EFFECT OF BIOFERTILIZER WITH *TRICHODERMA HARZIANUM* ON GROWTH AND YIELD OF TOMATO (*LYCOPERSIUM ESCULENTUM* M.)

Thet Su Min[1], Khaing Sanda Wint[2], Zaw Naing[3]

Abstract

In the present work, biofertilizer was prepared from farm waste materials such as cow dung, rice straw and rice bran with bioinoculant *Trichoderma harzianum* (Yezin Isolate). All materials such as cow dung, rice straw, rice bran, bioinoculant *Trichoderma harzianum* and water were used in composting process for preparation of biofertilizer by open heap layering method. Using the compost products mixed with pure soil at different rates, pot experiment was conducted to test the effect of *Trichoderma harzianum* as inoculated compost on the growth of tomato plant. The experiment was laid out in Randomized Complete Block Design (RCBD) with four treatments and five replications. Physical parameters and chemical compositions of soil samples before sowing and after harvesting were analysed by conventional methods and modern techniques. Statistical analysis was carried out using International Rice Research Institute (IRRISTAT Version 5.0) in this study. The results from this study indicated that the prepared biofertilizer is a suitable and effective replacement for chemical fertilizers for the growth and production of tomato.

Keywords: biofertilizer, *Trichoderma harzianum* (Yezin Isolate), composting process, tomato

Introduction

Tomato (*Lycopersicum esculentum* M.) is one of the most important vegetables in Asia and Africa and these continents account for more than 65% of global tomato production. Tomato is a warm season crop that originated in South America. Tomato is rich in nutrients such as vitamins, minerals and antioxidants which are important to well-balanced human diets. Tomato is also an important dietary component because it contains high levels of lycopene, an antioxidant that reduces the risks associated with several cancers and neurodegenerative diseases (Srinivasan, 2010).

1. 4PhD, Candidate, Department of Chemistry, University of Yangon
2. Associate Professor, Department of Chemistry, Taung Goke Degree College
3. Associate Professor, Department of Chemistry, Maubin University

Tomato is one of the promising main vegetables in Myanmar. Nowadays, the commercial production of vegetables is increasing as a result of rapid population growth. Moreover, improper use of chemical fertilizers have adverse effect on the environment and costly (Oguzar, 2007). These day's people are trying to reduce the use of inorganic fertilizers to sustain the natural resources. Organic manures improve the physical and chemical condition of the soil. Uptake of macro and micro nutrients improved with application of organic manures (Maskina *et al*., 1988).

The term biofertilizer, represent everything from manures to plant extracts. Biofertilizers are microbial preparations containing living cells of different microorganisms which have the ability to mobilize plant nutrients in soil from unusable to usable form through biological process. Biofertilizers are used in live formulation of beneficial microorganism which on application to seed, root or soil, mobilize the availability of nutrients particularly by their biological activity and help to build up the lost microflora and in turn improve the soil health in general. The microorganisms used for the biofertilizer are bacteria of *Bacillus*, *Pseudomonas*, *Lactobacillus*, photosynthetic bacteria, nitrogen fixing bacteria, fungi of *Trichoderma* and yeast. Biofertilizers have shown great potential as a renewable and environmental friendly source of plant nutrient (Ismail *et al*., 2014).

Trichoderma species present in nearly all agricultural soils and in other environment (Harman, 2000). The fungus *Trichoderma harzianum* (Figure 1) is a biological control organism against a wide range of soil-borne pathogens and has plant growth-promote capacity. It has been shown that *T.harzianum* stimulated the growth of tomato (Datnoff and Pernezny, 1998). In the present study, growth and yield of tomato under pot experiment was studied by using biofertilizer with *Trichoderma harzianum.*

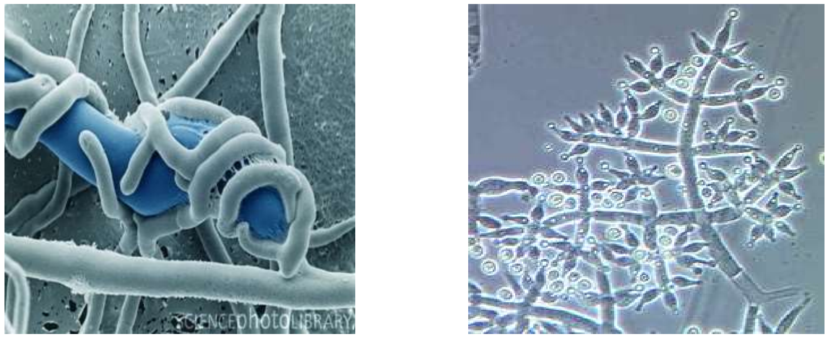


Figure 1 : Trichoderma harzianum

Materials and methods

Sample Collection and Preparation

Cow dung, rice straw and rice bran were collected from Sein Sar Pin Village, Maeutaw village group, Zaeyarthiri Township, Naypyitaw. All materials such as cow dung, rice straw, rice bran, bioinoculant *T. harzianum* (200: 40: 4: 1) and water (18L) were to be used in composting process for preparation of biofertilizer by open heap layering method. Size of box used in composting (pile size) was 8' × 4' × 2.5'. After about 75 days, the compost was ready to be used. The fertilizers were packed in bags, stored in a cool place. By using inoculated biofertilizer, the pot experiment was conducted at the Department of Agricultural Research, Yezin. The experiment was laid out in Randomized Complete Block Design (RCBD) with four treatments and five replications. The treatments used in this study were :

Treatment 1 (T1) = Soil treated with 10 % inoculated biofertilizer

Treatment 2 (T2) = Soil treated with 20 % inoculated biofertilizer

Treatment 3 (T3) = Soil treated with 30 % inoculated biofertilizer

Treatment 4 (T4) = control

Weekly plant height measurements were taken from two weeks after transplanting and shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and fruit yield per plant were recorded after the harvest. Land preparation was done by cutting the vegetation of scraping the soil surface.

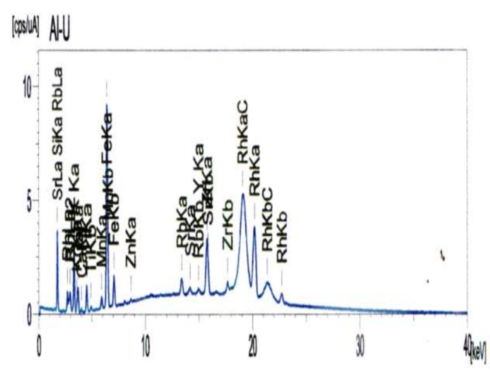
Methods

Four treatments were analysed by conventional and modern techniques before sowing and after harvesting. Qualitative elemental composition of biofertilizer was determined by EDXRF technique. Soil texture was determined by international pipette method. Moisture content was determined by oven drying method and soil pH was measured with a glass electrode using a 1:2.5 soil to water ratio. Organic carbon was determined by Tyurin's method, electrical conductivity was determined by conductivity meter, available nitrogen content was determined by alkaline permanganate method and available phosphorous content by Olsen's method. Available potassium, exchangeable calcium, magnesium and potassium were determined by AAS. In the analytical procedures of the experiments, recommended methods and techniques were applied (FAO, 2008; AOAC, 1980). Statistical analysis was carried out using International Rice Research Institute (IRRISTAT version 5.0) in this study.

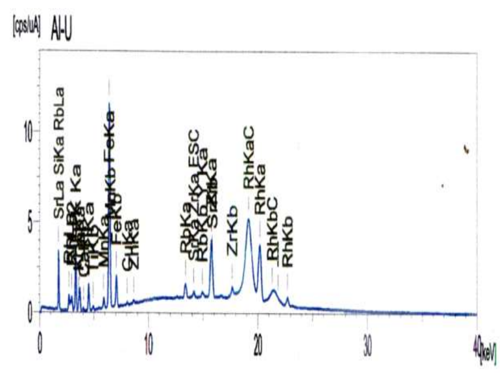
Results and Discussion

Elemental Analysis of Prepared Fertilizer by EDXRF

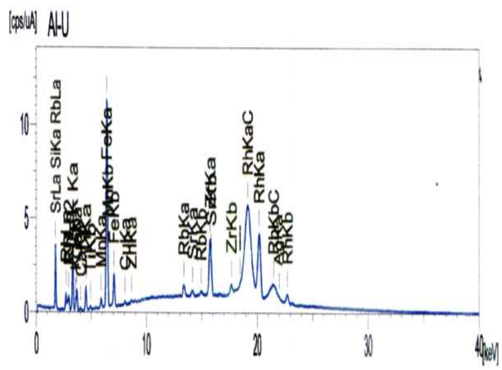
Qualitative elemental compositions in soil fertilized with biofertilizers were detected by EDXRF. These spectra are shown in Figure 2 and the data are presented in Table 1. According to EDXRF spectra, essential elements for plants and no toxic elements were found in the four treatments. All the treatments, silicon peaks were the most prominent and so it showed the highest content of silicon.



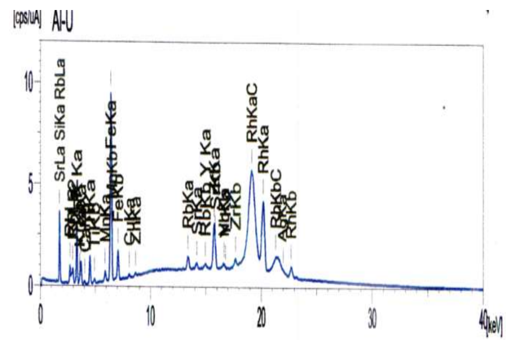
(T1)



(T2)



(T3)



(T4)

Figure 2 : EDXRF spectra of soil treated with biofertilizer

Table 1: Elemental Composition of Soil Treated with Biofertilizer by EDXRF

Element	Elemental Composition (%)			
	T1	**T2**	**T3**	**T4**
Si	74.448	71.612	70.115	74.699
K	12.159	13.517	13.774	11.266
Al	3.914	4.677	4.483	4.474
Ca	3.469	3.816	3.278	3.460
Fe	3.476	4.212	4.018	3.349
Ti	1.576	1.873	1.574	1.480
S	0.380	0.382	0.485	0.377
Mn	0.285	0.294	0.248	0.264
Zr	0.128	0.119	0.107	0.130
Ag	-	-	0.134	0.128
P	0.175	0.245	0.321	0.108
Rb	0.085	0.087	0.071	0.071
Zn	0.024	0.040	0.032	0.033
Cu	-	0.028	0.033	0.032
Sr	0.035	0.033	0.030	0.029
Nb	-	-	-	0.023
Y	0.012	0.015	-	0.011

T1 = Soil treated with 10 % biofertilizer T2 = Soil treated with 20 % biofertilizer

T3 = Soil treated with 30 % biofertilizer T4 = Control

Analysis of Soil for Four Treatments before Sowing

Soil is one of the most important natural resources for agricultural production, which heavily depends on soil fertility. It is therefore an essential mandate survey and to classify soil, and to apply fertilizers in order to increase yield. Table 2 shows the physical parameters and chemical compositions of four treatments before sowing. Soil texture depends on the relative proportions of sand, silt and clay in the soil. It indicates high percentage amount of sand in four treatments. This type of soil falls in the domain of loamy sand soil. This type of soil's texture has less capacity to retain water but afford to penetration of roots and being exposed to good aeration and retention of plant nutrients. The pH values of the fertilizer treated soils were found to be slightly increased than that of the original control soil. The pH values of treated soils were found to be in the range of 6.61-6.84. The observed pH values were suitable for plant growth. The values of electrical conductivity of the treated soils were higher than that of the original free soil. Electrical conductivity of soils informs the ionic nature of the soluble compound to supply the needs of plants. Soil humus is also important in increasing the water holding capacity of the soil and it plays a part in the retention of plant nutrients. The humus content increased from 1.79 % to 2.05 % and organic carbon increased from 1.04% to 1.19 %.

As for the total N content in the case of four treatments, T3 has the highest N content. It is a common fact that for plant growth nitrogen (N) is required to promote development of stem and leaf, phosphorous (P) acts to stimulate growth, accelerate fruits and seed formation and the function of potash (or) K is essential to development of starches, sugar and fibers. The highest content of available nitrogen, phosphorous and potassium in T3 were 38.00 ppm, 48.03 ppm and 796.69 ppm and the amounts of exchangeable Ca, Mg and K were about 8.50 me/100g, 0.55 me/100g and 2.68 me/100g respectively. On the context of what has been described above, T4 (control) has the lowest N, P and K contents. Regarding the observed values, it was considered that the four treatments can be used in crop production to enhance the soil fertility.

Table 2: Analysis Data of the Soil Before Sowing

Analytical Item	**T1**	**T2**	**T3**	**T4**
Texture - Sand (%)	83.92	83.80	83.76	83.96
Silt (%)	7.96	7.40	7.84	7.44
Clay **(%)**	8.12	8.80	8.40	8.60
Moisture (%)	1.14	1.44	1.64	0.74
pH	6.66	6.76	6.84	6.61
Electrical Conductivity (dS/m)	0.58	0.86	1.19	0.23
Organic Carbon (%)	1.05	1.15	1.19	1.04
Humus (%)	1.81	1.98	2.05	1.79
Total N (%)	0.11	0.23	0.32	0.06
C/N ratio	9.54	5.00	3.71	17.33
Available N (ppm)	22.00	25.00	38.00	20.00
Available P (ppm)	21.83	39.30	48.03	18.73
Available K (ppm)	350.41	605.79	796.69	272.48
Exchangeable Ca (me/100g)	7.28	7.63	8.50	7.13
Exchangeable Mg (me/100g)	0.45	0.48	0.55	0.34
Exchangeable K (me/100g)	1.76	1.89	2.68	0.47

T1 = Soil treated with 10 % biofertilizer T2 = Soil treated with 20 % biofertilizer

T3 = Soil treated with 30 % biofertilizer T4 = Control

Figure 3 : View of pot experiment for tomato

Analysis of the Soil used for Tomato at Harvesting

The texture, moisture percent, electrical conductivity, organic carbon content, humus percent, total nitrogen content, C/N ratio, available nitrogen, phosphorous and potassium, exchangeable calcium, magnesium, potassium and pH values of soil after harvesting tomato are shown in Table 3. The soils were subjected to different treatments by using inoculated compost (biofertilizer). Comparison for all cases is made with respect to the physicochemical composition of the soil samples. After harvesting, all type of soils are loamy sand type.

The soil organic carbon contents under the biofertilizer application were higher than these under the control. The C/N ratio of T3 is lower than that of other treatments for all crops because large amount of organic fertilizer utilization can cause nitrogen depletion and decrease the C/N ratio. A variety of treated soils was compared with the control soil after harvesting stage. Moreover, it was observed that the pH of the soil before sowing and after harvesting stages lie between 6.61 and 7.61.These can be considered as the slightly acidic and neutral type of soil.

The fertility status of the soil is expected to benefit from poultry manure application since the manure is known to improve soil organic matter, macro-nutrient status and micro nutrient qualities of the soil (Akande and Adediran, 2004). On reviewing the results of the data presented in Table 3 even after the harvesting stage nitrogen, phosphorous, potassium and as well as the organic carbon and humus contents in the case of soil T1, T2 and T3 were significantly changed under cultivation because most of them were frequently removed from the soil permanently by the crop produced.

Treatment 1

Treatment 2

Treatment 3

Treatment 4

Figure 4: The growth of four treated tomato plants

Table 3: Analysis Data of the Soil Using for Tomato After Harvesting

Analytical Item	**T1**	**T2**	**T3**	**T4**
Texture - Sand (%)	84.52	81.40	79.44	86.84
Silt (%)	10.48	12.56	16.04	8.08
Clay **(%)**	5.00	6.04	4.52	5.08
Moisture (%)	0.51	0.55	0.62	0.45
pH	7.59	7.61	7.27	7.01
Electrical Conductivity (dS/m)	0.30	0.32	0.34	0.20
Organic Carbon (%)	0.87	1.08	1.10	1.01
Humus (%)	1.49	1.86	1.89	1.74
Total N (%)	0.10	0.14	0.15	0.05
C/N ratio	8.70	7.71	7.33	20.20
Available N (ppm)	15.30	15.60	22.20	12.70
Available P (ppm)	84.76	105.68	116.59	60.26
Available K (ppm)	174.38	176.86	189.26	155.37
Exchangeable Ca (me/100g)	5.89	6.07	7.19	5.46
Exchangeable Mg (me/100g)	0.36	0.39	0.45	0.32
Exchangeable K (me/100g)	0.21	0.28	0.30	0.14

T1 = Soil treated with 10 % biofertilizer T2 = Soil treated with 20 % biofertilizer
T3 = Soil treated with 30 % biofertilizer T4 = Control

Effect of *T. harzianum* on Growth and Yield of Tomato

From the pot experiment investigation, it was observed that *T.harzianum* inoculated biofertilizer promoted plant growth and also enhanced the growth of tomato. Time frame of sowing to the harvested time was of 120 days. The growth factors of the crops were evaluated in terms of the plant height (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), number of total fruit per plant and total yield (g plant^{-1}).

There was no significant difference in plant height for all treatments (Table 4). There was significant difference in shoot fresh weight, root fresh weight, shoot dry weight, root dry weight between either treated soil and the original free soil. A major feature of that *T.harzianum* is its capability to grow along roots during their elongation, thus colonizing the whole root system and benefiting the crop for its entire life (rhizosphere competence). *T.harzianum* has beneficial effects on plant growth and vigour and on the development efficiency of the root systems of several crops (Bjorkman *et al*, 1998).

Number of total fruits of T3 was significantly higher than those of T1, T2 and T4. The total yield (g plant^{-1}) in pot experiment are presented in Table 4. There were highly significantly different in total yield of T3. This study revealed that application of *T. harzianum* showed positive results in growth and yield of tomato.

Fruits (T1) **Fruits (T2)**

Fruits (T3) **Fruits (T4)**

Figure 5: Yield of tomato fruits by treated biofertilizers and control

T1 = Soil treated with 10 % biofertilizer T2 = Soil treated with 20 % biofertilizer
T3 = Soil treated with 30 % biofertilizer T4 = Control

Table 4: Effect of Biofertilizer on Growth and Yield of Tomato

Treatment	Life time (day)	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of total fruit plant^{-1}	Total yield (g plant^{-1})
T1	120	35.48	70.42	10.31	20.95	3.07	44.20	867.68
T2	120	36.58	106.45	9.40	23.83	3.49	50.20	1055.52
T3	120	37.02	162.97	11.88	34.44	5.07	59.00	1242.46
T4	120	30.62	62.74	7.75	19.35	3.30	39.20	750.80
F-test		ns	**	**	**	**	**	**
LSD (5%)		9.78	6.91	0.94	1.98	0.38	5.37	160.90
CV (%)		20.30	5.00	6.90	5.80	7.40	8.10	11.90

T 1= 10 % Biofertilizer, T 2= 20 % Biofertilizer, T 3= 30 % Biofertilizer, T 4= Control
** ($P < 0.01$), ns= non significant

Conclusion

In this research work, it was conducted to know the effect of biofertilizer with *T.harzianum* on plant growth and yield of tomato. Based on the specific properties such as total carbon and nitrogen content, the value of macro and micronutrients, pH value and soil texture, EDXRF spectra of prepared biofertilizers and the growth and yield of tomato are reliable to enhance the soil fertility and soil productivity. It was observed that T3 (30% biofertilizer) was found to be enhanced better growth and yield than other treatments. Therefore, this study creates a platform to encourage the agricultural industry to use biofertilizer with *T.harzianum* as a substitute to the commercial chemical fertilizer which is more economical and environmental friendly.

Acknowledgements

The authors would like to express their profound gratitude to the Department of Higher Education (Lower Myanmar), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research and Myanmar Academy of Arts and Science for allowing to present this paper.

References

Akande, M.O. and Adediran, J.A. (2004). "Effects of Terralyt Plus Fertilizer on Growth Nutrients Uptake and Dry Matter Yield of Two Vegetable Crops". *Moor J. of Agric.Res.,* vol. 5, pp. 12-17

AOAC. (1980). "*Official Methods of Analysis*". Washington DC: 13th Ed, Association of Official Analytical Chemists, pp. 3-27

Bjorkman, T., Blanchard L. M. and Harman, G.E. (1998). "Growth Enhancement of Shrunken-2(sh2) Sweet Corn by *Trichoderma harzianum* 1295-22 : Effect of Environmental Stress". *J. Am. Soc. Hortic. Sci.,* vol. 125, pp 35-40

Datnoff, L. E. and Pernezny, K. L. (1998). "Effect of Bacterial and Fungal Microorganisms to Colonize Tomato Roots, Improve Transplant Growth and Control of Fusarium Crown and Root Rot". *Florida Tomato Institute Proceedings* PRO, vol. 111, pp. 26-33

FAO. (2008). *Guide to Laboratory Establishment for Plant Nutrient Analysis.* Rome: FAO, pp. 30-44

Harman, G. E. (2000). "Myths and Dogmas of Biocontrol, Changes in Perceptions Derived from Research on *Trichoderma harzianum* T-22". *Plant Dis*., vol. 84, pp. 377-393

Ismail, E.G., Salah, W.M, Salah, K. and Fadia,E.S. (2014). "Effect of Manure and Biofertilizers on Growth, Yield, Silymarin Content and Protein Expression Profile of *Silybum marianum*". *Adv Agric. Biol*., vol. 1 (1), pp. 36-44

Maskina, M.S., Singh, B., Singh, Y. and Meelu, O. P. (1988). " Fertilizer Requirement of Rice-Wheat and Maize-Wheat Rotations on Coarse Textured Soils Amended with Farmyard Manure". *Fert. Res*., vol. 17, pp. 153-164

Oguzar, N.S. (2007). "Effect of Palm Bunch Ash on Growth of Groundnut". *J. Agronomy*., vol. 1 (2), pp. 59-61

Srinivasan, R. (2010). *Safer Tomato Production Techniques*, *A Field Guide for Soil Fertility and Pest Management*, Taiwan: The World Vegetable Centre, Shanhua, AVRDC Publication, vol. 97, pp. 10-74