in HORTICULTURE**, embodies the result of a piece of authentic research work carried out by **NAIMA SABRIN**, Registration No. **12-04961** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

*I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.*

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*It is a fact that the remembrance of Allah brings peace in the heart. It is better to ponder over the verses to bring us even closer to Allah (swt).*

***DEDICATED TO-  
ALL MY WELL WISHERS***

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*The Author*

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**ABSTRACT**

An experiment was accomplished in the Horticulture farm of Sher-e-Bangla Agricultural University, Dhaka during the period from October 2017 to March 2018 to evaluate the bio efficacy of spore concentration of *Trichoderma harzianum* on tomato. Three tomato variety viz.  $V_1$  = Roma -VF,  $V_2$  = BARI Tomato-2,  $V_3$  = Apple Netherland, and *Trichoderma harzianum*  $T_0$  = No *Trichoderma* application,  $T_1$  =  $10^6$  spores/ ml, (7 and 15DAT),  $T_2$  =  $10^7$  spores/ml (7 and 15DAT) were used in this experiment arranged in a Randomized Complete Block Design with three replications. Data on different growth and yield attributes parameters were taken in which all the treatment showed significant variations. Among varieties, maximum plant height (111.2 cm), cluster number (7.6/plant), flower number (52.6/plant) and yield/ plant (2.9 kg) were found from variety  $V_1$  (Roma VF) whereas minimum in variety  $V_2$  (BARI Tomato-2) and among *Trichoderma* application, maximum plant height (110.6 cm), cluster number (7.4/ plant), flower number (54.2/ plant) yield/ plant (2.9 kg) found in spore concentration  $10^7$ / ml ( $T_2$ ). Maximum yield per hectare (91.5 t) were found in  $V_1T_2$  and minimum (84.7 t) in  $V_2T_0$ . Chlorophyll content showed significantly positive correlation with all growth and yield parameters. In view of overall performances, Variety  $V_1$  with  $10^7$  spores/ ml *Trichoderma harzianum* ( $T_2$ ) solution application has potentiality for increased tomato production.

## ABBREVIATIONS AND ACCORONYMS

AEZ	=	Agro-ecological Zone
Agric.	=	Agricultural
ANOVA	=	Analysis of Variance
BARI	=	Bangladesh Agricultural Research Institute
<i>Biol.</i>	=	Biology
<i>Biotechnol.</i>	=	Biotechnology
<i>Curr.</i>	=	Current
Cv	=	Coefficient of variance
DAT	=	Days after transplanting
<i>Environ.</i>	=	Environmental
<i>et al.</i>	=	And others
FAO	=	Food and Agriculture Organization
Hort.	=	Horticulture
i.e.	=	That is
<i>Intel.</i>	=	International
<i>J.</i>	=	Journal
LSD	=	Least Significance difference
Micribiol.	=	Microbiology
Pl.	=	Plant
RCBD	=	Randomized Complete Blocked Design
Res.	=	Research
SAU	=	Sher-e-Bangla Agricultural University
Sci.	=	Science
spp.	=	Species
Technol.	=	Technology
UNDP	=	United Nations Development Programme
Viz.	=	Namely

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## CHAPTER I

### INTRODUCTION

Tomato (*Lycopersicon esculantum* L.) is an important solanaceous vegetable crop all over the world including Bangladesh. It is popularly known as *wolf apple*, *love of apple* or *Vilayati baingan* is one of the most commonly grown solanaceous vegetable crop of the world due to its wide adaptability under various agroclimatic conditions, (Saravaiya *et al* ,2014). Its origin is the Andean zone particularly Peru-Ecuador-Bolivian areas (Salunkhe *et al.*, 1987) but cultivated tomato originated in Mexico (Ugwuanyi P.O. *et al.* 2016). It is a mildly acid, pulpy fruit, commonly red, sometimes yellow used as vegetable. Tomato is rich in vitamin A, C, calcium and phosphorus. It is a widely grown and versatile vegetable for taste, color, high nutritive value and diversified use. 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 (Bhutani and Kallo, 1983). Thus tomato has attained the uppermost position among the most consumed fresh vegetables in the world (Perveen *et al.*, 2015). In Bangladesh, tomato is cultivated all over the country due to its adaptability to wide range of soil and climate (Ahmed, 1995). The area of cultivation is about 13,066 ha with the production of about 74,000 m tons. It ranks next to potato and sweet potato in respect of vegetable production in the world (Hossain *et al.*, 2010). It is cultivated in almost all home gardens and also in the field for its adaptability to wide range of soil and climate in Bangladesh. The best growing areas of tomato in Bangladesh are Chittagong, Comilla and Rajshahi (Sharfuddin and Siddque, 1985).

Tomato plants are affected by several diseases like fungal, bacterial, insects and so on. To control these diseases chemical pesticides are used that is concerning issue for human health and environment (Weaver *et al.*, 1992). On that issue, biological control should be a great solve. Biological control of tomato diseases includes the use of resistant varieties (general or specific



resistance). Pathogens are highly variable; specific resistance has therefore contributed little to control disease. So other biologic methods including antagonistic micro-organisms, bio-fungicides or induced resistance have been attracting considerable interest in recent years.

*Trichoderma harzianum* is a fungus that is also used as a fungicide. The genus *Trichoderma* comprises several species of filamentous fungi that have their natural habitat in soil. It is used for foliar application, seed treatment and soil treatment for suppression of various disease causing fungal pathogens. It is also used as an industrial workhorse for several decades (reviewed in Mäntylä *et al*, 2003 and Peterson and Nevalainen, 2012).

*Trichoderma* is very common antagonistic soil fungi present in soils. It contains many species and strains, of which some are saprophytic while others are pathogenic to other fungi such as *Phytophthora*, *Pythium*, *Fusarium*, etc. *Trichoderma* are widely used in agricultural biotechnology and have been already used as biocontrol agents against numerous plant root diseases. Moreover, *Trichoderma* has also been found to be the dominant early recolonizer of soil after fumigation by various chemicals (Evans 1955; Saxena 1960; Warcup 1952) which are generally used to control root-diseases. *Trichoderma* species have shown biocontrol potential against many plant pathogens. Antagonists belonging to the genus *Trichoderma* are among the most commonly isolated soil fungi. Due to their ability to protect plants and contain pathogen populations under different soil conditions, these fungi have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments Vinale *et al.* (2008).

The productivity and yield of tomato is low as compared to other developed countries due to low quality seeds, poor crop husbandry and pests and diseases (Heuvelink *et al.* 2003). In Dhaka, Bangladesh upper stated diseases are occurring frequently. To control these diseases, more chemicals are applied

annually to the tomato field than to any other food plant we grow today. Success in controlling the disease has been due to the application of large quantities of chemical fungicides, their extensive use is causing a serious pollution problem in the environment (Ragunathan and Divakar, 1996). Biological control of plant diseases for its less or no pollution to environment is more and more concerned. So to develop and use biotic method to control in tomato is now demand of time. The aim of this study is to experiment the Bio efficacy of *Trichoderma harzianum* on growth, yield and disease resistance on different tomato varieties in Dhaka condition. Hence the main aim of this study is-

- To evaluate the growth and yield performance of tomato varieties
- To evaluate the influence of foliar application of different spore concentration of *Trichoderma harzianum* on growth and yield of tomato

## CHAPTER II

### REVIEW OF LITERATURE

Tomato crop is very important in terms of diet and economy in. Its production is hampered by various hazards. Among them nutritional imbalance of soil and chemical use is robust. Furthermore chemical is harmful to our world.

Rao *et al.* (1997) experimented on Neem cake (*Azadirachta indica*) and a biocontrol fungus, *Trichoderma harzianum*. They were evaluated either singly or in combination for the management of *Meloidogyne incognita* on tomato. Significant increase in plant growth and reduction in root galling and final population of *M. incognita* were observed in tomato seedlings transplanted in neem cake-amended soil incorporated with *T. harzianum*. Increase in colonization of *T. harzianum* on roots of tomato was also observed in the above treatments which indicated favorable effects of neem cake amendment on the growth of *T. harzianum*.

Sahar *et al.* (2016) investigated the effect of the rhizobacteria *Serratia proteamaculans* against *R. solani* comparing to *Trichoderma harzianum* and examining the ability of the two biocontrol agents to induce the plant defense system as a mechanism to suppress *R. solani* infection. Preliminary screening showed that *S. proteamaculans* was able to inhibit *R. solani in vitro* while *T. harzianum* over grown the pathogen. The two biocontrol agents were able to suppress *R. solani* infection by 61.4, 62.6, 61.6 % *in vivo* when plants treated with *T. harzianum*, *S. proteamaculans* and their combination respectively. In addition, plant fresh weight and length measurements indicated that both biocontrol agents either alone or in combination were able to increase tomato seedling growth compared to control plants with no significant differences among treatments. Examinations bio control agents capability to induce the antioxidants enzymes in plants under stress revealed challenges against pathogen protection provided by two bio control agents by APX, GPX and etc.

The obtained results suggest that *T. harzianum*, and *S. proteamaculans* – mediated protection against *R. solani* may be associated with alleviation of oxidative burst in host cells.

Bulluck *et al.* (2002) indicated that some organic amendments, such as cotton-gin trash, *Trichoderma* reduced the incidence of southern blight in processing tomato and also enhanced populations of beneficial soil microbes. Propagule densities of antagonistic soil fungi in the genus *Trichoderma* were highest in soils amended with composted cotton-gin trash or swine manure. Propagule densities of fluorescent pseudomonads in soil were higher in plots amended with organic amendments than with synthetic fertilizers in both years. Propagules densities of enteric bacteria were elevated in soils amended with raw swine manure biosolids in both years.

Abd-El-Khair *et al.* (2010) tested the antagonistic effect of four *Trichoderma* species, i.e. *Trichoderma album*, *Trichoderma hamatum*, *Trichoderma harzianum* and *Trichoderma viride*, against *F. solani* and *R. solani* in vitro, in greenhouse and in field. In greenhouse experiment, *T. album*, *T. hamatum*, *T. harzianum* and *T. viride*, as soil treatments, significantly reduced the pre- and post-emergence damping off disease incidence under artificial infection with *F. solani* and *R. solani*. The best protection to damping off disease was obtained by *T. hamatum*, followed by *T. viride*, *T. album* and *T. harzianum*, respectively. The treatments gave the highest plant survival (%) and improved the growth and yield parameters. The macro- and micro-elements content in treated bean plants was affected by *Trichoderma* species treatments compared to elements content in untreated plants. The relationship between plant nutrient content and some plant enzymes activity was studied.

Uddin *et al.* (2015) stated the beneficial effects of *Trichoderma* on growth and yield of tomato. *Trichoderma* concentrations were showed significant variation among the growth and yield characteristics of tomato. Highest yield per plant of tomato (3.0 kg) obtained in T1 (100 g/m<sup>2</sup>) treatment and lowest

(1.4 kg) was in control. Results also revealed that T1 (87.1%) showed the higher seedling survival rate than the control (57.9%).

Abedin *et al.* (2018) investigated the efficacy of Trichocompost utilizing *Trichoderma* as biological agent. Three doses of Trichocompost viz. 500g/m<sup>2</sup>, 750g/m<sup>2</sup>, 1000g/m<sup>2</sup> were applied including untreated control treatment. All the yield parameters were significantly higher when they were applied in different doses of Trichocompost. However, growth parameters did not show significant variation although luxuriant and lavish growth was noticed. Best performances were recorded by applying Trichocompost application @1000g/m<sup>2</sup>. Economic analysis of the treatments indicates that Trichocompost is profitable for farmers. Benefit Cost Ratio (BCR) was maximum (2.26) in Trichocompost treated plot @1000g/m<sup>2</sup>. It can be revealed that Trichocompost can be applied in the field for better tomato production.

Martínez- medina *et al.* (2013) reported that *Trichoderma* isolates with defense-related hormones jasmonic acid (JA), ethylene (ET), salicylic acid (SA), and abscisic acid (ABA) had systemic defense response in Root colonization and broad-spectrum of plant pathogens. They also involved in wider variety of signaling routes, interconnected in a complex network of cross-communicating hormone pathways.

Alexandru *et al.* (2013) posed that root colonization with *Trichoderma* spp. induces changes in physiological processes like photosynthesis or other metabolism processes. They analyzed the influence of *Trichoderma* spp. on the intensity of photosynthesis and on the assimilatory pigment content in tomato plants. They found positive results on strains of *Trichoderma* able to significantly increase the photosynthesis intensity in the leaves of tomato plantlets. A large variation of the chlorophyll content was observed between tomato plants treated with the two tested strains of the *T. harzianum*.

Azarmi *et al.* (2011) mentioned that *Trichoderma* species are very useful as biological control agents against phytopathogenic fungi and some isolates are

able to improve plant growth. . Seed germination rate was not affected by *Trichoderma* application, but shoot height, shoot diameter, shoot fresh and dry weight and root fresh and dry weight in tomato seedlings were interestingly ( $p \leq 0.05$ ) increased when sown in *Trichoderma* sp. and *T. harzianum* T969 fortified soil and when compared to the control. Chlorophyll content increased in seedling grown in *Trichoderma* sp. A dramatic increase ( $p \leq 0.05$ ) in the concentrations of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , P and  $\text{K}^+$  were recorded in the seedling shoot and root among *T. harzianum* T447.  $\text{Na}^+$  concentration.

Bal and Aldintas (2006) stated that the main effect of *T. harzianum* on early yield was the highest at 4 g/m<sup>2</sup>, producing 527 g/plant in comparison to the control with 374 g/plant. It was, therefore, hypothesised that the positive effect of *T. harzianum*, observed in the early yield, may be extended to the whole growing period by further applications, that is, by periodical renewal of the *T. harzianum* population at the root zone. *Trichoderma harzianum* was applied to seedling growing media at the time of sowing, at transplanting to vials and at planting. The factorial experiment using 2 seedling growing media, 2 cultivars and 4 dosages of *T. harzianum* at 0 (control), 4 g/m<sup>2</sup>, 10 g/m<sup>2</sup> and 24 g/m<sup>2</sup> showed that the *Trichoderma* dosage had significant effect on the yield or fruit quality. The 3 factor interactions between the criteria studied, that is, total yield, marketable yield, early yield, individual fruit weight, total soluble solids and titratable acidity, fruit diameter were significant. The 3 factor combination of Peat  $\times$  Belleveu F1  $\times$  10 g/m<sup>2</sup> resulted in the highest total yield, marketable yield and early yield with 1552, 1373 and 681 g/plant, respectively. The interaction between media and cultivars was significant for all the yield characteristics studied.

Brotman *et al.* (2013) 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.

Borrero *et al.* (2011) worked on the behavior of *Fusarium oxysporum* f.sp. *lycopersici* (Fol) and the effectiveness of the microbial control agent *Trichoderma asperellum* strain T34 in hydroponically grown tomato plants under five ammonium/nitrate ratios. They showed that disease severity was reduced by the action of T34 under increasing concentrations of ammonia. Furthermore, rhizosphere *F. oxysporum* populations decreased with T34 application. The presence of T34 augmented leaf nitrogen concentration in treatments infested with Fol.

Rini and Sulochana (2007) reported that different strains of *Trichoderma* and *Pseudomonas* showed varying degrees of antagonism against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato under in vitro conditions.

Cai *et al.* (2013) reported that harzianolide ((secondary metabolite from) *Trichoderma harzianum* strain SQR-T037) significantly promoted tomato seedling growth by up to 2.5-fold (dry weight) at a concentration of 0.1 ppm compared with the control. *Trichoderma* secondary metabolites may influence the early stages of plant growth through better root development for the enhancement of root length and tips. Both of the purified harzianolide and crude metabolite extract increased the activity of some defense-related enzymes to response to oxidative stress. Further experiment showed that a subsequent challenge of harzianolide-pretreated plants with the pathogen *Sclerotinia sclerotiorum* resulted in higher systemic resistance by the reduction of lesion size. These results indicate that secondary metabolites of *Trichoderma* spp., like harzianolide, may play a novel role in both plant growth regulation and plant defense responses.

Chacon *et al.* (2007) analyzed the capacity of the fungus *Trichoderma harzianum* CECT 2413 to colonize roots and stimulate plant growth. Analysis of the *T. harzianum*-tomato interaction in soil indicated that the contact between *T. harzianum* and the roots persisted over a long period of time. This interaction was characterized by the presence of yeast-like cells, a novel and previously undescribed developmental change. To study the molecular

mechanism underlying fungal ability to colonize the tomato-root system, the *T. harzianum* transcriptome was analyzed during the early stages of the plant-fungus interaction. The expression of fungal genes related to redox reactions, lipid metabolism, detoxification, and sugar or amino-acid transport increased when *T. harzianum* colonized tomato roots. These observations are similar to those regarding the interactions of mycorrhiza and pathogenic fungi with plants.

Cuevas (2006) evaluated that presence of the fungus (*Trichoderma*) in the soil in sufficient population resulted to more mineral nutrients especially P and Zn available for plant use that increased crop growth and yield. The fungus soil application at 0 N fertilizer in farmer's field before transplanting of rice seedling resulted to 18% mean yield increase compared to treatments without fungus application. Treatment with fungus application yielded grain significantly higher than that treated with 90 kg N/ha without the fungus. It seems that plots with higher yields, or those applied with fungus, had greater uptake of P and Zn. Other yield components such as tiller number, panicle number, and tiller biomass were affected by the fungus soil application.

Naseby *et al.* (2000) assessed effectiveness of *Trichoderma* upon pea growth and their antagonistic activity against large *Pythium ultimum* inocula. The effect of *Trichoderma* inocula upon the indigenous soil microflora and soil enzyme activities in the presence and absence of *Pythium* is assessed. In the absence of *Pythium*, *Trichoderma* strain N47 significantly increased the wet shoot weight by 15%. Strains TH1 and N47 resulted in significantly greater root lengths. All the *Trichoderma* strains reduced the number of lesions caused by *Pythium* and increased the number of lateral roots. Inoculation with *Trichoderma* strains TH1 and T4 resulted in significantly greater wet root weights (62% and 57%, respectively) in the presence of *Pythium* compared to the *Pythium* control. Inoculation with *Trichoderma* strains T4, T12 and N47 significantly reduced *Pythium* populations. Overall, strains T4 and N47 had the greatest beneficial characteristics, as both these strains improved plant growth



in the absence of *Pythium* and reduced plant damage in the presence of *Pythium*. The dual properties of these strains improve the commercial application, giving them an advantage over single action inocula, especially in the absence of plant pathogens.

Dababat *et al.* (2006) experimented on *Trichoderma harzianum* and *Trichoderma viride*. They tested for their capacity to reduce the incidence of the root-knot nematode *Meloidogyne incognita* on tomato. In vitro studies demonstrated that all tested isolates were effective in causing nematode mortality compared with the control. *Trichoderma* slightly reduced nematode damage to tomato in vivo. Treatment of the soil with the biocontrol agents before transplanting, improved control over treatment directly at transplanting. The *Trichoderma* isolates could not be re-isolated from the endorhiza, but were successfully re-isolated from the rhizosphere 45 days after fungal inoculation. Only slight increases in plant growth could be measured. The mutualistic endophyte *F. oxysporum* 162, used as positive control, was more effective in root-knot nematode biocontrol than the *Trichoderma* isolates.

Datnoff *et al.* (1995) conducted field experiments to evaluate commercial formulations of two beneficial fungi, *Trichoderma harzianum* and *Glomus intraradices*, for the control of *Fusarium* crown and root rot of tomato, caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Tomato seeds cv. "Sunny" were planted into soil nonamended or amended with the biocontrol agents, the former treatment serving as the control. After 6-7 weeks, plants were transplanted into beds fumigated with methyl bromide-chloropicrin in commercial tomato fields with a previous history of *Fusarium* crown and root rot. Disease incidence and severity were recorded at harvest maturity. Large and extra-large fruits (greater than or equal to 6.27 cm) also were harvested, counted, and weighed at maturity. Total marketable fruit yield was also determined. Compared to the controls, significant decreases in disease incidence were obtained with treatments of *T. harzianum* (1993), *G. intraradices* (1991), and *T. harzianum* + *G. intraradices* (both years).

Significant decreases in disease severity were obtained with the treatments of *T. harzianum* (1993), *G. intraradices* (1991), and *T. harzianum* + *G. intraradices* (1993). Yields of large and extra-large fruit or total marketable yield were not significantly different over the controls. These data suggest that commercial biological control agents may be effective in reducing Fusarium crown and root rot and that further evaluation of these agents is justified.

Dutta *et al.* (2002) The inoculum of *S. ralfsii* in sclerotial form was obtained by growing in 4 percent maize meal sand medium (MSM) in polypropylene bags (28 x 26 cm) incubating at  $28 \pm 1^\circ\text{C}$  for 21 days and applied in the field @ 500g/m<sup>2</sup> Mycelial culture of the antagonists was prepared by mass culture on well decomposed farm yard manure (FYM) and applied to the soil @ 300g/m<sup>2</sup> (4.5 x 10<sup>7</sup> propagules/ g) at the time of transplanting of tomato seedlings. Two chemicals viz., Dithane M-45 75 WP (0.1%) and Thiram 75 WP (0.1%) were used as seedling root dip treatment. The seedlings were uprooted carefully from the nursery bed and dipped in the 0.1% solution of both the chemicals for Y:Z hand were then transplanted in the main field.

Cai *et al.* (2014) studied that the capacity of *Trichoderma harzianum* strain SQR-T037 to colonize tomato roots work as bio-organic fertilizer stimulate plant growth and increase yields under field conditions. Field trials were conducted with a reduced application of chemical fertilizer (75 % of the recommended application) plus *Trichoderma* -enriched bio organic fertilizer (BF) or organic fertilizer (OF) or *Trichoderma* spore suspension (SS), while 100 % of the recommended chemical fertilizer (CF) was used as control.

Furthermore Nzanza and Puffy Soundy (2012) conducted an experiment to investigate the interactive effect of the plant growth promoting fungi *Trichoderma harzianum* and the arbuscular mycorrhizal fungi (AMF) in growth and development of tomato (*Solanun lycopersicum*) seedlings grown under greenhouse conditions. *T. harzianum* and/or AMF treated plants improved shoot length, root length, dry shoot mass and dry root mass. Pre-

inoculation with AMF increased shoot N, P and S content of tomato seedlings, whereas pre-sowing with *T. harzianum* alone increased the shoot N. Generally, shoot Zn and Mn content were affected by both fungi, with the best result observed when AMF was applied 2 weeks after *T. harzianum*. In conclusion, they suggested that *T. harzianum* and AMF have the potential to improve tomato seedling growth and development.

Hansen *et al.* (2010) reported that bioaerosol exposure increases during the growth season and that exposure to fungi, bacteria, and endotoxin can reach levels during the harvest period that may cause respiratory symptoms in growers that can be replaced by *Trichoderma harzianum*.

Haque *et al.* (2012). *Trichoderma* enriched biofertilizers when supplemented with N fertilizer significantly boosted up the growth and yield of mustard and tomato. The results suggested that *Trichoderma* -enriched biofertilizer could save at least 50% N fertilizer uses for mustard and tomato and could reduce excessive uses of NPK for crop cultivation.

Hermosa *et al.* (2013) reported that the complex plant defense signaling network that allows the recognition of fungi as non-hostile microbes, including microbial-associated molecular patterns (MAMPs), damage associated molecular patterns (DAMPs) and secreted elicitors. They examined how fungal interactions with plant receptors can activate induced resistance by priming and balancing plant defense and growth responses. Their observations were integrated into a model describing *Trichoderma* -plant hormone signaling network interactions.

In another study Affokpon *et al.* (2011) mentioned that tomato yields improved by over 30% following the application of biocontrol agents, especially *T. asperellum* T-16. They studied on seventeen isolates of the free-living soil fungus *Trichoderma* spp., collected from *Meloidogyne* spp. infested vegetable fields and infected roots in Benin, were screened for their rhizosphere competence and antagonistic potential against root-knot

nematodes, *Meloidogyne incognita*, in greenhouse pot experiments on tomato. The five isolates expressing greatest reproductive ability and nematode suppression in pots were further assessed in a typical double-cropping system of tomato in the field in Benin. In pots, a number of isolates provided significant nematode control compared with untreated controls. Field assessment demonstrated significant inhibition of nematode reproduction, suppression of root galling and an increase of tomato yield compared with the non-fungal control treatments. *Trichoderma asperellum* T-16 suppressed second stage juvenile ( $J_2$ ) densities in roots by up to 80%; *Trichoderma brevicompactum* T-3 suppressed egg production by as much as 86%, soil  $J_2$  densities were suppressed in treated plots, by as much as 94% (*T. asperellum* T-12), compared with the non-fungal controls. . This study provided the first information on the potential of West-African *Trichoderma* spp. isolates for use against root-knot nematodes in vegetable production systems. Their results are highly encouraging, demonstrating their strong potential as an alternative and complementary crop protection component.

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

Menzies (1993) found *Trichoderma viride* work as pathogen in the surface of an apparently healthy tomato root. *T. viride* were found to contain a heat-stable factor that caused a decrease in the growth of the roots of young cucumber, pepper and tomato seedlings. In greenhouse experiments, the fungus did not affect germination or seedling fresh weight of young cucumber seedlings in soil- peat- or rockwool-based germinating media, but 10% of seedlings germinated in rockwool showed signs of infection. This is the first report of *T. viride* being pathogenic on pepper and tomato.

Khan *et al.* (2017) conducted an experiment to evaluate the efficacy of *Trichoderma* -enriched biofertilizer (BioF/compost) on antioxidants and minerals in ripe tomatoes and soil health improvements in terms of nutrient availability and microbial populations. It was found that the application of BioF/compost enhanced plant growth, leaf greenness, and produced 12.9% higher yield compared to the recommended doses of NPK and other treatments. Mineral contents (P, K, Ca, Mg, Cu, Fe, Mn and Zn) in tomato roots, shoots and fruits and antioxidant compounds, i.e., ascorbic acid, ( $\beta$ -carotene, and lycopene) were increased significantly in fruits fertilized with 100% BioF/compost. The high efficiency of *Trichoderma* compost might be the result of its potential of nutrient solubilization and harboring soil microorganisms. *Trichoderma* also contributed to higher yield in tomatoes. Thus, *Trichoderma* -enriched biofertilizer may reduce application of chemical fertilizers and therefore, can be considered as a noble practice in sustainable

Khiareddine *et al.* (2009) tested three endogenous *Trichoderma* species *in vitro*, *in vivo* and *in situ* for their antagonistic activity against *Verticillium* spp. causing tomato vascular wilt in Tunisia. *Trichoderma harzianum*, *T. viride* and *T. virens* isolates reduced the radial growth of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* in comparison to the untreated controls. Antagonistic potential of *Trichoderma* spp. against tested wilt agents showed intra- and inter-specific variations. All tomato cv. 'Ventura' plants, when treated at planting with a *Trichoderma* spp. spore suspension and inoculated with *V. dahliae*, showed a reduced severity of *Verticillium* wilt in comparison to inoculated and untreated control plants. In addition, plants treated with *Trichoderma* spp. showed increased height and root and stem fresh weights in comparison to the inoculated and untreated control. *T. harzianum*, *T. viride* and *T. virens* significantly reduced the discoloration index compared to the untreated control. Plants treated with *Trichoderma* spp. Showed an increase of more than 50% of their roots and stem fresh weights in comparison to the untreated control.

Lee *et al.* (2008) carried out a test to examine the effects on tomato growth of application of purple non-sulfur bacterium *Rhodopseudomonas* sp. which had enhanced germination and growth of tomato seed under axenic conditions without affecting the native bacterial community in tomato rhizosphere soil,. The shoot length of tomato plant inoculated by *Rhodopseudomonas* sp. KL9 increased by 34.6% compared to that of control. The formation ratio of tomato fruit from flower was also raised by inoculation of KL9. In addition, *Rhodopseudomonas* sp. KL9 treatment enhanced the fresh weight and lycopene content in the harvested tomato fruits by 98.3 and 48.3%, respectively compared to those of the uninoculated control. This bacterial capability may be applied as an environment-friendly biofertilizer to cultivation of high quality tomato and other crops including lycopene-containing vegetables and fruits.

Margaret *et al.* (2011) showed *Trichoderma harzianum* (P52) and arbuscular mycorrhizal fungi (AMF) enhance growth in tomato seedlings. It was also found that a wilt pathogen caused by *Fusarium oxysporum* f. sp. *lycopersici* in tomato seedlings controlled by them.

Tucci *et al.* (2011) reported that Rhizosphere-competent fungi of the genus *Trichoderma* are widely used as biofertilizers and biopesticides in commercial formulates because of the multiple beneficial effects on plant growth and disease resistance. In this work, they demonstrated that genetic variability among wild and cultivated tomato lines affects the outcome of the interaction with two 'elite' biocontrol strains of *T. atroviride* and *T. harzianum*. The beneficial response included enhanced growth and systemic resistance against *Botrytis cinerea*. Expression studies on defence-related genes suggested that the fungus is able to trigger, in the responsive lines, a long-lasting up-regulation of the salicylic acid pathway in the absence of a pathogen, possibly activating a priming mechanism in the plant. They showed that, at least in tomato, the *Trichoderma* induced systemic resistance mechanism is much more complex than considered so far, and the ability of the plant to benefit from this symbiotic-like interaction can be genetically improved.

Mastouri *et al.* (2012) suggested that *Trichoderma* colonize roots and induce profound changes in plant gene expression that lead to enhanced growth, especially under biotic and abiotic stresses oxidized (lower ratios of reduced to oxidized forms), whereas colonized plants maintained redox state as high as or higher than unstressed and untreated plants. Enzymes were induced in uncolonized plants in response to water-deficit stress but to a lower extent when compared with colonized plants.

Molla *et al.* (2012) reported the impact of *Trichoderma* -enriched biofertilizer (BioF), i.e., BioF/compost (household/kitchen wastes composted by *Trichoderma harzianum* T22) and BioF/liquid (*T. harzianum* T22 grown in liquid media, i.e., broth culture) together with Nitrogen: Phosphorus: Potassium to recognize their roles in growth, yield and nutritional quality of tomato (*Lycopersicon esculentum* Mill.) in field studies. Total soluble solids, sugar, ascorbic acid,  $\beta$ -carotene, lycopene, phosphorus and manganese content in tomato were significantly higher when fertilized with BioF/compost. In addition, protein content and some essential minerals were increased in 50 % BioF/compost + 50 % N:P:K treatment. *Trichoderma* composted kitchen wastes can serve as prospective biofertilizer for improvement in yield and quality of tomato cultivation.

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

Montealegre *et al.* (2005) exposed that *Trichoderma harzianum* can be great bio weapon against root and crown rot in tomatoes. *T. harzianum* 650 (Th650) and *Paenebacillus lentimorbus* 629 (Pl629) selected earlier for their ability to control *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum* *in vitro*, were applied alone or combined with solarization (summer assay) and/or with methyl bromide (MeBr) (summer and winter assays) to a soil with a high

inoculum level, for the control of tomato root rot caused by the complex *F. oxysporum* f. sp. *lycopersici* - *Pyrenochaeta lycopersici* - *Rhizoctonia solani*. MeBr decreased tomato root damage caused by the complex from 88.7% to 21.2% and from 78.4% to 35.7% in the summer and in the winter assay, respectively. None but Th650 and Pl629 reduced root damage caused by this complex in the summer assay. Independent evaluations showed that the positive control of Th650 towards *R. solani* and the lack of effect on *P. lycopersici* correlate well with the endochitinase pattern expressed by Th650 in response to these phytopathogens. Thus represented an alternative use of this chemical fungicide for the control of this phytopathogen.

Montealegre *et al.* (2010) reported that *Trichoderma* strains used in tomato affected by *Rhizoctonia solani* did not have a major preventative effect on the damage as *T. harzianum* strains, has higher resistance to *R. solani*. In addition, it is good alternative to chemical fungicides for the control of *R. solani*. 100% mortality of tomato plants cv. 92.95 caused by *R. solani*, and the 40% mortality in tomato plants cv. Gondola (greenhouse assays). Mortality reduction was reflected in *Trichoderma* and in plant parameters increases (development, fresh and dry weights).

Morsy *et al.* (2009) stated tomato as one of the important economic vegetable crops which is attacked by several serious diseases such as root rot. They suggested that *Trichoderma* and *Bacillus* genera are most feasible biocontrol microorganisms suppress several pathogens like *Fusarium solani*. The efficiency of these antagonistic' treated plant by strains was evaluated using an *in vitro* assay. Their obtained results showed that, these treatments favored greater proliferation of rhizosphere microflora and higher dehydrogenase activity in the rhizosphere. The dual inoculation gave the highest records of growth parameters, fruit yields and plant nutrient content than individual one. Moreover, the dual treatment by *T. viride*+*B. subtilis* decreased the percentage of infection and increased survival rate than individual one. They



recommended to use these strains as a common biocontrol practice in agriculture.

Nazir Uddin *et al.* (2018) evaluated the ability of *Trichoderma harzianum* on plant growth and inhibitory activity against *Phythium ultimum* and *Phytophthora capsici* under laboratory and greenhouse conditions. *T. harzianum* reduced the size of lesions caused by the two pathogens. Observation of hyphae interaction of the *T. harzianum* with pathogens demonstrated that it inhibited the entry of both pathogens to the vascular bundle of the host tissue. Furthermore, they observed on the vascular bundle, pith and cortex of treated host plant inoculated with *T. harzianum* and pathogens

Naznin *et al.* (2015) conducted an experiment to determine the appropriate dose and combination of organic and chemical fertilizers and to assess the effect of bio-control agent (*Trichoderma*) on qualitative and quantitative characteristics of tuberose (*Polianthes tuberosa* L. cv. Single), including stem length, rachis length, spike length, floret number, flower yield, flower durability, number of bulb etc. Maximum growth, yield and yield contributing characters were recorded in Tricho-compost + $\frac{1}{4}$  RDF which were statistically superior to other treatments. Maximum plants emergence (93.3%) recorded in (Tricho-compost +  $\frac{1}{4}$  RDF). In case of plant height, number of leaves per plant, plant spread, days to flowering, number of florets, flower yield, bulb production, Tricho-compost (3 t/ha) +  $\frac{1}{4}$  RDF gave superior results over control (Recommended doses of fertilizer). The data obtained from the experiment showed that Tricho-compost with fertilizer enhanced qualitative and quantitative characters of tuberose flowers.

Nzanza *et al.* (2011) investigated on tomato (*Solanum lycopersicum* L.) with *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* on fungal root colonization, plant growth, yield and quality of field-grown tomato. The four treatments included *T. harzianum*, AMF, *T. harzianum*+AMF, and uninoculated control. Inoculation with AMF alone or in combination with *T. harzianum* increased dry shoot weight by 35% and 30%,

respectively, during the first season, and by 30% and 21%, respectively, during the second growing season. *Trichoderma harzianum* increased the percentage of large fruit by 76%. Similarly, AMF increased total soluble solids by 10%. Inoculated tomato seedlings with *T. harzianum* and/or AMF significantly increased early yield of tomato, by 10%, 65% and 70%, respectively and by 27%, 36% and 37%, respectively. In conclusion, results of the study suggested that *T. harzianum* and AMF have the potential to improve growth, early yield and fruit quality of field-grown tomato.

Nzanza *et al.* (2012) suggested that fungal inoculants such as *Trichoderma harzianum* or arbuscular mycorrhizal fungi (AMF) have the potential to improve yield and fruit quality of crops. They investigated the effect of inoculating tomato (*Solanum lycopersicum* L.) with *T. harzianum* and the AMF (*Glomus mosseae*) on yield and nutrient content of tomato fruit. They conducted experiment in a greenhouse. Both *T. harzianum* and AMF increased total yield and marketable yield of tomato ( $P > 0.05$ ). Inoculating tomato with AMF before sowing significantly increased the percentage of extra-large fruit, while inoculation with *T. harzianum* two weeks after sowing lowered the Ca and Mg contents of tomato fruit. *T. harzianum* and AMF inoculation increased the lycopene content, but did not affect the antioxidant activity, total flavonoids or vitamin C of the tomato fruit.

Ozbay and S.E. Newman (2004) performed an Experiment on *Trichoderma harzianum* and tomato seedlings to test whether *Trichoderma harzianum* strains have any effect on the growth of tomato seedlings. In green house, the seedlings were sampled for growth comparisons on seedling emergence, number of true leaves, fresh and dry weights of roots and shoots, stem caliper and shoot height. The results demonstrated that *Trichoderma harzianum* strains improved tomato seedling growth. There were differences between the untreated control and the treatments for all of the growth parameters at 4 weeks after inoculation with the exception of root fresh and dry weight.

Ozbay *et al.* (2004) experimented to know the performance of *Trichoderma harzianum* on growth of tomato seedlings. 18 days old tomato seedlings were treated with *Trichoderma* grown in green house. At six weeks, the seedlings were sampled for growth comparisons on seedling emergence, number of true leaves, fresh and dry weights of roots and shoots, stem caliper and shoot height. The results demonstrated that *Trichoderma harzianum* strains improved tomato seedling growth. There were differences between the untreated control and the treatments for all of the growth parameters at 4 weeks after inoculation with the exception of root fresh and dry weight.

Patal and Saraf (2017) studied to evaluate the efficacy of *Trichoderma asperellum* MSST to promote the growth and yield parameters of tomato S-22, a susceptible variety. This study was also undertaken to manage fusarium wilt disease under *in vitro* and *in vivo* conditions. Significant increase in vegetative parameters like root length, shoot length, plant weight and chlorophyll content was observed. There was reduction in the incidence of fusarium wilt in tomato up to 85%. Increase in the level of total phenol, peroxidase, polyphenoloxidase and phenylalanine ammonium lyase activity at 10th day of pathogen inoculation showed enhancement of plant defence mechanism by *T. asperellum* MSST against FOL. Overall study revealed that isolate MSST was proven to be potential biocontrol agent showing induced resistance against FOL.

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.

Rui-Xia Li *et al.* (2015) reported that *Trichoderma harzianum*, a biocontrol agent; enhance the uptake of nutrients (macro- and microelements) by plants in fields and also P and microelement (Fe, Mn, Cu and Zn) nutrition in tomato plants grown in soil and in hydroponic conditions. It significantly improved the biomass and nutrient uptake of tomato seedlings grown in a nutrient-limiting

soil. They also suggested through this study that the induction of increased or suppressed plant growth occurs through the direct effect of *T. harzianum* on root development, in combination with indirect mechanisms, such as mineral solubilisation (including solubilisation via acidification, redox, chelation and hydrolysis).

Muhammad and Amusa (2003) studied the compost-inhabiting bacteria for their effect on seedling blight inducing pathogens. *Trichoderma harzianum*, *Aspergillus niger* etc were the microbes found associated with cow dung, sawdust and rice husk composted soils. They are highly effective against *Sclerotium rolfsii*, *Fusarium oxysporum* etc are responsible for seedlings blight of tomato and so on crops.

Sundaramoorthy and Balabaskar (2013) conducted a research on efficacy of *Trichoderma* spp. against wilt of tomato. The dominating pathogen, which causes Fusarium wilt of tomato, was isolated and identified as *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Fifteen native *Trichoderma* antagonists were isolated from healthy tomato rhizosphere soil in different geographical regions. Under in vitro conditions, the results revealed that *Trichoderma harzianum* (ANR-1) isolate was found to effectively inhibit the radial mycelial growth of the pathogen (by 53%) when compared to all other isolates. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (by 15.33%). Also tomato plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62 cm) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other isolates and untreated control.

Saman Abeysinghe (2007) reported that *Trichoderma* inoculation increased the root and shoot length, and total fresh weight of bean plant over the uninoculated control. For tomato plants, significantly higher ( $p \leq 0.05$ , LSD) average root growth was observed when inoculated with *T. harzianum* strain CE262 over the control

Segarra *et al.* (2010) suggested using *Trichoderma asperellum* strain T34 to control the disease caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) on tomato plants. The reduction of the *Fusarium*-infected shoot by T34 was only significant. They hypothesized that Fe competition is one of the key factors in the biocontrol activity exerted by T34 against Fol, as an increase in Fe concentration over 10  $\mu$ M would lead to the suppression of T34 siderophore synthesis and thus inhibition of Fe competition with Fol. Nevertheless, several plant physiological parameters like net CO<sub>2</sub> assimilation (A), stomatal conductance etc demonstrated the protection against Fol damage by treatment with T34 at 100  $\mu$ M Fe. T34-treated plants had significantly greater heights and dry weights than control plants. Furthermore, T34 enhanced plant height even at the optimal Fe concentration (10  $\mu$ M) compared to control plants. In conclusion, *T. asperellum* strain T34 protected tomato plants from both biotic (Fusarium wilt disease) and abiotic stress [Fe (III) toxic effects].

Siddiqui *et al.* (2004) determined the influence of soil-borne fungus *Trichoderma harzianum* on the biocontrol performance of *Pseudomonas fluorescens* strain CHA0 and its 2, 4-diacetylphloroglucinol (DAPG) overproducing derivative CHA0/pME3424 against *Meloidogyne javanica*.

Singh *et al.* (2013) mentioned that *Trichoderma harzianum* and *Pseudomonas* and their combination if applied as seed and seedlings treatment in tomato, consortium treatment showed the greater plant growth promotion activity in comparison to solo treatment. Consortium treatment showed highest chlorophyll content (1.05mgg<sup>-1</sup> fresh weight of leaves) while in the pathogen inoculated control lowest amount was recorded (0.8mgg<sup>-1</sup> fresh weight of leaves). The lowest mean disease rating (MDR) 1.96 and maximum percent disease reduction (PDR), 53.23% recorded in consortium treatment. The yield was also significantly higher in bio agents treated treatments, T3 (37.41 tons h<sup>-1</sup>), T4 (36.60 tons h<sup>-1</sup>), T5 (43.84 tons h<sup>-1</sup>) and untreated unchallenged treatment (45.29 tons h<sup>-1</sup>) than untreated challenged control (24.07 tons h<sup>-1</sup>).

From these results it can be concluded that the application of consortium of compatible bioagents will enhance the plant growth and biological control of in contrast to treatment with single bioagent.

Singh *et al.* (2014) *Trichoderma harzianum* solubilizes mineral nutrients and inorganic fertilizers, increasing availability and uptake of nutrients to the plant. The application of *Trichoderma* in consortium form increased mineral nutrient uptake, reduced disease incidence and obtained a greater yield with reduced chemical pesticide loads, benefitting farmers and consumers. The aim of this study is to evaluate the effect of *Trichoderma harzianum* (BHU-51), *Trichoderma harzianum* (BHU-105) and their consortium *Trichoderma harzianum* (BHU-51+BHU-105) on management of *R. solani* and nutrient levels in the plants and they found the best against the pathogen. Field trials also showed that the consortium produced better results in terms of shoot length, chlorophyll content and yield than the control.

Sivan and Chet (1993) had combined *Trichoderma harzianum* with soil solarization or with a reduced dose of methyl bromide, under field conditions, resulted in significant ( $p = 0.05$ ) disease control of fusarium crown and root rot of tomato induced by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). Maximum disease control (48%) was obtained with a combination of *T. harzianum*. The highest yield improvement (105% over the control) was recorded in plots where the antagonist had been applied in combination with soil solarization. In plots not treated with *T. harzianum*, the rhizosphere soil contained low levels of *Trichoderma* spp. [ $10^2$ - $10^3$  colony-forming units (c.f.u.)  $g^{-1}$  rhizosphere soil], and tomato root segments showed no evidence of colonization by the antagonist. High levels of *Trichoderma* ( $10^4$ - $10^5$  c.f.u.  $g^{-1}$ ) were recovered from rhizosphere soil or crown segments of plants treated with the antagonist in combination with methyl bromide or soil solarization. This study reveals that combination of *T. harzianum* with a sub-lethal dose of methyl bromide or with soil solarization is effective in controlling FORL in tomato.

Sivan *et al.* (1987) experimented during 2 successive growing seasons in fields naturally infested with *F. oxysporum* f.sp. *radicis-lycopersici* in which *T. harzianum* was applied as a seed coating or as a wheat-bran/peat(1:1,v/v) preparation introduced into the tomato rooting mixture, *T.*-treated transplants were better protected ( $P=0.05$ ) against crown rot than untreated controls when planted in methyl bromide-fumigated or nonfumigated infested fields. The total yield of tomatoes in the treated plots was increased by up to 26.2% over controls. When *T. harzianum* was applied to the root zone of tomato transplants, it proliferated successfully in the rhizosphere. Soil samples taken from the crown area 5-10 cm from the plant stem showed an increase in *T. harzianum* population levels during the growing season; however, no significant decline was found in the soil population density of *Fusarium* spp. in the same soil samples. When tomato seeds previously treated with conidia of *T. harzianum* were sown in a naturally infested field, the antagonist was detected on root segments from plants sampled 20 weeks after planting. The highest counts of the antagonist were detected on the root tips, resulting in the complete reduction of *Fusarium* spp. recovered from these segments

Srivastava *et al.* (2010) had an experiment on *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.). An effort was made to develop an eco-friendly approach to control *Fusarium* wilt in tomato using fluorescent *Pseudomonas*, *Trichoderma harzianum* and *Glomus intraradices*, an arbuscular mycorrhizal fungus (AMF). Besides direct interaction with plant pathogens, bioagents have been reported to induce systemic resistance in plants. Application of *T. harzianum* and fluorescent *Pseudomonas* by seed bio-priming significantly increased seed germination (22–48%) and reduced the days required for germination (2.0–2.5 days). The combination of fluorescent *Pseudomonas*, *T. harzianum* and AMF increased yield by 20%. Comparing to control (-CDC), the combination of all three bioagents with CDC significantly reduced disease by 81 and 74% in pots and field, respectively and enhanced the yield by 33%.

Strashnov *et al.* (1985) experimented on application of *Trichoderma harzianum*, to soil or by coating tomato fruits, reduced *Rhizoctonia solani* fruit rot by up to 43% and 85%, respectively, under laboratory conditions. When mixed with naturally infested soil, *Trichoderma* reduced *R. solani* inoculum potential by 86% in field trials. It also significantly reduced fruit rot by 27–51%.

Tanwar *et al.* (2013) assessed the effectiveness of two arbuscular mycorrhizal fungi (AMF) (*Funneliformis mosseae* and *Acaulospora laevis*) and *Trichoderma viride* against tomato wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* under pot condition. All the bioagent showed appreciable results in increasing plant growth. Combined inoculation of *F. mosseae*, *A. laevis* and *T. viride* showed maximum increases in plant height, shoot fresh weight, root dry weight, number of leaves and number of branches per plant while dual inoculation of *F. mosseae* and *T. viride* increased rest of the growth parameters like shoot dry weight, root fresh weight, root length and leaf area. Bio agent application increase remarkable in photosynthesis, chlorophyll content and nutrient content. The findings of this study concludes that soil inoculation with *F. mosseae* along with root inoculation with conidial suspension of *T. viride* before transplantation offered better survival and resistance to tomato seedlings against Fusarium wilt.

Gravel *et al.* (2007) demonstrated that *Pseudomonas putida* or *Trichoderma atroviride* expose their promoting effect on the growth of mature healthy tomato plants grown under hydroponic conditions. *P. putida* and *T. atroviride* were shown to improve fruit yields in rockwool and in organic medium. *P. putida* and *T. atroviride* also increased the fresh weight of both the shoot and the roots of tomato seedlings grown in the presence of increasing concentrations of L-tryptophan (up to 0.75 mM).

Vinale *et al.* (2013) mentioned that *Trichoderma* spp. produce numerous biologically active compounds, including cell wall degrading enzymes, and secondary metabolites. They studied the three-way relationship established



with *Trichoderma*, the plant and the pathogen are aimed at unravelling the mechanisms involved in partner recognition and the cross-talk used to maintain the beneficial association between the fungal antagonist and the plant. This review presents recent advances and findings regarding the biocontrol-resulting events that take place during the *Trichoderma*–plant–pathogen interaction.

Vitti *et al.* (2016) reported the ability of *Trichoderma harzianum*, strain T-22 (T22) to control *Cucumber mosaic virus* (CMV) in *Solanum lycopersicum* var. *cerasiforme* plants and the changes in the physiology of tomato treated/infected with T22/CMV were examined. Plant growth-promoting effects, photosynthetic performance, reactive oxygen species scavenging enzymes, and phyto hormones were investigated. T22 improved tomato growth in terms of plant height and improved photosynthesis, total chlorophyll content and plant gas exchange. The analysis of plant hormones demonstrated that treating with T22 before or simultaneously to CMV infection, led to a systemic resistance by jasmonic acid/ethylene and salicylic acid signaling pathways. Conversely, systemic resistance was abscisic acid-dependent when T22 treatment was administered after the CMV infection. In conclusion, the data reported that the T22-based strategy may be the most effective measure against CMV.

Wokocha (1990) showed the effect of simultaneous applications of *T. viride* with PCNB, captan or Aldrex T on the basal stem rot of tomato was studied in artificially inoculated field plots in Samaru (Northern Guinea Savanna Zone) during 1978–1980. The results showed that the addition of *T. viride* with PCNB gave excellent disease control and plants treated with the combination showed no symptoms of basal stem rot during both dry- and wet-season trials. The combined effects of *T. viride* and PCNB were, however, not significantly different from those obtained in separate applications with *T. viride*. When applied alone, PCNB was less effective giving a disease severity index (DSI) of 15.6%, whereas captan and Aldrex T were ineffective, with a DSI of 84.9% and 79.2% respectively, during wet-season disease indices were

generally high. Combined applications with *T. viride*, however, significantly enhanced the three fungicides in disease control, giving maximum DSIs of 0.0% (*T. viride* + PCNB), 16.4% (*T. viride*+captan) and 19.0% (*T.viride*+Aldrex T. *Trichoderma viride* thus provided a synergistic effect which substantially increased the disease-control capabilities of PCNB, captan with a Aldrex T. Marketable tomato fruit yield was highest (18.75–19.37 tonnes ha<sup>-1</sup>) in plots treated with a combination of *T. viride* and PCNB.

## CHAPTER III

### MATERIALS AND METHODS

This chapter illustrates information concerning methodology that was used in the execution of the experiment. It comprises a short description of experimental site, climatic condition, materials used for the experiment, treatments, data collection, procedure, and statistical analysis.

#### 3.1. Experimental site

The experiment was conducted at Horticulture farm, Sher-e-Bangla Agricultural University, Dhaka during the period from October 2017-March 2018 to study the effect of foliar application of *Trichoderma harzianum* on some tomato varieties.

#### 3.2. Geographical Location

The location of the experimental site is 23<sup>o</sup> 74 N latitude and 90<sup>o</sup>35 E longitude and at an elevation of 8.2 m from sea level (Anon. 1989) in Agro-Ecological Zone of Madhupur Tract (AEZ 28) (UNDP-FAO, 1988). This is a region of complete relief and soils developed over the Modhupur clay, where floodplain sediments buried the dissected edges of the Modhupur Tract leaving small hillocks of red soils as 'islands' surrounded by floodplain.

#### 3.3. Climatic condition

Experimental site was located on the subtropical monsoon climatic zone, where heavy rainfall occurs during the month of April to September (kharif season) and exiguous of rainfall during the rest of the year (Rabi season). Moderately low temperature and plenty of sunshine remain during October to March (Rabi season), which is suitable for tomato cultivation in Bangladesh. A detail of the meteorological data during the period of the experiment was collected from the Bangladesh Meteorological Department, Agargoan, Dhaka.

### **3.4. Characteristics of soil**

The experimental site belongs to general soil type, which is Shallow Red Brown Terrace Soils under Tejgaon Series where top soil is olive gray with common fine to medium distinct dark yellowish brown mottles and were clay loam in texture. Organic matter content 0.84%. Soil pH ranged from 6.0-6.6. Experimental area was flat having good drainage system and available irrigation and it is above flood level. From experimental field soil sample was collected from 0-15 cm depths and analyzed by Soil Resources and Development Institute (SRDI), Dhaka. Physiochemical properties were present in the soil appropriately.

### **3.5. Planting materials**

Seeds of three tomato varieties were collected from the Advanced Seed Research and Biotech Centre (ASRBC), ACI Limited. All the varieties are determinate type.

### **3.6. Treatments of the experiment**

The two factorial experiment was conducted to evaluate the effect of *Trichoderma* on growth, yield and disease resistance of some tomato varieties. Factors are follows:

#### **Factor A: Tomato varieties**

In the experiment, three tomato varieties were used. These were:

BARI Tomato-2 ( $V_1$ )

Roma -VF ( $V_2$ )

Apple Netherland ( $V_3$ )

#### **Factor B:**

***Trichoderma* spore concentration:**

*Trichoderma harzianum* was applied as three different treatments in this study

### **Treatment**

Control (T<sub>0</sub>)

T<sub>1</sub>= 10<sup>6</sup> spores/ml

T<sub>2</sub>= 10<sup>7</sup> spores/ml

### **3.7. Design and layout of the experiment**

The experiment was laid out following Randomized completely block design (RCBD) having two factors with three replications. There were 27 unit plots in the experiment. The size of each plot was 3 sq m. and distance 0.5 m from block to block.

### **3.8. Preparation of *Trichoderma* spore solution**

*Trichoderma harzianum* mother culture in barley was collected from BARI. New cultures were prepared from the mother culture in PDA media at Advanced Seed Research and Biotech Centre (ASRBC), ACI Limited. 25-30 days old new cultured spore was counted under light microscope. Estimated spore culture was mixed in autoclaved water to make the solution (Plate. 1 c)

### **3.9. Seedbed preparation and raising of seedlings**

Tomato seedlings were raised in seed trays at Advanced Seed Research and Biotech Centre (ASRBC), ACI Limited (plate. 1 a). The soil was well prepared and converted into loose friable. All weeds and stubbles were removed. Ten gram of seeds was shown on trays on October 2017. After showing seeds in trays are covered with light soil. Germination was visible 3 to 4 days after seed sowing. Within 5 to 6 days after sowing emergence of the seedlings took place. 25 days old seedlings were transplanted in the main field.

### 3.10. Land preparation

The experimental area was ploughed to open direct sunshine to kill soil borne pathogens and soil inhabitant insects. Then the plot was prepared by several plowing and cross plowing followed by laddering and harrowing with power tiller to bring about to good tilth in the second week of October 2017. The corners were shaped and the clods were broken into pieces. Weeds and other stubbles were removed carefully from the experimental plot. Then the area was divided into plots of 3m x 1m according to the layout of the experiment.

### 3.11. Manure and fertilizers applications

Total cow dung and triple super phosphate (TSP) were applied in the field during final land preparation. After three weeks of transplanting half of the urea and half of the muriate of potash (MOP) were applied in the plot. Remaining urea and muriate of potash (MOP) were applied five weeks after transplanting. Dose of manure and fertilizers used in the study are showing in Table 1.

**Table 1. Doses of manures and fertilizers with BARI recommended dose along with plot**

SL No.	Manures/ fertilizers	Recommended Dose
1	Cowdung	10 t/ha
2	Urea	300 kg/ha
3	TSP	200 kg/ha
4	MoP	220 kg/ha

### 3.12. Transplanting of seedlings

The seed tray was watered before uprooting the seedlings to minimize the damage of roots. At the time of uprooting, care was taken so that root damage become minimum and some soil remained with the roots. Twenty one days-old healthy seedlings were transplanted at the spacing of 60cm × 40cm in the

experimental plots on 5 December 2017 at noon to avoid the detrimental effect of sunlight to the tender seedlings. The transplanted seedlings were lightly watered at regular basis to make a firm relation with roots and soil to stand along.

### **3.13. Application of *Trichoderma* solution**

Application of *Trichoderma* was done to the tomato field soil along with the leaf and the whole plant. First application was done at 7 days after transplanting and twice application was done at 14 days after transplanting (Plate 1 e).

### **3.14. Intercultural operations**

After transplanting the seedlings, various kinds of intercultural operations were accomplished for better growth and development of the plants, which are as follows:

#### **3.14.1. Gap filling**

When seedlings were well established, the soil around the base of each seedling was pulverized. A few gaps filling was done by healthy seedlings of the same stock where initial planted seedlings failed to survive.

#### **3.14.2. Weeding**

Weeding was done uniformly in all the plots when the seedlings were well established. After 20 days of the first one second weeding was done.

#### **3.14.3. Staking**

When the plants were well established, staking was done to each plants using bamboo sticks with rope to keep the plants erect (Plate 1 d). As the plants grew up within a few days of staking, other cultural operations were carried out.

#### **3.14.4. Irrigation**

After transplanting the seedlings were properly irrigated and it was for 4 consecutive days. After each top dressing of urea, flood irrigation was given to the plants. When plants were reached to active fruiting stage final irrigation was given to the plant.

#### **3.14.5. Pesticide application**

As the experiment regarded with *Trichoderma*, no pesticide was applied.

#### **3.14.6. Insecticide application**

As the experiment regarded with *Trichoderma*, no insecticide was applied.

#### **3.15. Harvesting**

Harvesting of fruits were done on the basis of horticultural maturity, size, color and age being determined for the purpose of consumption as the fruit grew rapidly and soon get beyond the marketable stage.

#### **3.16 Data collection**

Data were recorded on the following parameters from the sample plants during the course of experiment. Ten plants were selected randomly from each plot for data collection in such a way that the border effect could be avoided for the highest precision.

##### **Growth related parameter**

- Plant height (cm)
- No. of leaves per plant
- No. of cluster per plant
- No. of branches per plant

##### **Duration related parameter**

- Days to first flowering from transplanting



- Days to first fruit setting from transplanting
- Days to first fruit maturity from transplanting

#### **Yield related parameter**

- No. of flower per cluster
- No. of flower per plant
- No. of fruit per cluster
- No. of fruit per plant
- Single fruit weight (g)
- Fruit length (cm)
- Fruit diameter (mm)
- Pericarp thickness (mm)
- Yield per plant (kg)
- Yield per hectare (t)

#### **3.16.1. Plant height (cm)**

Plant height was measured from sample plants in centimeter from the ground level to the tip of the longest stem and the mean value for each treatment was calculated. Plant height was recorded at 20, 40, and 60 days after transplanting.

#### **3.16.2. Number of leaves plant<sup>-1</sup>**

Leaves number was manually counted from selected plants and each leaf was counted from bottom to top maintaining certain days interval (20, 40, and 60 days after transplanting) and their average was computed as average number of leaves per plant.

#### **3.16.3 Number of branches plant<sup>-1</sup>**

The total number of branches was counted from selected plants from each treatment. Data was recorded maintaining certain days interval starting from 20 days of planting up to 60 days.

#### **3.16.4. Days to first flowering**

Days to first flowering was counted from DAT by observing the first flower at top of the plant (Plate. 1 f).

#### **3.16.5. Chlorophyll percentage (SPAD value)**

Leaf chlorophyll was measured by using SPAD-502 plus. The chlorophyll was measured from three different portion of the leaf and then averaged for analysis. Chlorophyll content was expressed in percentage.

#### **3.16.6. Number of cluster per plant**

Number of clusters was taken from selected plants after certain days interval of transplanting. Each cluster was counted manually and the average was expressed as the number of cluster/ plant.

#### **3.16.7. Number of flower per cluster**

The number of flower per cluster was counted manually from every cluster of the selected plant at a certain days of interval and the average was computed and as expressed in average number of flower per cluster.

#### **3.16.8. Number of flower per plant**

The number of flower per plant was counted manually from every cluster of the selected plant at a certain days of interval and the average was computed and as expressed in average number of flower per plant.

#### **3.16.9. Days to first fruit setting**

First fruit setting was observed visually and fruit maturity was counted the days from the date of tomato plant transplanting.

#### **3.16.10. Number of fruit per cluster**

The number of fruit in every cluster was counted manually from selected plant, and then the average was computed and expressed as the average number of fruit per cluster (Plate. 1 g).

#### **3.16.11. Number of fruit plant<sup>-1</sup>**

The number of fruit from selected plant was counted and then the average was computed and expressed as the average number of fruit per plant.

#### **3.16.12. Days to first fruit maturity**

First fruit maturity was counted the days from the date of tomato plant transplanted.

#### **3.16.13. Fruit length (cm)**

Fruit length and diameter were measured by using Digital Calipers -515 (DC-515) in centimeter (cm) from the neck of the fruit to the bottom.

#### **3.16.14. Fruit diameter (mm)**

Fruit diameter was measured by using Digital Calipers -515 (DC-515) in millimeter (mm).

#### **3.16.15. Pericarp thickness (mm)**

Pericarp thickness was measured by using Digital Calipers-515(DC-515) in millimeter (mm).

#### **3.16.16. Single fruit weight (g)**

Fruit weight was measured by Electronic Precision Balance in gram. Total fruit weight of each pot was obtained by addition of weight of the total fruit number and average fruit weight was obtained from division of the total fruit weight by total number of fruit.

### **3.16.17. Yield plant<sup>-1</sup> (kg)**

Yield per plant was calculated in kilogram (kg) by a balance from the total weight of fruits per selected plant.

### **3.16.18. Yield /hectare (t)**

Yield per hectare was calculated from the yield obtained in each of the experimental unit and was expressed in tons per hectare.

### **3.17. Statistical analysis**

Collected data were statistically analyzed using STATISTIX-10 computer package programme. Mean for every treatments were calculated. Difference between treatments was assessed by Least Significant Difference (LSD) test at 0.05% level of significance (Gomez and Gomez, 1984).



a



b



c



d



e



f



g

Plate 1: Pictorial presentation of different methodological works. **a.** Seedlings in tray, **b.** Growing plant **c.** growing *Trichoderma* in PDA, **d.** Staking of plant, **e.** Foliar application of *Trichoderma* solution, **f.** Flowering of tomato plants, **g.** Fruits of tomato plant

## Chapter IV

### RESULTS AND DISCUSSION

The research work on 'Bio efficacy of *Trichoderma harzianum* spore concentrations on growth and yield of tomato' was undertaken in the Department of Horticulture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The experimental results on growth and yield contributing parameters recorded during the entire period of study are presented as follows:

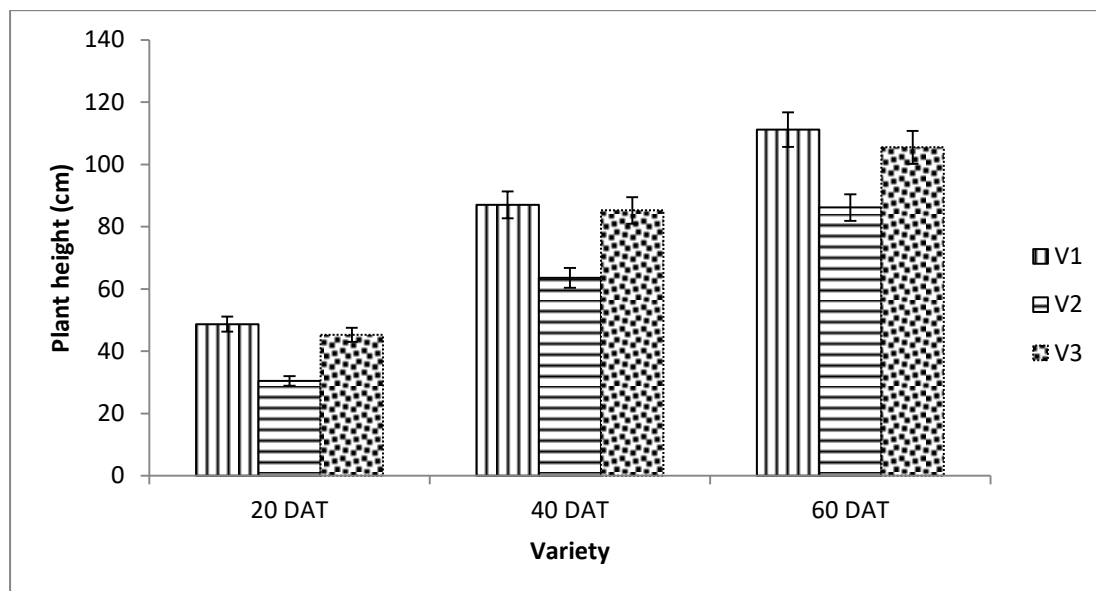
#### 4.1 Plant Height (cm)

Plant height is one of the most important growth parameters in tomato which is positively correlated with yield and the growing conditions significantly influenced this trait. Significant differences exist among varieties with regard to plant height at 20 days, 40 days and 60 days after transplanting. The Tallest plant was found from V<sub>1</sub> (111.2 cm) which is statistically similar with V<sub>3</sub> (105.7 cm) whereas the shortest from V<sub>2</sub> (86.3 cm) at 60 days after transplanting (Fig. 2). Significant increase in plant height was observed from 20-40 DAT in all the varieties which then slowed down at 40-60 DAT because indicating it reaching maturity.

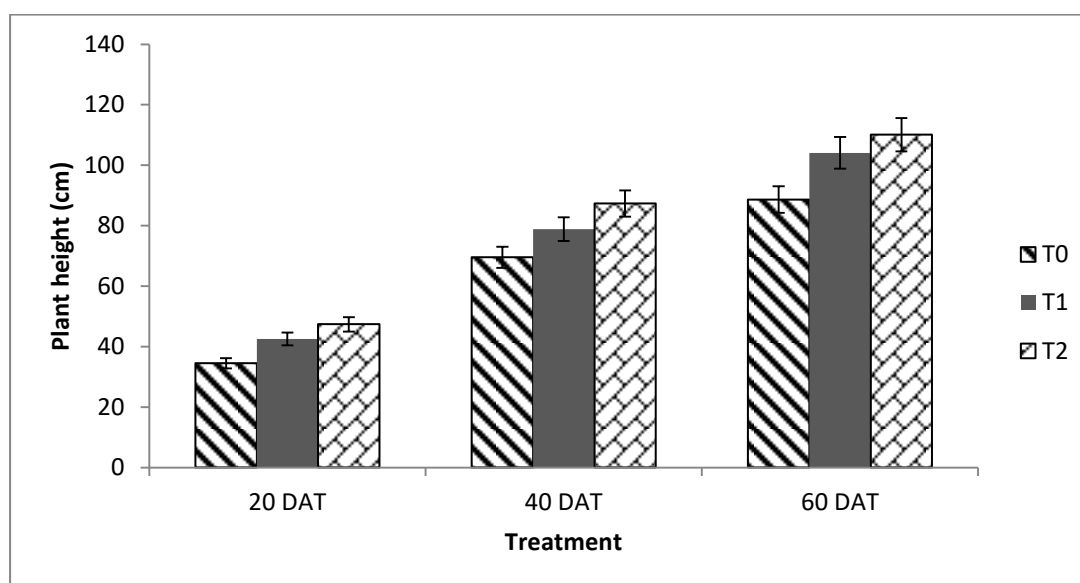
In case of different spore concentration of *Trichoderma harzianum*, significant variation in plant height was observed. (Appendix II). The tallest plant (81.1 cm) was found under T<sub>2</sub> (110.6 cm) and the shortest plant (88.64 cm) was found from control T<sub>0</sub> at 60 days after transplanting (fig. 3).

The antagonism effect of *Trichoderma* increases the plant height. Vascular discoloration by pathogen is lower in *Trichoderma* inoculated plant Khiareddine *et al.* (2005). In tomato plant *Trichoderma* alone can increase the plant height whether in case of mustard *Trichoderma* along with Nitrogen is required to increase the plant height (Hauqe *et al.* 2012). *Trichoderma* enhanced plant height even at the optimal Fe concentration (10 µM)

compared to control plants (Segarra *et al.*, 2010). Increased plant height with application of *Trichoderma* also founded by Abedin *et al.* 2018, Ozbay *et al.* (2004) and Sundaramoorthy *et al.* (2013).



**Fig.2.** Performance of three tomato varieties on plant height (cm) at different days after transplanting;  
i.e. V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>=Apple Netherland



**Fig.3.** Effect of foliar application of *Trichoderma harzianum* on plant height (cm);  
i.e. T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

In case of combination treatment significant variation in plant height (cm) was observed which indicated the influence of concentration of *Trichoderma* spore on plant height of different varieties (Appendix II). Roma VF variety produced tallest plant (114.8 cm) under T<sub>2</sub> condition (V<sub>1</sub>T<sub>2</sub>) whereas shortest plant (75.7 cm) was found in V<sub>2</sub>T<sub>0</sub> at 60 days after transplanting (Table 2).

**Table 2.** Combined effect of varieties and foliar application of *Trichoderma* on plant height at different days after transplanting of tomato

<sup>x</sup> Combinations	Plant height (cm)		
	20 DAT	40 DAT	60 DAT
V <sub>1</sub> T <sub>0</sub>	39.3 d	80.3 c	88.8 f
V <sub>1</sub> T <sub>1</sub>	49.7 b	87.1 b	95.8 b
V <sub>1</sub> T <sub>2</sub>	57.3 a	93.5 a	114.8 a
V <sub>2</sub> T <sub>0</sub>	25.5 g	55.3 f	75.7 h
V <sub>2</sub> T <sub>1</sub>	30.7 f	62.7 e	98.9 d
V <sub>2</sub> T <sub>2</sub>	35.3 e	73.7 d	96.47 e
V <sub>3</sub> T <sub>0</sub>	38.6 d	73.3 d	91.8 f
V <sub>3</sub> T <sub>1</sub>	47.4 c	87.3 b	86.7 g
V <sub>3</sub> T <sub>2</sub>	49.7 b	95.7 a	109.7 c
LSD <sub>(0.05)</sub>	0.53	1.23	0.86
CV (%)	1.54	1.30	1.15

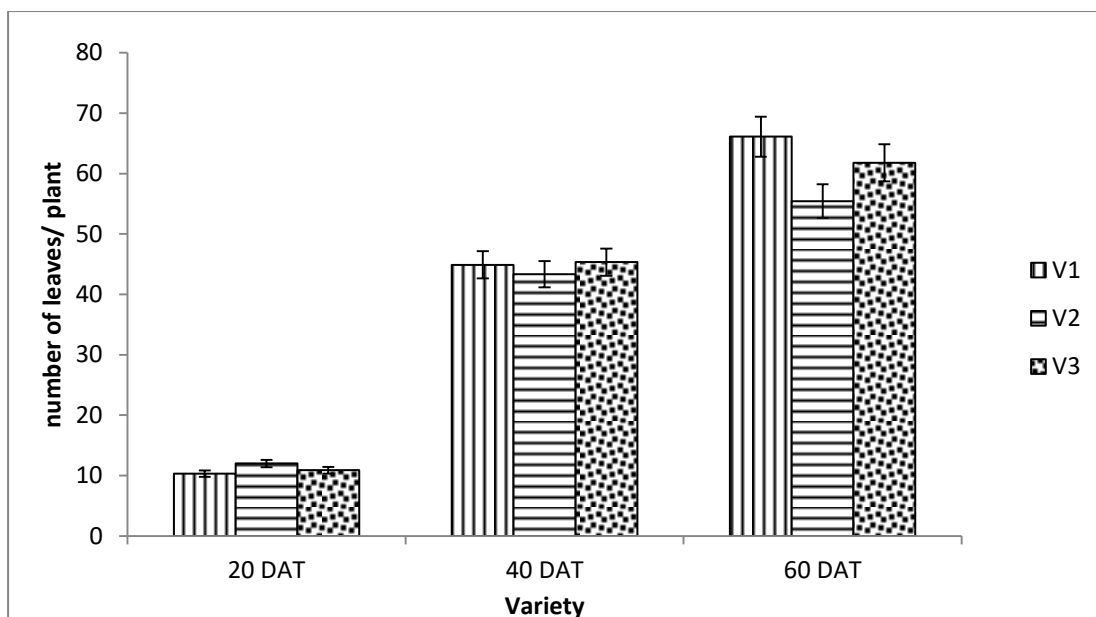
<sup>x</sup> Here, V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>= Apple Netherland. T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

#### 4.2 Number of leaves plant<sup>-1</sup>

The data with respect to the number of leaves per plant was recorded at 20, 40 and 60 DAT are presented in Appendix VII. Significant difference was observed in terms of number of leaves per plant at different DAT due to the application of different concentration of *Trichoderma*. At 20 DAT, the highest number of leaves (12.0) per plant was recorded from the variety V<sub>1</sub> which was superior to all other varieties. This was followed by variety V<sub>3</sub> (10.9) and V<sub>2</sub> (10.33) (Fig. 4). On the contrary, the minimum number of leaves (9.4) per plant was recorded in the control (T<sub>0</sub>).

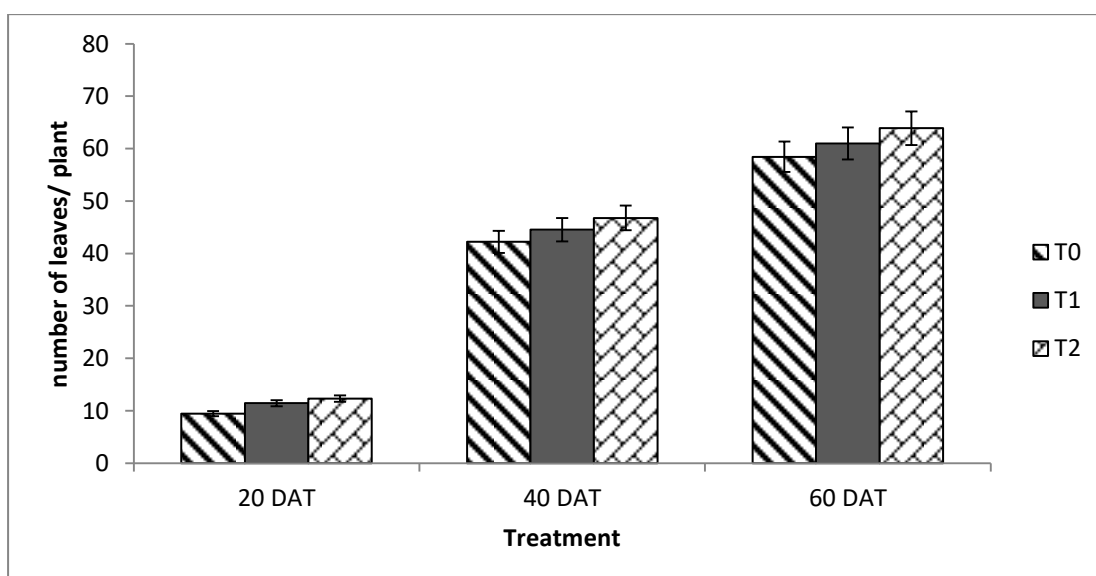




**Fig.4.** Performance of three tomato varieties on number of leaves at different days after transplanting;

i.e.  $V_1$ = Roma -VF,  $V_2$ = BARI Tomato-2,  $V_3$ = Apple Netherland

At 40 DAT, the highest number of leaves (45.33) per plant was found from  $V_3$  which was statistically similar to  $V_1$  and minimum number of leaves was found with  $V_2$  (43.33). On the other hand, the minimum number of leaves (42.22) per plant was found from the treatment  $T_0$  (control). The result further showed that other treatments exerted a significant increase in the number of leaves per plant that is statistically similar to the 40 DAT plant heights.



**Fig.5.** Effect of foliar application of *Trichoderma harzianum* on number of leaves;

i.e.  $T_0$ = Control,  $T_1$ = spore concentration  $10^6$ ,  $T_2$ = spore concentration  $10^7$

As regard number of leaves per plant  $T_2$  was found significantly superior to all other treatments with variety  $V_1$ . The maximum number of leaves (69.0) was found from variety  $V_1$  and  $T_2$  followed by  $V_1T_1$  (66.0),  $V_3T_2$  (64.3) and  $V_3T_1$  (62.0) and the minimum number was found from  $V_2T_0$  (53.0) at 60 days after transplanting. The result further showed that  $T_2$  was found superior to all other treatments in every stage of observations with variety  $V_1$  (Table 3). This might be due the enhanced nutrient uptake in plants that results in increased plant growth and fitness. Uptaking of major nutrients, such as nitrogen (N), phosphorus (P) and potassium (K) increased in tomato plant treated with *Trichoderma* significantly observed from several reports (Sudisha *et al.*, 2018).

**Table 3.** Combined effect of varieties and foliar application of *Trichoderma* on number of leaves at different days after transplanting of tomato

<sup>x</sup> Combinations	Number of leaves (cm)		
	20 DAT	40 DAT	60 DAT
$V_1T_0$	8.7 e	42.3 d	63.3 d
$V_1T_1$	11.0 c	45.0 b	66.0 b
$V_1T_2$	11.3 bc	47.3 a	69.0 a
$V_2T_0$	10.3 cd	41.3 e	53.0 h
$V_2T_1$	12.3 ab	43.3 c	55.0 g
$V_2T_2$	13.3 a	45.3 b	58.3 f
$V_3T_0$	9.3 de	43.0 cd	59.0 f
$V_3T_1$	11.0 c	45.3 b	62.0 e
$V_3T_2$	12.3 ab	47.7 a	64.3 c
LSD <sub>(0.05)</sub>	0.55	0.49	0.36
CV (%)	5.70	1.23	0.72

<sup>x</sup> Here,  $V_1$ = Roma -VF,  $V_2$ = BARI Tomato-2,  $V_3$ =Apple Netherland  
 $T_0$ = Control,  $T_1$ = *Trichoderma harzianum* spore concentration  $10^6$ ,  $T_2$ = *Trichoderma harzianum* spore concentration  $10^7$

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

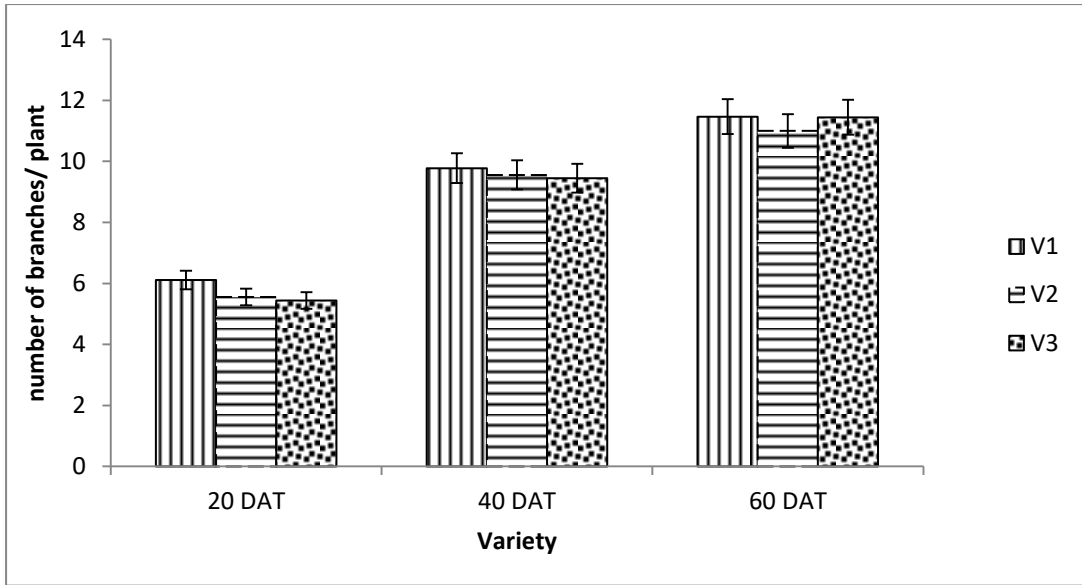
#### 4.3. Number of branches plant<sup>-1</sup>

Number of branch per plant of tomato exposed statistically significant difference among three tomato varieties ( $V_1$ ,  $V_2$  and  $V_3$ ) at 20, 40, 60 days after transplanting (Appendix IV). Maximum number of branches was found in  $V_1$  (11.7) at 60 days after transplanting and minimum number of branches was found in  $V_2$  (11.0) at 60 days after transplanting (Fig. 4).

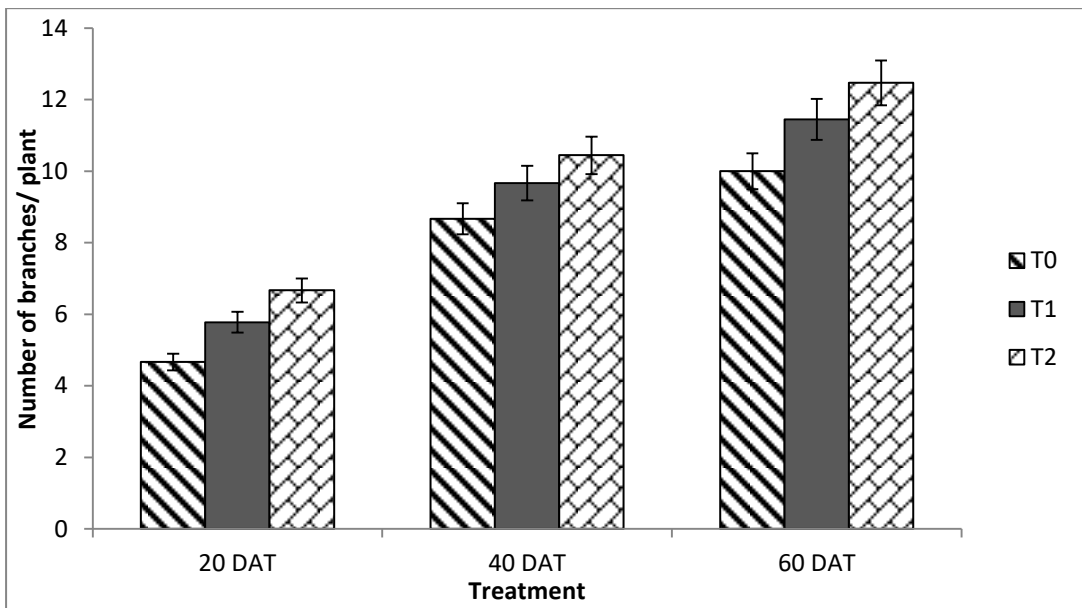
Branch number of tomato varieties exposed statistically significant inequality among different concentration of application of *Trichoderma*. The number was significantly affected by *Trichoderma* application (Appendix IV). In terms of *Trichoderma* application highest number of branches was found at T<sub>2</sub> (12.7) and lowest number of branches was found at T<sub>0</sub> (10.0) (Fig. 5).

This might be due to the increase in the height of plant due to *Trichoderma*. *Trichoderma* act indirectly in plant growth factors and the environmental conditions including nutrient availability, pH, temperature and iron concentration thus activation of specific compounds and metabolites such as pathogenesis related lytic enzymes, siderophores, antibiotics, and carbon and nitrogen permeases (Harsukh Gajera *et al.*, 2013; Kabdwal *et al.*, 2019). Abd-El-Khair *et al.* (2010) also found the average number of branches per plant as a result of application of *Trichoderma* spp. in the range of 5.0-6.3 branch/plant, compared to 3.7 branch/ plant in control treatment and this is similar to the experimental result.

Branch number of tomato influenced significantly among the combinations of tomato varieties and foliar application of *Trichoderma*. In combined effect highest number of branches were found at V<sub>1</sub>T<sub>2</sub> (12.7) treatment which was statistically similar with V<sub>3</sub>T<sub>2</sub>, V<sub>2</sub>T<sub>2</sub> and V<sub>3</sub>T<sub>1</sub> treatments respectively and lowest at V<sub>1</sub>T<sub>0</sub> (9.7). (Table. 4)



**Fig.6.** Performance of three tomato varieties on number of branches at different days after transplanting;  
 i.e. V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>= Apple Netherland



**Fig.7.** Effect of foliar application of *Trichoderma harzianum* on number of branches at different days after transplanting;  
 i.e. T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

**Table 4.** Combined effect of varieties and foliar application of *Trichoderma* on number of branches at different days after transplanting of tomato

<sup>x</sup> Combinations	Number of branches (cm)		
	20 DAT	40 DAT	60 DAT
V <sub>1</sub> T <sub>0</sub>	4.3 g	8.3 g	9.7d
V <sub>1</sub> T <sub>1</sub>	5.3 de	9.3 de	10.7 c b
V <sub>1</sub> T <sub>2</sub>	7.0 a	11.0 a	12.7 a
V <sub>2</sub> T <sub>0</sub>	5.0 ef	9.0 ef	10.3 cd
V <sub>2</sub> T <sub>1</sub>	6.3 b	10.0 bc	11.7 b
V <sub>2</sub> T <sub>2</sub>	7.0 a	10.3 b	12.3 ab
V <sub>3</sub> T <sub>0</sub>	4.7 fg	8.7 fg	10.0 cd
V <sub>3</sub> T <sub>1</sub>	5.7 cd	9.7 cd	12.0 ab
V <sub>3</sub> T <sub>2</sub>	6.0 bc	10.0 bc	12.4ab
LSD <sub>(0.05)</sub>	0.28	0.28	0.42
CV (%)	6.08	3.62	4.60

<sup>x</sup> Here, V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>=Apple Netherland

T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

#### 4.4 Chlorophyll (%)

Chlorophyll influences the growth of a plant which is correlated with the yield. Significant variation was observed for SPAD values due to the application of different concentration *Trichoderma* in three tomato varieties at growth stage.

Chlorophyll content of leaves (SPAD reading) was also significantly affected by three tomato varieties and showed significant inequality among the tomato varieties; V<sub>1</sub>, V<sub>2</sub> and V<sub>3</sub> at 60 days after transplanting of tomato. The Highest chlorophyll content was found from V<sub>1</sub> (40.47%) which is statistically identical with V<sub>3</sub> (40.28%). The lowest percentage of chlorophyll was found from V<sub>2</sub> (37.37%).

Significant variation was observed in case of chlorophyll percentage of leaves due to various *Trichoderma* concentration solutions (Appendix V). Maximum chlorophyll percentage (41.378) was found under T<sub>2</sub> and minimum (37.344) was observed in (T<sub>0</sub>)

Chlorophyll content increased in seedling grown in *Trichoderma* sp Azarmi *et al.* (2011). *Trichoderma* overcomes the effect of decrease in photosynthesis, chlorophyll content and nutrient content by pathogenic attack and a remarkable increase in the plant phosphorus and nitrogen content was recorded by Tanwar *et al.* (2013), Singh *et al.* (2013), Singh *et al.* (2014) and Alexandru *et al.* (2013) Khan *et al.* (2017) Patal S. and Saraf M. (2017) and Azami *et al.* 2011

In case of combination treatment significant variation was observed (Appendix IV). Maximum chlorophyll content of leaves (43.10%) was found in V<sub>1</sub>T<sub>2</sub> and minimum chlorophyll content of leaves (35.77 %) was found from (V<sub>2</sub>T<sub>0</sub>).

#### **4.5. Days to first flowering (Visual observation)**

Significant variation was received among the varieties in respect of days to flowering after transplantation of tomato varieties (Appendix V). Longest period was required for flowering in variety V<sub>1</sub> (41.6 days) while shortest period was in V<sub>2</sub> (36.8 days) (Table 5). The result showed that V<sub>2</sub> is early flowering variety and V<sub>1</sub> is late flowering variety.

Days to flowering were significantly affected by foliar application of *Trichoderma* on tomato (Appendix V). There was significant variation among the three treatments, control (T<sub>0</sub>), 10<sup>6</sup> concentrated *Trichoderma* (T<sub>1</sub>), 10<sup>7</sup> concentrated *Trichoderma* (T<sub>2</sub>). Early flowering was recorded in T<sub>2</sub> (38.2) and delayed in control T<sub>0</sub> (41.9) (Table 6).

*Trichoderma* might be helpful for the antagonists for its faster multiplication and prevention of many pathogenic diseases. Chet (1987) reported induction early flowering when *Trichoderma* spp. was used for disease management in vegetable crops. Uddin *et al.* (2015) found induction of early flowering when *Trichoderma* spp. was used for disease management in vegetable crops. Dutta *et al.*, 2002; Chet (1987) reported increased germination, leaf area, and dry weight of root and shoots and induction early flowering when *Trichoderma* spp. was used for disease management in vegetable crops.

Combination of different varieties and application of *Trichoderma* at different levels effects on days taken for flowering (Appendix V). V<sub>2</sub>T<sub>2</sub> (35.8) required minimum period for flower bud initiation whereas maximum from V<sub>1</sub>T<sub>0</sub> (43.0) (Table. 7)

#### **4.6. Number of cluster plant<sup>-1</sup>**

The observations on number of cluster per plant in tomato as influenced by three varieties of tomato are given in Table 5 and Appendix V. The number of flower cluster varied from a low of 6.2 in V<sub>2</sub> to as high as of 7.6 in V<sub>1</sub>.

Significant variation was found in terms of different *Trichoderma* concentrations (Appendix V). Cluster number per plant show statistically significant inequality among T<sub>0</sub>, T<sub>1</sub>, and T<sub>2</sub>. Maximum number of cluster was found at T<sub>2</sub> (7.4) and lowest at control, T<sub>0</sub> (5.9) treatment (Table 6).

Number of clusters per plant (7.33) was founded by Khan *et al.* (2012). According to Haque *et al.* (2012) flower number almost remains same when only *Trichoderma* is applied and when *Trichoderma* +Nitrogen are applied.

In case of combination treatment the number of cluster per plant varied significantly. The highest number of cluster was found from V<sub>1</sub>T<sub>2</sub> (7.8) combination at 60 days after transplanting of tomato varieties and minimum was found from V<sub>3</sub>T<sub>0</sub> (5.7) which is statistically almost similar with V<sub>1</sub>T<sub>0</sub> (6.0) and V<sub>2</sub>T<sub>0</sub> (6.0) (Table 7).

#### **4.7. Number of Flower Cluster<sup>-1</sup>**

The significant difference was observed due to different tomato varieties (Appendix V). The maximum number of flower per cluster (8.9) was found from V<sub>1</sub> (Roma VF) and followed by (8.7) V<sub>3</sub> (Apple Netherland). On the other hand, minimum number of flower cluster<sup>-1</sup> (7.4) was recorded from V<sub>2</sub> (BARI Tomato-2) (Table. 5).

In case of application of different concentration of *Trichoderma* significant difference was found (Appendix V). The maximum number of flower per cluster (8.6) was found from T<sub>2</sub> (10<sup>7</sup>) and followed by (7.9) T<sub>1</sub> (10<sup>6</sup>). On the other hand, minimum number of flower cluster<sup>-1</sup> (7.5) was recorded from control, T<sub>0</sub>. (Table. 6)

Numerous hypothesis have been proposed to explain this observation including the improvement of chemical solubilisation, sequester, availability (i.e. siderophores production) (WZ *et al.*, 2013; Vinale *et al.* 2009,; Vinale *et al.* 2013) and nutrient uptake by the plant (Altomare *et al.*, 1999). The result was similar with Woo *et al.* (2014).

The significant difference was observed due to interaction effect of different tomato varieties and *Trichoderma* application. The maximum number of flower per cluster (8.9) was found from V<sub>1</sub>T<sub>2</sub> and followed by (8.8) V<sub>3</sub>T<sub>2</sub>. On the other hand, minimum number of flower cluster<sup>-1</sup> (6.7) was recorded from V<sub>2</sub>T<sub>0</sub> (Table. 7).

#### **4.8. Number of flower per plant**

In case of different tomato varieties the number of flower per plant varied significantly (Appendix V). Maximum number of flower (52.6) was found in V<sub>1</sub> (Roma VF variety) and minimum number of flower (47.9) was found in V<sub>2</sub> (Table 5 and Appendix V). Similar results were reported by Sk. Rahul *et al.* (2017). significant difference among the tomato varieties in case of the number of flowers per plant.

In case of different *Trichoderma* concentration the number of flower per plant varied significantly (Appendix V). Maximum number of flower (54.2) was found in T<sub>2</sub> condition and minimum number of flower (46.1) were found in control (T<sub>0</sub>) treatment (Table 6).

This experiment showed higher no of flowers over the control plot which is similar to the result of (Naznin *et al.*, 2015 and Abedin *et al.*, 2018).



In case of combined treatment the number of flowers per plant varied significantly (Appendix V). Maximum number of flowers (58.3) was found in  $V_1T_2$  treatment combination and minimum number of flowers (45.0) was found in  $V_2T_0$  (Table 7).

**Table 5.** Performance of three tomato varieties on days to 1st flowering number of cluster/plant, number of flower/cluster, number of flower/plant

<sup>x</sup> Varieties	Days to 1st flowering	Number of cluster/plant	Number of flower /cluster	Number of flower/plant
V <sub>1</sub>	41.6 a	7.6 a	8.9 a	52.6 a
V <sub>2</sub>	36.8 c	6.2 b	7.4 b	47.9 c
V <sub>3</sub>	40.7 b	6.9 a	8.8 a	50.1 b
LSD <sub>(0.05)</sub>	0.28	0.19	0.18	0.25
CV (%)	1.30	5.97	4.47	0.99

<sup>x</sup>three varieties coded from V<sub>1</sub> to V<sub>3</sub>, V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>= Apple Netherland

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

**Table 6.** Influence of different *Trichoderma harzianum* concentration on days to 1st flowering number of cluster/plant, number of flower/cluster, number of flower/plant

<i>Trichoderma</i> application	Days to 1st flowering	Number of cluster/plant	Number of flower /cluster	Number of flower/plant
T <sub>0</sub>	41.9 a	5.9 c	7.5 c	46.1 c
T <sub>1</sub>	39.1 b	6.9 b	7.9 b	50.2 b
T <sub>2</sub>	38.2 c	7.4 a	8.6 a	54.2 a
LSD <sub>(0.05)</sub>	0.28	0.19	0.18	0.25
CV (%)	1.30	5.97	4.47	0.99

T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

**Table 7.** Combined effect of varieties and *Trichoderma harzianum* spore concentration on days to 1st flowering number of cluster/plant, number of flower/cluster, number of flower/plant

<sup>X</sup> Combinations	Days to 1st flowering <sup>Y</sup>	Number of cluster/plant <sup>Y</sup>	Number of flower /cluster <sup>Y</sup>	Number of flower/plant <sup>Y</sup>
V <sub>1</sub> T <sub>0</sub>	43.0 a	6.0 cd	7.8 bc	47.0f
V <sub>1</sub> T <sub>1</sub>	41.3 b	7.2 ab	8.3 b	52.3 c
V <sub>1</sub> T <sub>2</sub>	40.3 c	7.8 a	8.9 a	58.3 a
V <sub>2</sub> T <sub>0</sub>	38.3 d	6.0 cd	6.7 d	45.0 g
V <sub>2</sub> T <sub>1</sub>	36.3 e	7.6 bc	7.7 c	48.0 e
V <sub>2</sub> T <sub>2</sub>	35.8 f	7.7 ab	7.9 b	50.7 d
V <sub>3</sub> T <sub>0</sub>	42.3 a	5.7 d	7.7 c	46.3 f
V <sub>3</sub> T <sub>1</sub>	39.7 c	6.7 cd	8.7 ab	50.3 d
V <sub>3</sub> T <sub>2</sub>	38.5 d	6.8 bc	8.8 ab	53.7 b
LSD <sub>(0.05)</sub>	0.45	0.33	0.29	0.45
CV (%)	1.30	5.97	4.47	0.99

<sup>X</sup>Here, V<sub>1</sub>= Roma -VF, V<sub>2</sub> = BARI Tomato-2, V<sub>3</sub>= Apple Netherland. T<sub>0</sub>= Control, T<sub>1</sub>=*Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>=*Trichoderma harzianum* spore concentration 10<sup>7</sup>

<sup>Y</sup> In a column, means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

#### 4.9. Days to first fruit setting

There was significant difference among the different treatments of *Trichoderma* on different varieties of tomato in case of fruit setting. Most protracted period (54.9) days was noticed in V<sub>3</sub> whereas most concise period (45.9) days was required from V<sub>2</sub> (Table 8). The results indicate that V<sub>2</sub> was early fruiting variety and V<sub>3</sub> was late one. Early fruiting is required to increase cropping intensity (Shiam I. H., 2013).

Days to first fruit setting was significantly affected by foliar application different concentration of *Trichoderma* solution (Appendix VI). Early fruit setting was recorded in T<sub>2</sub> (47.6) and late fruiting was recorded in T<sub>0</sub> (52.6) (Table 9).

Gravel *et al.* (2007) also mentioned the performance of *Trichoderma* for early and developed fruit setting. The experimental result also supports the findings of Datnoff *et al.* (1995).

The combined effect of tomato varieties and concentration of *Trichoderma* spores on first fruit setting was significant (Appendix VI). Longest period (56.7) was found for fruiting in V<sub>1</sub>T<sub>0</sub> whereas shortest period (43.4) was recorded from V<sub>2</sub>T<sub>2</sub> (Table 10). It indicates that V<sub>2</sub>T<sub>2</sub> combination is superior for days to first fruit initiation whereas V<sub>1</sub>T<sub>0</sub> is inferior in this regard.

#### **4.10. Number of fruit cluster<sup>-1</sup>**

Significant variation was found among the varieties of tomato in terms of number of fruit per cluster (Appendix VI). Number of fruit per cluster exposed statistically significant variation among the three tomato varieties V<sub>1</sub>, V<sub>2</sub> and V<sub>3</sub>.

Number of fruits per cluster differed significantly due to application of *Trichoderma* at 15 days interval. From the table it was found that the maximum number of fruits per cluster was recorded from variety V<sub>1</sub> (6.8) and lowest was found from variety V<sub>2</sub> (5.4) (Table 8).

Fruit number per cluster was significantly affected by the application of different *Trichoderma* concentration (Appendix VI). Fruit number of tomato exposed significant inequality among T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> treatment. Maximum number of fruits per cluster was recorded in T<sub>2</sub> (6.8) which is statistically similar with T<sub>1</sub> (6.7) and lowest was recorded from T<sub>0</sub> (5.9) (Table. 9). Haque *et al.* (2012) mentioned in case of tomato *Trichoderma* alone can increase the fruit number and fruit size. Gravel *et al.* (2007) and Montealegre *et al.* (2010) had shown similar result.

Combined effect of three tomato varieties and treatments in terms of number of fruits per cluster also exposed significant variation (Appendix VI). Number of fruit per cluster of different tomato varieties exposed significant inequality among different treatments. Highest number of fruit per cluster was recorded in

V<sub>1</sub>T<sub>2</sub> (7.4) which is statistically similar with V<sub>3</sub>T<sub>2</sub> (7.3) and lowest was recorded in V<sub>2</sub>T<sub>0</sub> (5.0) (Table. 10).

#### **4.11. Number of fruit plant<sup>-1</sup>**

In case of three different tomato varieties, number of fruit/plant varied significantly (Appendix VI). Maximum number of fruits per plant (37.9) were found in V<sub>1</sub> (Rom VF) and V<sub>2</sub> produced minimum number of fruits (35.6) (Table. 8)

In case of different *Trichoderma* spore concentration the number of fruits per plant varied significantly (Appendix VI). Maximum number of fruits per plant (37.8) was found in T<sub>2</sub> and minimum number of fruits per plant (35.0) was found in T<sub>0</sub> (Table. 9).

It was reported that application of *Trichoderma* spp dramatically increased the number of fruits per plant in pepper and tomato grown in greenhouse than untreated control (Vinale *et al.*, 2008).

In case of combination treatment the number of fruits per plant varied significantly (Appendix VI). Maximum number of fruits per plant (39.0) was found in V<sub>1</sub>T<sub>2</sub> and minimum number of fruit per plant (33.7) were found in V<sub>2</sub>T<sub>0</sub> (Table 11).

#### **4.12. Days to first fruit maturity**

Significant variation was found among the varieties in respect of days to first fruit maturity (Appendix VI). Most prolonged period was required for first fruit maturity in V<sub>3</sub> (68.9) and shortest period in V<sub>2</sub> (57.1) (Table 8). The results indicate that V<sub>2</sub> is early fruit maturity variety whereas V<sub>3</sub> is late one.

Days to fruit maturity were significantly affected by different *Trichoderma* spore concentration at different stages. Early fruit maturity was recorded in T<sub>2</sub> (46.1) and delayed in control T<sub>0</sub> (54.2) (Table. 9).

Combination of tomato varieties and foliar application of *Trichoderma* at different levels affect significantly on days taken to first fruit maturity

(Appendix VI). Earlier fruit maturity was observed in V<sub>2</sub>T<sub>3</sub> (56.0) and delayed fruit maturity was observed in V<sub>3</sub>T<sub>0</sub> (71.3) (Table 10).

This might be due to the enhancement of photosynthesis, deposition of photo assimilates, translocation of carbohydrates, improvement in physiological and other metabolic activity which led to an increase in various plant metabolites responsible for actively cell division and elongation results improvement in growth characters (Hatwar *et al.*, 2003).

**Table 8.** Performance of three tomato varieties on Days to first fruit setting, number of fruit cluster, number of fruit plant<sup>-1</sup>, Days to first fruit maturity

<sup>x</sup> Varieties	Days to first fruit setting	Number of fruit cluster <sup>-1</sup>	Number of fruit plant <sup>-1</sup>	Days to first fruit maturity
V <sub>1</sub>	48.8 b	6.4 b	37.9 a	63.3 b
V <sub>2</sub>	45.9 c	5.4 c	35.6 c	57.1 c
V <sub>3</sub>	54.9 a	6.8 a	36.8 b	68.9 a
LSD <sub>(0.05)</sub>	0.22	0.29	0.14	0.18
CV (%)	0.94	8.66	0.83	0.62

<sup>x</sup>three varieties coded from V<sub>1</sub> to V<sub>3</sub>, V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>= Apple Netherland

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

**Table 9.** Influence of different *Trichoderma harzianum* concentration on Days to first fruit setting, number of fruit cluster, number of fruit plant<sup>-1</sup>, Days to first fruit maturity

<i>Trichoderma</i> application	Days to first fruit setting	Number of fruit cluster <sup>-1</sup>	Number of fruit plant <sup>-1</sup>	Days to first fruit maturity
T <sub>0</sub>	52.6 a	5.9 b	35.0 c	54.2 a
T <sub>1</sub>	49.7 b	6.7 ab	36.6 b	50.2 b
T <sub>2</sub>	47.6 c	6.8 a	37.8 a	46.1 c
LSD <sub>(0.05)</sub>	0.22	0.29	0.14	0.18
CV (%)	0.94	8.66	0.83	0.62

T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

**Table 10.** Combined effect of varieties and *Trichoderma harzianum* spore concentration on Days to first fruit setting, number of fruit cluster, number of fruit plant<sup>-1</sup>, Days to first fruit maturity

<sup>X</sup> Combinations	Days to first fruit setting <sup>Y</sup>	Number of fruit cluster <sup>-1Y</sup>	Number of fruit plant <sup>-1 Y</sup>	Days to first fruit maturity <sup>Y</sup>
V <sub>1</sub> T <sub>0</sub>	56.7 a	6.5 abc	36.7 c	65.0 d
V <sub>1</sub> T <sub>1</sub>	54.7 b	6.9 ab	38.1 b	68.5 b
V <sub>1</sub> T <sub>2</sub>	53.3 c	7.4 ab	39.0 a	66.8 c
V <sub>2</sub> T <sub>0</sub>	48.2 e	5.0 d	33.7 f	58.3 g
V <sub>2</sub> T <sub>1</sub>	46.3 f	5.4 d	35.0 e	57.0 h
V <sub>2</sub> T <sub>2</sub>	43.4 g	5.8 cd	36.7 c	56.0 i
V <sub>3</sub> T <sub>0</sub>	52.2 d	6.7 bc	36.0 d	71.3 a
V <sub>3</sub> T <sub>1</sub>	48.7 e	6.7 abc	36.8 c	63.0 e
V <sub>3</sub> T <sub>2</sub>	46.3 f	7.3 a	37.8 b	62.0 f
LSD <sub>(0.05)</sub>	0.39	0.45	0.28	0.32
CV (%)	0.94	8.66	0.83	0.62

<sup>X</sup> Here, V<sub>1</sub>= Roma -VF, V<sub>2</sub> = BARI Tomato-2, V<sub>3</sub>= Apple Netherland. T<sub>0</sub>= Control, T<sub>1</sub>=*Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>=*Trichoderma harzianum* spore concentration 10<sup>7</sup>

<sup>Y</sup> In a column, means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

#### 4.13. Fruit length (cm)

Significant difference was revealed on fruit length with different tomato varieties (Appendix VI). Among the varieties of tomato V<sub>1</sub> (Roma VF) gave the longest fruit (6.3 cm) while V<sub>2</sub> gave the shortest fruit (4.5 cm) length which is statistically dissimilar with V<sub>3</sub> (5.5 cm) (Table 11).

Significant variation was found for fruit length in case of different *Trichoderma* spore concentration (Appendix VI). Maximum fruit length (9.7cm) was observed under T<sub>2</sub> condition and minimum fruit length (5.3cm) was observed in T<sub>0</sub>. (Table. 12)

Significant variation was found for fruit length in case of combined effect (Appendix VI). Maximum fruit length (6.7 cm) was found in V<sub>1</sub>T<sub>2</sub> and minimum fruit length (5.0 cm) was found in V<sub>2</sub>T<sub>0</sub> (Table 13).

#### **4.14. Fruit Diameter (mm)**

The difference in varieties for fruit diameter was found significant (Appendix VII). Where, maximum fruit diameter was recorded (57.9 mm) in  $V_3$  and the smallest diameter of fruit was (36.7 mm) in  $V_1$  (Table. 11).

The growing conditions *viz.*, different *Trichoderma* spore concentration significantly influenced the fruit diameter in tomato (Appendix VII). Where, maximum fruit diameter (53.6 mm) found in  $T_2$  and minimum fruit diameter (45.8 mm) found in  $T_0$  (Table 12).

The interaction between varieties and *Trichoderma* spore concentration was also significant, which indicated that the influence of *Trichoderma* spore concentration on fruit diameter of different tomato varieties (Appendix VII). Maximum fruit diameter (60.8 mm) found in  $V_3T_2$  and minimum fruit diameter (33.7) found in  $V_1T_0$ .

#### **4.15. Pericarp thickness (mm)**

Significant variation was observed for pericarp thickness among three tomato varieties  $V_1$ ,  $V_2$  and  $V_3$  (Appendix VII). Maximum thickness was recorded from  $V_3$  (8.1 mm) and minimum was recorded from  $V_2$  (6.8 mm) (Table. 11).

Pericarp thickness was significantly affected by the foliar application of *Trichoderma* after transplanting of tomato. Pericarp thickness exposed significant inequality among  $T_0$ ,  $T_1$  and  $T_2$  treatment. Maximum thickness was recorded in  $T_2$  (7.7 mm) and lowest was recorded from  $T_0$  (6.2 mm) (Table. 12).

Combined effect of three tomato varieties and treatments in terms of pericarp thickness also exposed significant variation (Appendix VII). Pericarp thickness was recorded maximum in  $V_1T_2$  (8.3 mm) which is statistically identical with  $V_1T_1$  (8.1 mm) and minimum was recorded in  $V_2T_0$  (6.5 mm) (Table. 13).

#### **4.16. Single fruit weight (g)**

Significant variation was among the varieties of tomato in terms of single fruit weight/plant (Appendix VII). V<sub>3</sub> (94.2 g) tomato variety exposed highest single fruit weight per plant while minimum was obtained from V<sub>1</sub> (71.1 g) (Table. 11).

Single fruit weight per plant was significantly affected by the foliar application of *Trichoderma* (Appendix VII). Single fruit weight per plant exposed significant inequality among T<sub>0</sub>, T<sub>1</sub>, and T<sub>2</sub> treatment. Maximum single fruit weight was recorded in T<sub>2</sub> (84.5 g) and lowest was recorded from T<sub>0</sub> (82.9 g) (Table. 12).

Combined effect of three tomato varieties and treatments in terms of single fruit weight per plant also exposed significant variation (Appendix VII). Single fruit weight per plant of different tomato lines exposed significant inequality among different treatments. Single fruit weight per plant was recorded maximum in V<sub>3</sub>T<sub>2</sub> (94.7 g) which is statistically identical with V<sub>3</sub>T<sub>1</sub> (94.1 g) and lowest was recorded in V<sub>1</sub>T<sub>0</sub> (70.7 g) (Table. 13).

#### **4.17. Yield plant<sup>-1</sup> (kg)**

Yield per plant was significantly affected by tomato variety. Yield per plant of tomato exposed significant inequality among V<sub>1</sub>, V<sub>2</sub> and V<sub>3</sub> (Appendix VII). Maximum yield per plant was recorded from V<sub>1</sub> (2.9 kg) and minimum was recorded from V<sub>2</sub> (2.6 kg) (Table 11).

Yield per plant was significantly affected by the foliar application of *Trichoderma*. Yield per plant exposed significant inequality among T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> (Appendix VII). Maximum yield per plant was recorded in T<sub>2</sub> (2.9 kg) and lowest was recorded from T<sub>0</sub> (2.7 kg) (Table 12).



The beneficial effect of *Trichoderma harzianum* on tomato was reported to be modulated by plant genotype (Tucci *et al.* 2010). Thus the efficiency *Trichoderma* may depend on variety of tomato and concentration of spore of *Trichoderma* used in this experiment. Abedin *et al.* 2018 and Abd-El-Khair *et al.* 2010 observed similar result.

Combined effect of three tomato varieties and treatments in terms of yield per plant per plant also exposed significant variation (Appendix VII). Yield per plant was recorded maximum in V<sub>1</sub>T<sub>2</sub> (3.0 kg) which is statistically followed by V<sub>1</sub>T<sub>1</sub> (2.9 kg) and lowest was recorded in V<sub>2</sub>T<sub>0</sub> (2.5 kg) (Table 13).

#### **4.18. Yield hectare<sup>-1</sup> (t)**

The yield per hectare significantly influenced by three tomato varieties V<sub>1</sub>, V<sub>2</sub> and V<sub>3</sub> (Appendix VII). Maximum yield per hectare was recorded from V<sub>1</sub> (90.3 t) and minimum was recorded from V<sub>2</sub> (85.7 t) (Table. 11)

Yield per hectare was significantly affected by the foliar application of *Trichoderma*. Yield per plant exposed significant inequality among T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> (Appendix VII). Maximum yield per hectare was recorded in T<sub>2</sub> (89.8 t) and lowest was recorded from T<sub>0</sub> (86.7 t) (Table. 12).

Srivastava *et al.* (2006) reported highest yield of tomato 81.9 t/ha with *Trichoderma* application. Haque *et al.* (2012) and Sundaramoorthy (2013) reported the similar result when *Trichoderma* is applied. Molla *et al.* (2012), Sivan *et al.* (1993) and Datnoff *et al.* (1995) also supported the results in field condition. Gravel *et al.* (2007) and Bal *et al.* (2006) studied on tomato and *Trichoderma* in green house and recorded the increased yield of tomato. *T. harzianum* increased total yield and marketable yield of tomato Nzanza *et al.*, (2012) and Wokocha (1990). 336.5 % yield increase was recorded over control Molla *et al.* (2012). Abd-El-Khair *et al.* (2010) supported the result. Sivan *et al.* (1993) mentioned the highest yield improvement (105%) over control by *Trichoderma*.

Combined effect of three tomato varieties and treatments in terms of yield per hectare also exposed significant variation (Appendix VII). Yield per hectare was recorded maximum in  $V_1T_2$  (91.5 t) and lowest was recorded in  $V_2T_0$  (84.7 t) (Table.13).

**Table 11:** Performance of three tomato varieties on Fruit length, Fruit Diameter, Pericarp thickness, Single fruit weight, Yield per plant, Yield per hectare

<sup>x</sup> Varieties	Fruit length (cm)	Fruit Diameter (mm)	Pericarp thickness (mm)	Single fruit weight (g)	Yield plant <sup>-1</sup> (kg)	Yield hectare <sup>-1</sup> (t)
V <sub>1</sub>	6.3 a	36.7 c	7.8 b	71.1 c	2.9 a	90.3 a
V <sub>2</sub>	4.5 c	54.3 b	6.8 c	86.7 b	2.6 c	85.7 c
V <sub>3</sub>	5.5 b	57.9 a	8.1 a	94.2 a	2.8 b	87.9 b
LSD <sub>(0.05)</sub>	0.16	0.29	0.18	0.16	0.19	0.86
CV (%)	0.61	1.00	5.13	0.39	1.43	0.20

<sup>x</sup>three varieties coded from V<sub>1</sub> to V<sub>3</sub>, V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>= Apple Netherland

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

**Table 12.** Influence of different *Trichoderma harzianum* concentration on Fruit length, Fruit Diameter, Pericarp thickness, Single fruit weight, Yield per plant, Yield per hectare

<i>Trichoderma</i> application	Fruit length (mm)	Fruit Diameter (mm)	Pericarp thickness (mm)	Single fruit weight (g)	Yield plant <sup>-1</sup> (kg)	Yield hectare <sup>-1</sup> (t)
T <sub>0</sub>	5.3 c	45.8 c	6.2 b	82.9 c	2.7 c	86.7 c
T <sub>1</sub>	7.3 b	50.6 b	7.6 ab	83.7 b	2.8 b	87.8 b
T <sub>2</sub>	9.7 a	53.6 a	7.7 a	84.5 a	2.9 a	89.8 a
LSD <sub>(0.05)</sub>	0.16	0.23	0.18	0.16	0.19	0.86
CV (%)	0.61	1.00	5.13	0.39	1.43	0.20

T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

**Table 13.** Combined effect of varieties and *Trichoderma harzianum* spore concentration on Fruit length, Fruit Diameter, Pericarp thickness, Single fruit weight, Yield per plant, Yield per hectare

Combinations <sup>x</sup>	Fruit length <sup>y</sup> (cm)	Fruit Diameter <sup>y</sup> (mm)	Pericarp thickness <sup>y</sup> (mm)	Single fruit weight <sup>y</sup> (g)	Yield plant <sup>-1y</sup> (kg)	Yield hectare <sup>-1y</sup> (t)
V <sub>1</sub> T <sub>0</sub>	5.7 c	33.7 g	7.6 bc	70.7 g	2.8 bc	89.7 c
V <sub>1</sub> T <sub>1</sub>	6.0 b	37.6 f	8.1 a	70.9 g	2.9 b	90.2 b
V <sub>1</sub> T <sub>2</sub>	6.7 a	39.6 e	8.3 a	71.5 f	3.0 a	91.5 a
V <sub>2</sub> T <sub>0</sub>	5.0 f	49.9 d	6.5 e	85.0 e	2.5 e	84.7 i
V <sub>2</sub> T <sub>1</sub>	5.3 e	54.7 c	6.7 de	86.3 d	2.7 d	85.6 h
V <sub>2</sub> T <sub>2</sub>	5.4 de	59.0 b	7.3 cde	87.7 c	2.7 cd	86.8 f
V <sub>3</sub> T <sub>0</sub>	5.1 f	54.7 c	7.1 bcd	93.3 b	2.8 c	86.2 g
V <sub>3</sub> T <sub>1</sub>	5.5 d	58.5 b	7.7 abc	94.1 a	2.8 bc	87.9 e
V <sub>3</sub> T <sub>2</sub>	5.7 c	60.8 a	7.8 ab	94.7 a	2.9 b	88.9 d
LSD <sub>(0.05)</sub>	0.29	0.45	0.33	0.27	0.39	0.19
CV (%)	0.61	1.00	5.13	0.39	1.43	0.20

<sup>x</sup> Here, V<sub>1</sub>= Roma -VF, V<sub>2</sub>= BARI Tomato-2, V<sub>3</sub>= Apple Netherland

T<sub>0</sub>= Control, T<sub>1</sub>= *Trichoderma harzianum* spore concentration 10<sup>6</sup>, T<sub>2</sub>= *Trichoderma harzianum* spore concentration 10<sup>7</sup>

<sup>y</sup> In a column, means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

## CHAPTER V

### SUMMARY 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 March 2018 to study the bio efficacy of *Trichoderma harzianum* spore concentrations on growth and yield of tomato. The double factor experiment consisted of  $V_1$ = Roma- VF,  $V_2$ = BARI Tomato 2,  $V_3$ = Apple Netherland and  $T_0$ = Control (No Trichoderma),  $T_1$ =  $10^6$  spores/ ml,  $T_2$ =  $10^7$  spores/ ml *Trichoderma*. The experiment was laid out in Completely Randomized Design (CRD) with four replications. Data on growth and yield parameter were recorded in field.

In case of plant height, among the three varieties of tomato plant the tallest plant was found from  $V_1$  (111.2 cm) and the shortest from  $V_2$  (86.3 cm) at 60 days after transplanting. Observing the effect of *Trichoderma* spore concentration on plant height the highest plant was found from  $T_2$  (110.6 cm) and the shortest from  $T_0$  (88.64 cm). In combined effect  $V_1$  produced the tallest plant (114.8 cm) under  $T_2$  condition ( $V_1T_2$ ) whereas the shortest plant (75.7 cm) was found in  $V_2T_0$  at 60 days after transplanting.

In counting leaves number more number of leaves found from  $V_1$  (66.1) and less number of leaves found from  $V_2$  (55.4) at 60 days after transplanting. When *Trichoderma* is applied among the varieties, the effect was superior on  $T_2$  (63.9) and the lowest in control  $T_0$  (58.4) at 60 days after transplanting. In combination the maximum number of leaves (69.0) was found from  $V_1T_2$  and the minimum number was found from  $V_2T_0$  (53.0) at 60 days after transplanting.

Monitoring the number of branches, the maximum branch number (11.7) was found from  $V_1$  at 60 days after transplanting and the minimum number of branches was found in  $V_2$  (11.0) at 60 days after transplanting. In case of

*Trichoderma* spore effect maximum number of branches found from T<sub>2</sub> (12.7) and minimum number of branches was found at T<sub>0</sub> (10.0). In amalgamation, the highest number of branches was found at V<sub>1</sub>T<sub>2</sub> (12.7) and the lowest at V<sub>1</sub>T<sub>0</sub> (9.7).

Observing the chlorophyll content the highest chlorophyll content was found from V<sub>1</sub> (40.8%) and the lowest percentage of chlorophyll was found from V<sub>2</sub> (37.7%). Maximum chlorophyll percentage (41.8) was found under T<sub>2</sub> and minimum (37.4) was observed in (T<sub>0</sub>) in case of *Trichoderma* effect. In combination outcome maximum chlorophyll content of leaves (43.1%) was found in V<sub>1</sub>T<sub>2</sub> and minimum chlorophyll content of leaves (35.7 %) was found from (V<sub>2</sub>T<sub>0</sub>).

Among three varieties of tomato, the longest period was required for flowering in variety V<sub>1</sub> (41.6 days) while the shortest period was in V<sub>2</sub> (36.8 days). In *Trichoderma* application early flowering was recorded in T<sub>2</sub> (38.2 days) and delayed in control T<sub>0</sub> (41.9 days). Combined effect showed the result that V<sub>2</sub>T<sub>2</sub> (35.8 days) required minimum period for flower bud initiation whereas the maximum from V<sub>1</sub>T<sub>0</sub> (43.0 days).

Considering the different variety of tomato plant, lower number of cluster (6.2) in V<sub>2</sub> and higher from (7.6) in V<sub>1</sub>. Inequality was found from *Trichoderma* spraying. Maximum number of cluster was found at T<sub>2</sub> (7.4) and lowest at control, T<sub>0</sub> (5.9). In combined effect highest number of cluster was found from V<sub>1</sub>T<sub>2</sub> (7.8) and minimum was found from V<sub>3</sub>T<sub>0</sub> (5.7).

Among the tomato varieties, maximum number of flower per cluster (8.9) was found from V<sub>1</sub> and minimum number of flower per cluster (7.4) was recorded from V<sub>2</sub>. In case of application of different concentration of *Trichoderma* maximum number of flower per cluster (8.6) was found from T<sub>2</sub> and minimum number of flower cluster<sup>-1</sup> (7.5) was recorded from T<sub>0</sub>. Interaction effect of different tomato varieties and *Trichoderma* application, the maximum number

of flower per cluster (8.9) was found from  $V_1T_2$  and minimum number of flower per cluster (6.7) was recorded from  $V_2T_0$ .

Regarding different tomato variety, maximum number of flower (52.6) was found in  $V_1$  and minimum number of flower (47.9) was found in  $V_2$ . Different *Trichoderma* concentration gave significant result. Maximum number of flower (54.2) was found in  $T_2$  and minimum numbers of flower (46.1) were found in  $T_0$ . In case of combination treatment maximum number of flowers (58.3) was found in  $V_1T_2$  and minimum number of flowers (45.0) was found in  $V_2T_0$ .

In case of fruit setting, most prolonged period was required for first fruit maturity in  $V_3$  (68.9) and the shortest period in  $V_2$  (57.1). *Trichoderma* spore concentration showed early fruit maturity was recorded in  $T_2$  (46.1) and delayed in control  $T_0$  (54.2). Combined effect showed earlier fruit maturity in  $V_2T_2$  (56.0) and delayed fruit maturity in  $V_3T_0$  (71.3).

Among tomato varieties, the longest fruit were given from  $V_1$  (6.3 cm) and  $V_2$  gave the shortest fruit (4.5 cm). *Trichoderma* spraying gave maximum fruit length (9.7 cm) was observed under  $T_2$  and minimum fruit length (5.3 cm) was observed in  $T_0$ . In combination maximum fruit length (6.7cm) was found in  $V_1T_2$  and minimum fruit length (5.1cm) was found in  $V_3T_0$ .

Maximum fruit diameter was recorded 57.9mm in  $V_3$  and the smallest diameter of fruit was 36.7mm in  $V_1$  among tomato variety. In *Trichoderma* application maximum fruit diameter (53.6mm) found in  $T_2$  and minimum fruit diameter (45.8mm) found in  $T_0$ . In combined effect maximum fruit diameter (60.8mm) found in  $V_3T_2$  and minimum fruit diameter (33.7mm) found in  $V_1T_0$ .

Among the tomato varieties, premier pericarp thickness was recorded from  $V_1$  (8.1mm) and minimum was recorded from  $V_2$  (6.8 mm). Maximum thickness was recorded in  $T_2$  (7.7 mm) and lowest was recorded from  $T_0$  (6.2 mm) in *Trichoderma* application. Combined effect was recorded maximum in  $V_1T_2$  (8.3 mm) and minimum was recorded in  $V_2T_0$  (6.5 mm).

Tomato varieties, V<sub>3</sub> (94.2 g) exposed the highest single fruit weight per plant while minimum was obtained from V<sub>1</sub> (71.1 g). In *Trichoderma* application maximum single fruit weight was recorded in T<sub>2</sub> (84.5 g) and lowest was recorded from T<sub>0</sub> (82.9 g). In interaction of both factors maximum was recorded in V<sub>3</sub>T<sub>2</sub> (94.7 g) which is statistically lowest was recorded in V<sub>1</sub>T<sub>0</sub> (70.7 g).

In case of yield per plant maximum was recorded from V<sub>1</sub> (2.9 kg) and minimum from V<sub>2</sub> (2.7 kg). Maximum yield per plant was recorded in T<sub>2</sub> (2.9kg) and lowest was recorded from T<sub>0</sub> (2.7 kg) in *Trichoderma* application. Combined effect showed maximum in V<sub>1</sub>T<sub>2</sub> (3.0 kg) and minimum was recorded in V<sub>2</sub>T<sub>0</sub> (2.5 kg).

Regarding tomato varieties maximum yield per hectare was recorded from V<sub>1</sub> (90.3 t) and minimum was recorded from V<sub>2</sub> (85.7 t). In case of *Trichoderma* application maximum yield per hectare was recorded in T<sub>2</sub> (89.8 t) and lowest was recorded from T<sub>0</sub> (86.7 t). Combined effect showed maximum yield per hectare in V<sub>1</sub>T<sub>2</sub> (91.5 t) and lowest was recorded in V<sub>2</sub>T<sub>0</sub> (84.7 t).

Looking upon the above circumstances it can be easily enunciated that V<sub>1</sub> provided best result in terms of plant height, number of branches per plant, number of cluster per plant, number of flower per cluster, number of flower per plant, number of fruit per cluster, number of fruit per plant, fruit length, pericarp thickness, yield per plant, yield per hectare. V<sub>2</sub> exposed as early flowering and early fruiting variety and V<sub>3</sub> exposed as late one. In case of foliar application of *Trichoderma* T<sub>2</sub> provided best results in terms of all parameter and T<sub>0</sub> provide worst result in case of all parameter. In case of combinations, V<sub>1</sub>T<sub>2</sub> combination provided better performance in terms of plant height, number of branches per plant, number of cluster per plant, number of flower per cluster, number of flower per plant, number of fruit per cluster, number of fruit per plant, fruit length, pericarp thickness, yield per plant and yield per hectare over any other combinations.

## Conclusion

The present results revealed V<sub>1</sub>- Roma VF was the outstanding variety and T<sub>2</sub> (*Trichoderma* spore concentration 10<sup>7</sup>) was the most excellent foliar application for growth and yield attributes for tomato. Therefore, on the basis of the results it can be concluded that combination of Roma VF and *Trichoderma harzianum* considered as noble strategy for sustainable tomato production with higher yield and this is totally environment friendly.

## Suggestions

Further research in the subsequent areas may be suggested

- Further studies are needed to clarify the quality of tomato grown through application of *Trichoderma*.
- The soil quality may be tested to know the nutrient provided by *Trichoderma* to confirm whether the soil health enhanced by *Trichoderma*



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## APPENDICES

### Appendix I. Physio-chemical characteristics of the experimental soil

Physical characteristic

Textural class Silty clay loam to clay loam

Bulk density (g cm<sup>-3</sup>) 1.33 Particle density (g cm<sup>-3</sup>) 2.61 Porosity (%) 46.9

Chemical

<b>Appendix II. Analysis of variance on plant height at different days after transplanting of Tomato</b>				
Source of Variation	Degrees of freedom	Mean Square for plant height (cm)		
		20 DAT	40 DAT	60 DAT
Factor A (Tomato varieties)	2	847.888*	1531.03*	1552.48*
Factor B ( <i>Trichoderma</i> spore concentration)	2	382.263*	710.77*	1099.17*
Interaction (A×B)	4	16.029*	20.05*	12.61*
Error	16	0.409	1.05	1.35
*: Significant at 0.05 level of probability				

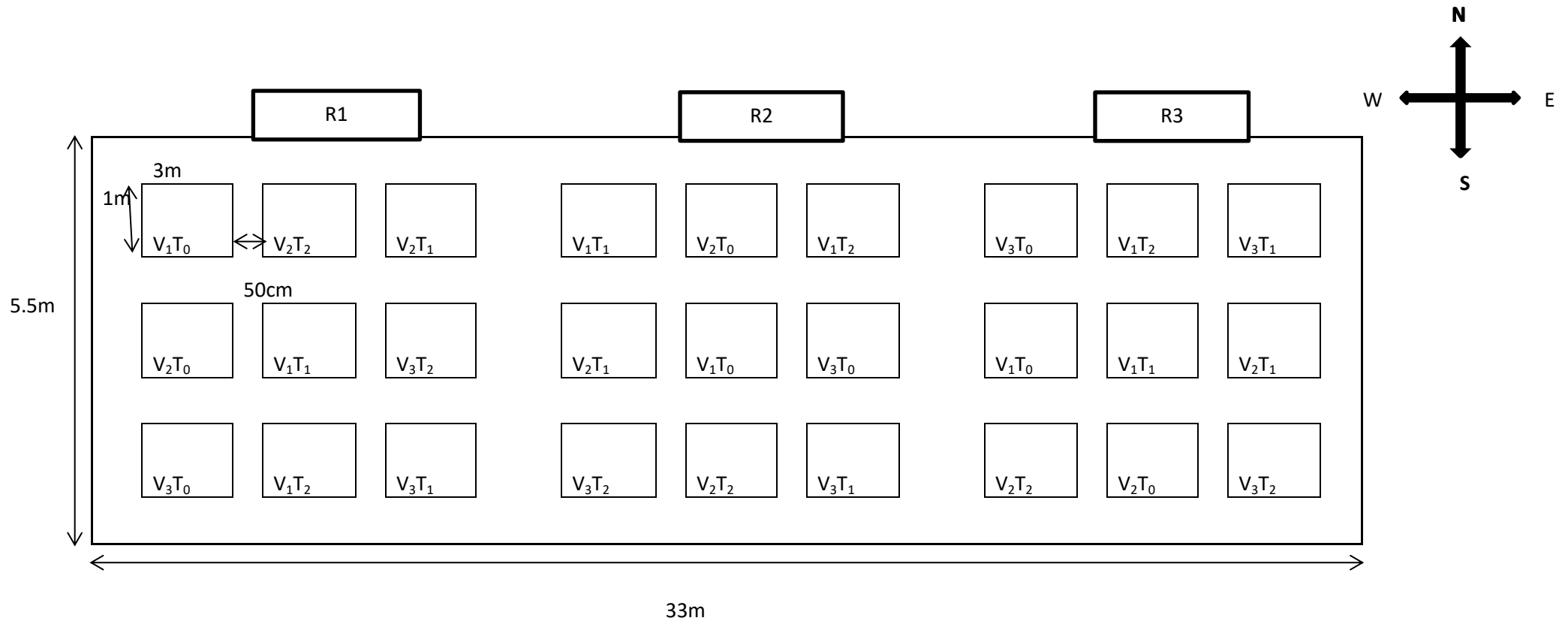
<b>Appendix III. Analysis of variance on leaf number of plant at different days after transplanting of Tomato</b>				
Source of Variation	Degrees of freedom	Mean Square for leaf number		
		20 DAT	40 DAT	60 DAT
Factor A (Tomato varieties)	2	6.4815*	9.9259*	259.00*
Factor B ( <i>Trichoderma</i> spore concentration)	2	6.4815*	46.7037*	66.778*
Interaction (A×B)	4	0.2037*	0.2037*	0.278*
Error	16	0.3981	0.3009	0.194
*: Significant at 0.05 level of probability				

<b>Appendix IV. Analysis of variance on branch number of plant at different days after transplanting of Tomato</b>				
<b>Source of Variation</b>	<b>Degrees of freedom</b>	<b>Mean Square for number of branches</b>		
		<b>20 DAT</b>	<b>40 DAT</b>	<b>60 DAT</b>
Factor A (Tomato varieties)	2	1.148*	2.592*	6.237*
Factor B ( <i>Trichoderma</i> spore concentration)	2	9.037*	7.148*	13.824*
Interaction (A×B)	4	0.482*	0.593*	0.624*
Error	16	0.120	0.120	0.270

**\*: Significant at 0.05 level of probability**

<b>Appendix V. Analysis of variance on the number of branch per plant, Chlorophyll %, Days to 1st flowering, Days to 1st fruiting and Days to 1st fruit maturity of tomato</b>						
<b>Source of Variation</b>	<b>Degrees of freedom</b>	<b>Mean Square for Number of</b>				
		<b>Number of flower plant<sup>-1</sup></b>	<b>Chlorophyll %</b>	<b>Days to 1<sup>st</sup> flowering</b>	<b>Number of flower cluster plant<sup>-1</sup></b>	<b>Number of Flower Cluster<sup>-1</sup></b>
Factor A (Tomato varieties)	2	49.037*	27.291*	53.818*	3.142*	3.992*
Factor B ( <i>Trichoderma</i> spore concentration)	2	148.037*	36.608*	20.863*	4.891*	2.703*
Interaction (A×B)	4	6.481*	1.229*	0.488*	0.265*	0.103*
Error	16	0.245	0.184	0.2652	0.164	0.128

**\*: Significant at 0.05 level of probability**



Plot size: 3m x 1m

Spacing: 60 cm x 40 cm

Spacing between plot: 50 cm

Spacing between replication: 1 m

**Factor A: Tomato varieties**

V<sub>1</sub> = Roma-VF

V<sub>2</sub> = BARI Tomato- 2

V<sub>3</sub> = Apple Netherland

**Factor B: *Trichoderma* spore concentration**

T<sub>0</sub> = No *Trichoderma* (control)

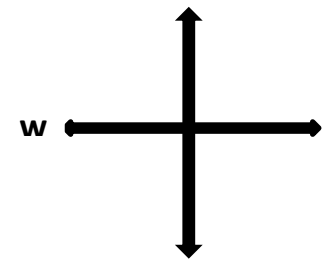
T<sub>1</sub> = 10<sup>6</sup> spores/ ml

T<sub>2</sub> = 10<sup>7</sup> spores/ ml

**Fig 1: Layout of experiment**



R1	R2	R3
$V_1T_0$	$V_2T_2$	$V_2T_1$
$V_2T_0$	$V_1T_1$	$V_3T_1$
$V_3T_2$	$V_3T_0$	$V_1T_0$
$V_2T_1$	$V_2T_0$	$V_3T_0$
$V_1T_1$	$V_3T_1$	$V_1T_2$
$V_3T_0$	$V_1T_2$	$V_3T_2$
$V_1T_2$	$V_2T_1$	$V_2T_0$



$V_3T_1$

$V_3T_2$

$V_2T_2$

$V_2T_2$

$V_1T_0$

$V_1T_1$