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Effects of Microbial Organic Fertilizer and Mulch to Population and Bioactivity of Beneficial Microorganisms in Tea Soil in Phu Tho, Viet Nam

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Currently, tea in Phu Tho is grown in nutrient-poor and acidic soils, with pH_{KCl} from 3.6 to 4.6, low total and digestible phosphate (P_2O_5); average organic matter (OM), potassium (K_2O) and protein, low CEC (from 3.36 to 4.24 meq/ 100g soil), low Ca and Mg (from 2.75 to 3.27 meq/100g soil). Using microbial organic fertilizer at level of 3.0 tons per hectare influenced significantly both the population and composition of microorganism species in tea soil.
Many soil microbes such as Pseudomonas, Bacillus, Azotobacter, Nitrosomonas and Nitrobacter which decompose organic matter, cellulose, insoluble phosphates have appeared in the soil as microbial organic fertilizers were provided. The filamentous fungi which are able to decompose insoluble phosphate, cellulose such as Aspergillus spp., Penicillium spp Trichoderma spp, and Fusarium spp. have been detected.
The total number of bacteria has increased rapidly in a short time as Guot (Gleichenia linearis Clarke) was used as mulch material. The number of bacteria reached to the highest level of 11.30 x 10^6 CFU/g (soil) after 180 days. Total numbers of actinomycetes have increased significantly. Many newly actinomycetes have appeared in the soil after 90 days of mulching, and continued to increase up to 11.05 x 10^6 CFU/g after 270 days. Fungi population also grew fast (from 5.35 x 10^3 CFU/g to 7.45 x 103 CFU/g) after 90 days of mulching then continue to rise sharply in the next 90 days and stabilized later. When using different materials, the percentage of microorganism groups that have bioactive are also different. Cellulose decomposing bacteria accounted for 40%, while the rate of bacteria that decompose insoluble phosphate made up for 44%.

Keywords: Microbial organic fertilizer, Compost, microbiology, microbial, tea, tea cultivation, mulching for tea

Introduction

Agriculture in the 21st century is towards safe and sustainability. Therefore, the strategy of use of fertilizers in this century is integrate crop nutrition, maintain and adjust soil fertility and provide nutrients to make

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optimum conditions for crop growth in order to stabilize the desired yield. Microbial organic fertilizers are producted that meet these requirements.

Microbial organic fertilizer products are produced from different organic materials in order to provide nutrients for plants and to improve the soil. These products also contain one or more alive microorganisms with a standard density, contributing to improve productivity and quality of agricultural products. Microbial organic fertilizers do not cause adverse effects on human health, animals, ecology and quality of agricultural products.

Although microbial organic fertilizers have an important role in improving productivity, soil fertility, their uses are limited in Vietnam in general, especially in Northern mountainous area. Moreover, microbial organic fertilizer is only produced for high-cost plants, and limitations in transportation to the remote areas. Tea farmers in midlands - northern mountainous area do not have many opportunities to approach and use this fertilizer.

For many years, previous results have showed that an increase of crop yields between 10-20% in areas where the microbial organic fertilizers were used as compared to areas, which only used chemical fertilizers.

Providing organic fertilizer also reduces the amount of mineral that anually added to the soil because the macronutrient, secondary and micronutrient elements exist in the organic fertilizers. In addition, organic fertilizer improves the physical, chemical and biological properties of the soil, limiting the toxic levels of some elements such as aluminum, iron, Organic fertilizer decreases drifting and evaporation of urea from inorganic sources, so the efficiency of inorganic nitrogen is increased.

With tea producers, applying integrated farming process is really necessary in the current situation due to over 80% of products are exported, and the importers have given more stringent criteria in chemical residue.

The aims of this study is to assess the influence of organic fertilizer on tea planting and beneficial microorganisms in the soil to complete the goal of safe agricultural production and sustainability.

Materials and methods

Material

Tea varieties LDP1 grown in 2000 were used as material in this study.

Microbial organic fertilizers used in the study are composted from straw, fresh plants which used biological products containing LKCDM49 and VGY strains to decompose cellulose, and added YB13 and YB6 strains to transform organics. (LKCDM49, VGY, YB13, YB6 which are new cellulose decomposing strains were isolated and named by Soils and Fertilizers Research Institute).

This study was carried out under field conditions in Northern mountainous agriculture and forestry science institute (NOMAFSI), from December 2012 to September 2015.

Methods

Research of soil microorganisms

Soil sampling method according to mechanical composition, time and humidity (Egorov, 1983). The soil was sampled for microbial analysis three times during the growing season at depth of 5 to 20cm. Isolate and quantify microbial cells as described by DUNG Nguyen Lan (DUNG Nguyen Lan *et al.*, 1972; 1978). Microorganisms were selected according to their ability to decompose cellulose, phosphates, stimulate growth and fix free nitrogen (DUNG Nguyen Lan *et al*., 1972; 1978).The experiments were set up as a randomized block design in three replications at the experimental field of Northern mountainous agriculture and forestry science institute.

Organic fertilizer

Organic fertilizers were supplied once at the beginning of the year, at the time of rain and wet soils. The trial involved four treatments with different level of microbial organic fertilizers used to evaluate the effect of the amounts of organic fertilizer on the variation of beneficial microorganisms in the soil. T0 treatment with inorganic fertilizers used as a control (N: 300, P_2O_5: 100, K_2O: 100 per year (Process of MARD)); T1 treatment with 1.0 ton/ha/year; T2 treatment with 2.0 tons/ha/year; and T3 treatment with 3.0 tons/ha/year.

Mulching

To study the effect of mulching material to the variation of beneficial microorganisms in the soil, fresh guots and tea tre wastes were used as material. They were spreaded along the tea rows, close to the stumps. The trial was involved in three treatments: no-mulch control (T0); T1 treament with Guot (30 tons/ha/year) and T2 treatment with tea tree wastes (30 tons/ha/year).

The experiments arranged in randomized block consist of four or three treatments (organic fertilizer or mulching, respectively), with 3 replicates for each treatment.

Area of one plot is 45 m^2 (10 m long x 1.5 m x 3 rows).

Statistical analysis

All statistical analyses were carried out using the R software. To determine whether treatments were statistically different, Tukey's multiple comparison tests ($P<0.05$) were used to test differences between the means.

Results and Discussion

Effect of microbial organic fertilizers on the variation of microorganisms in tea soil in Phu Tho, Viet Nam.

Analysis of physical and chemical compositions of the soil

Analysis of soils shows that most of the samples belong to group of yellow red soil on shale or mica which are poor nutrients and low fertility, especially, total organic matter in the soil is very low (Table 1).

Table 1. Physical properties of the soil in Phu Tho

	Content
Clay (<0.002mm), (%)	43.2
Limon (0.002 - 0.02) (%)	4.2
Fine sand (0.02 - 0.2 mm) (%)	30.9
Coarse sand (0.2 - 2mm) (%)	21.7
Density (g/cm^3)	1.36
Dry density (g/cm^3)	2.63
Porosity (%)	48.3
The field maximum moisture content (%)	33.5

As showing the Table 2, tested soils were acidic and poor nutrients. The pH_{KCl} ranged from 3.6 to 4.6 and soluble phosphate levels were low (0.04-0.05%). The organic matter (OM), K_2O and nitrogen were at average levels (0.06-0.23%). The Cation Exchange Capacity (CEC) index was from 3.36 - 4.24 meq/100g soil, Ca and Mg contents were also low, only from 2.75 to 3.27 meq/100g (soil).

Table 2. Chemical properties of the soil (layer 0 - 20cm)

Samples	**pH_{KCl}**	**%**				**mg/100g (soil)**		**meq/100g (soil)**		
		OM	**N**	**P_2O_5**	**K_2O**	**P_2O_5**	**K_2O**	**Ca^{2+}**	**Mg^{2+}**	**CEC**
1	3.6	2.52	0.06	0.04	0.06	0.90	1.41	0.57	0.08	6.69
2	4.6	2.58	0.08	0.05	0.23	4.0	10.3	2.16	0.87	4.24
3	4.4	2.58	0.10	0.05	0.18	3.8	10.3	2.05	1.22	4.12
Mean ± SD	4.2 ± 0.53	2.56 ± 0.03	0.08 ± 0.02	0.05 ± 0.01	0.16 ± 0.09	2.9 ± 1.73	7.34 ± 5.1	1.59 ± 0.89	0.72 ± 0.58	5.02 ± 1.45

These results are consistent with previous studies. KHUE Phan Van (2016) reported that generally soils in Phu Tho were yellow red soil on shale, Ferrlics (Fs) - Ferralic Acrisols (ACf). Top soil of this type has soil texture at average level, with a thick clay layer at the lower layer, from 24.1 % to 30.3%. It is also acidic soil (pH_{KCl} is from 3.55 to 3,7), with organic amount at average level (OC% =1.57%) and much lower according to the deep of the soil. Total phosphate is from average to relative level, P_2O_5% from 0.14 to 0.10%; poorly soluble phosphates, P_2O_5% from 3.4 to 1.5mg/100g (soil). Total K_2O increases according to the deep of the soil, average level at top soil, higher content at lower layers (1.64 to 2.57%) but soluble posstasium is poor (ranges from 6.7 – 7.6 mg/100g soil).

With light soil texture, low field maximum moisture content and low CEC, fertilizers need to be used in reasonable amounts to limit soil erosion in Phutho.

Effect of microbial organic fertilizers on the composition of microorganisms

Effect of microbial organic fertilizers on the population of bacteria.

Results in Figure 1 show that the numbers of bacteria in the soil increased significantly as the applied microbial organic fertilizer amount increased, ranging from 8.2 x 10^6CFU/g soil – 9.81 x 10^6CFU/g soil, after 60 days, compared to the control (7.9 x 10^6CFU/g soil). After 240 days, when microbial organic fertilizer was applied at the level of 3.0 tons/ha/year, the number of bacteria in soil had a dramatic increase, reached to 14.32 x 10^6CFU/g soil. Mealwhile, at the level of 1.0 and 2.0 tons/ha/year, the population of bacteria was relatively stable (9.85x10^6CFU/g and 9.95x10^6CFU/g (soil), respectively). These results indicate that microbial organic fertilizer could significantly enhance the number of bacteria in the soil, improving the soil quality and increasing tea's ability to absorb nutrients.

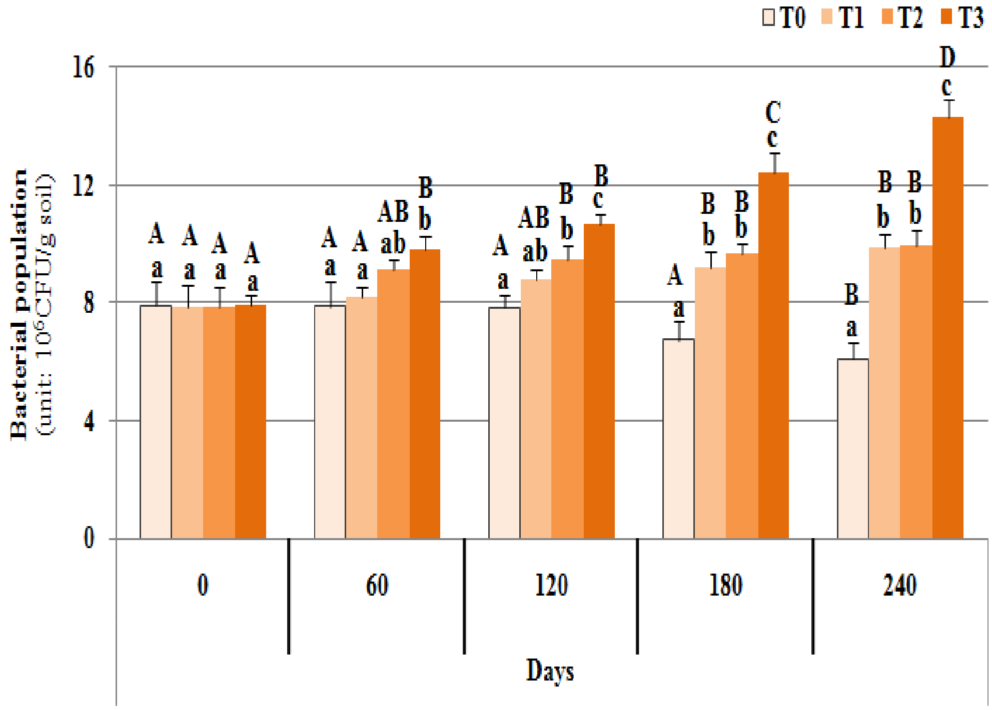

Figure 1: Effect of microbial organic fertilizer on bacterial population (unit: 10^6CFU/g soil).

Data are means ± SD of three samples from each treatment. Statistically significant differences were calculated by using Tukey HSD test. Different lower case letters indicate significant differences among treatments at the same day and different upper case letters indicate significant differences among days at $P < 0.05$.

Effect of microbial organic fertilizers on the population of actinomycetes

The number of actinomycetes is identified in the soil after microbial organic fertilizers were applied. As showing in the Table 3, all treatments, which applied microbial organic fertilizers, have higher number of actinomycetes than that of the control. The number of actinomycetes gradually increased and reached up to 10.67 x 10^6CFU/g soil, when microbial organic fertilizer was applied at level of 3.0 tons/ha/year, after 240 days. With lower amounts of organic fertilizers used (1.0 and 2.0 tons/ha/year), the number of actinomycetes slightly increased after 60 days but significantly increased after 240 days.

Table 3. The number of actinomycetes in the soils (unit: 10^5CFU/g soil).

Treatments	Days				
	0	**60**	**120**	**180**	**240**
T0 (Control)	6.24 ±0.42[a]	6.26 ±0.36[a]	6.12 ±0.25[a]	6.18 ±0.44[a]	6.12 ±0.41[a]
T1 (1.0 ton/ha/year)	6.30 ±0.43[a]	6.44 ±0.41[a]	7.06 ±0.43[b]	7.64 ±0.49[b]	8.73 ±0.2[b]
T2 (2.0 ton/ha/year)	6.25 ±0.48[a]	7.11 ±0.52[ab]	7.83 ±0.48[b]	9.02 ±0.56[b]	9.85 ±0.22[c]
T3 (3.0 tons/ha/year)	6.28 ±0.69[a]	8.07 ±0.47[b]	8.96 ±0.66[cb]	9.76 ±0.63[cb]	10.67 ±0.29[d]

Data are means ±SD (n=3). Statistically significant differences were calculated by using Tukey HSD test. Different letters indicate significant differences among treatments at the same day at $P < 0.05$.

Effect of microbial organic fertilizers on the number of microorganisms which have biological activity.

Biological activity of soil microorganisms was also evaluated in this study, and results are presented in the Table 4.

Table 4. Biological activity of some main microorganisms (after 240 days)

Treatments	Micro-organisms	Decomposition activity (%) per Total of strains isolate*							
		Cellulose		*Insoluble Phosphates*		*Starch*		*Mucous membrane*	
		Good	*Relatively good*	*Good*	*Relatively good*	*Good*	*Relatively good*	*Good*	*Relatively good*
T0 (Control)	Bacteria	5	18	12	5	15	2	0	0
	Actinomycetes	18	30	10	15	0	0	0	0
T1 (1.0 *ton/ha/year*)	Bacteria	20	24	15	27	14	23	0	0
	Actinomycetes	22	16	10	25	5	7	0	0
T2 (2.0 tons/ha/year)	Bacteria	24	19	14	30	21	15	15	3
	Actinomycetes	15	25	20	35	0	0	0	0
T3 (3.0 tons/ha/year)	Bacteria	46	23	32	20	22	20	15	18
	Actinomycetes	41	20	34	29	20	10	0	0

*The number of strains tested biological activity is 10 in all experiments

In the treatments that applied microbial organic fertilizers, the observed microorganisms exhibited better biological activities than that in the control. Of ten strains tested for biological activity in the treatment T3 (fertilized 3.0 tons of microbial organic fertilizers per ha per year), 46% of the strains were cellulose decomposition, while the number of the strains decomposing insoluble phosphates, starch and mucous membrane was 32%, 22% and 15%, respectively. The number of microorganisms decomposing cellulose, insoluble phosphates, starch and mucous membrane isolated in the treatments T1 and T2 was much lower than that in the treatment T3. In addition, many strains which are not able to decompose starch and mucous membrane were also detected in the treatments T1 and T2.

In the groups of soil microorganisms, cellulose decomposing bacteria play a vital role. These bacteria decompose trunk, leaves and other plant wastes into humus. The appearance of the cellulose decomposing bacteria helps tea trees to uptake faster nutrients which are supplied by fertilizers. In addition, free nitrogen fixing bacteria and Azotobacter were also detected in the soils in the treatment T3 after 240 days, but were not in the others. Detecting Azotobacter in the soils may be due to the highest amount of organic fertilizer added in the treatment T3 which provided high moisture and good environment needed for the growth of Azotobacter.

Effects of microbial organic fertilizer on bacterial diversity in soil

This work evaluated the diversity of bacteria in the soils in the tested treatments based on the analyses of morphology, physiology, chemical biology and identification of species and varieties. Results show that the diversity of microorganisms in the tested treatments was different. The diversity in composition of bacteria reflects the relationship between soil quality and microorganisms. Table 5 shows that higher level of bacterial diversity was observed in the tested treatments as compared to the control.

Table 5. The diversity of microorganisms in the soils after providing microbial organic fertilizers (240 days)

Treatments	**Genus total**	**Bacteria**		**Filamentous fungi**		**Lipomyces**		**Actinomycete**	
		Number	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**
T0 (Control)	8	4	50	0		0	0	4	50
T1 (1.0 *ton/ha/year*)	15	8	53.3	1	.67	2	13.33	4	26.67
T2 (2.0 *tons/ha/year*)	16	8	50	2	2.5	2	12.5	4	25
T3 (3.0 *tons/ha/year*)	24	14	58.33	6	5	2	8.3	2	8.33

The microorganism in the treatments, which applied microbial organic fertilizers, was much more diverse than that observed in the control. The most diversity of microorganisms was observed in the treatment T3, with 24 genera including 58% of bacteria, and 25% of filamentous fungi.

To verify the diversity of microorganisms in the soils, the composition of bacterial species was also considered. As shown in the table 6, the cellulose- and insoluble-phosphates decomposing bacteria such as *Pseudomonas, Bacillus, Azotobacter, Nitrosomonas* and *Nitrobacter* were observed to occur with higher frequency in the soils that were provided higher amount of microbial organic fertilizers. Notably, most of the detected filamentous fungi were capable of decomposing insoluble photphates and/or cellulose.

Table 6. Effect of microbial ogarnic fertilizers on composition and occurrence frequency of microorganisms the soil after 240 days.

Groups	**Composition of varieties**	**Frequency of occurrence of strains***			
		T1 (control)	**T2**	**T3**	**T4**
Cellulose - and insoluble phosphates - decomposing bacteria, and nitrogen-fixing bacteria	*Pseudomonas*	++	++	+++	++++
	Bacillus	+++	+++	++++	++++
	Cellulomonas	-	+++	++	++++
	Agrobacterium	-	+	-	+
	Enterobacter	+	+	++	+++
	Nitrosomonas	+	-	-	++++
	Nitrobacter	-	+	++	++++
	Azotobacter	-	-	-	+++
	Mycobacterium	-	++	+	++
	Rhizobium	+	+	++	++
Filamentous fungi decompose cellulose and insoluble phosphates	*Aspergillus*	+++	++	++++	++
	Penicillium	+++	+++	++++	++
	Trichoderma		+	++	++
	Fusarium		+	+++	+
	Mucor		-	++	+
	Mertahzium		++	+	+
Actinomycetes	*Actinomycetes producing antibiotics*		+	++	++
Lipomyces	*Lipomyces producing polysacharide*		+	+	-
Total	**18**				

* So many: (++++), many: (+++), relative: (++), less: (+), and none: (-)

The results indicate that providing organic fertilizers significantly impact composition and diversity of microorganisms in the soil. The increase in the number of beneficial microorganisms in the soils is important for region of yellow red soil on shale, as Phu Tho, due to the low levels of organic matter observed for our soils. The increase in the number and the diversity in the composition of microorganisms is important for improving the quality of the soil since that organic matter influence soil physical, chemical and biological properties (Araújo *et al*., 2009).

Previous investigations have demonstrated that organic fertilizers increased bacterial and fungi diversity by increasing the carbon pool of the soil, thus improving the living conditions for indigenous microbial populations (Wu *et al*., 2008; Helgason *et al.*, 2010). The positive effects of organic fertilizers on the soil quality have been also reported by Mandic (2011) and Nakhro (2010). They found that the application of organic fertilizers increased the organic carbon content of the soil and thereby increasing the microbial counts and microbial biomass carbon (Mandic *et al.*, 2011; Nakhro *et al*., 2010). Based on our data, the treatment T3 greatly improved microbial diversity, indicating that T3 is conducive to the establishment of a diverse microbial community structure (Table 5). These results are in agreement with findings of Zhen *et al.* who found that the application of bacterial fertilizer provided a variety of nitrogen-fixing bacteria and phosphorus-solubilizing bacteria that could improve the soil microbial community structure (Zhen *et al.*, 2014).

Effect of mulching materials on the variation of beneficial microorganisms in the soil.

Effect of mulching on the composition of microorganisms in the soils

Mulching materials slowly decays and this process also depends on the weather. In this study, therefore, evaluation of the mulching effects on the microorganisms in the soil was evaluated after 90 days.

Effect of mulching on the population of microorganisms

As presented in Table 7, the different treatments exhibited different the total number of bacteria in the soils. In the control (without mulch), the number of bacteria varied slightly, the highest level at $8.02 x 10^6$CFU/g soil. In contrast, the number of bacteria changed quickly at the stages of 180 days and 270 days in the treatment T1. An increase of bacteria in this experiment may be due to Guot which is decayed fast as in the good condition with high moisture. Notably, the number of bacteria was significantly higher in the treatment T1 ($11.3 x 10^6$CFU/g) than in the treatment T2 ($10.86 x 10^6$CFU/g) after 90 and 180 days. This can be explained by the distinct in the mulching materials. In the treatment T2, mulching materials were old trunk, leaves and other plant wastes which are high wood fiber content and bigger than Guot. Hence, the decay was more slowly than the Guot used in the treatment T1.

Table 7. The number of bacteria in the soils (unit: 10^6CFU/g soil).

Treatments	**Days**			
	0	**90**	**180**	**270**
T0 (Control)	7.92 ±0.22^{a}	7.90 ±0.26^{a}	8.02 ±0.42^{a}	8.00 ±0.38^{a}
T1 (mulching by Guot - 30 tons/ha/year)	7.90 ±0.53^{a}	8.45 ±0.2^{b}	9.56 ±0.56^{b}	11.30 ±0.41^{b}
T2 (mulching by tea tree wastes - 30 tons/ha/year)	7.90 ±0.45^{a}	8.00 ±0.58^{a}	8.43 ±0.45^{a}	10.86 ±0.44^{b}

Data are means ±SD (n=3). Statistically significant differences were calculated by using Tukey HSD test. Different letters indicate significant differences among treatments at the same day at P < 0.05.

Effect of mulching materials on the population of actinomycetes

As shown in Table 8, the number of actinomycetes were significantly different in the soils in the treatments. In contrast to an increase of bacteria number in the treatment T1 in which Guot was used as mulching material, the number of actinomycetes decreased in this treatment.

Table 8. Effect of mulching materials on the population of actinomycetes (10^5CFU/g (soil)).

Treatments	**Days**			
	0	**90**	**180**	**270**
T0 (Control)	6.24 ±0.25$^{a\mathbf{A}}$	6.20 ±0.17$^{a\mathbf{A}}$	6.14 ±0.35$^{a\mathbf{A}}$	6.18 ±0.36$^{a\mathbf{A}}$
T1 (mulching by Guot - 30 tons/ha/year)	6.30 ±0.35$^{a\mathbf{A}}$	7.05 ±0.11$^{b\mathbf{B}}$	8.40 ±0.23$^{b\mathbf{C}}$	9.17 ±0.37$^{b\mathbf{C}}$
T2 (mulching by tea tree wastes -30 tons/ha/year)	6.25 ±0.39$^{a\mathbf{A}}$	8.23 ±0.3$^{c\mathbf{B}}$	9.31 ±0.31$^{c\mathbf{C}}$	11.05 ±0.52$^{c\mathbf{D}}$

Data are means ±SD (n=3). Statistically significant differences were calculated by using Tukey HSD test. Different letters indicate significant differences among treatments at the same day, and different capital letters (in bold) indicate significant differences among days at the same treatments at P < 0.05.

The number of actinomycetes increased after 90 days and reached to the highest level after 270 days in both the treatment T1 and T2. Actinomycetes number was significantly higher in the treatment T2 than in the treatment T1 after 90, 180 and 270 days. In contrast, the population of actinomycetes did not change in the number in all the tested days.

Effect mulching materials on the population of fungi

In the previous part, the effect of organic fertilizer on the variation of fungi was not considered because the variation of fungi in the samples were ambiguous and insignificant. However, when analysing the effect of mulching materials on the bacteria, the changes in the number of fungi were observed.

Table 9. Effect mulching materials on the population of fungi (unit: 10^3CFU/g (soil)).

Treatments	Days			
	0	**90**	**180**	**270**
T0 (Control)	5.18 ±0.32[a]	5.30 ±0.27[a]	5.27 ±0.23[a]	5.24 ±0.43[a]
T1 (mulching by Guot - 30 tons/ha/year)	5.35 ±0.38[a]	7.45 ±0.42[b]	9.05 ±0.26[b]	8.04 ±0.37[b]
T2 (mulching by tea tree wastes - 30 tons/ha/year)	5.26 ±0.41[a]	6.12 ±0.21[c]	9.26 ±0.37[b]	9.34 ±0.24[c]

Data are means ±SD (n=3). Statistically significant differences were calculated by using Tukey HSD test. Different letters indicate significant differences among treatments at the same day at $P < 0.05$.

As shown in Table 9, the number of fungi increased in the treatments T1 and T2, in comparison with the control. Fungi thrived after 90 days, from 5.35x 10^3CFU/g (soil) to 7.45x 10^3CFU/g (soil) and they still developed the days after. As discussed above, Guot decays faster, and the early emergence of the fungus has proven for that. The number of fungi increased more slowly in the treatment T2 than that in the treatment T1 after 90 days. However, the fungi number was higher in the treatment T2 than that in the treatment T1 after 180 and 270 days. It is clear that, mulch material stimulated the development of fungi, increasing diversity of microorganism in the soils.

Effect of mulch on the bioactivity of some microorganism groups

Results (Table 10) show that ratio of microorganism groups which have bioactivity was distinct as using different mulching materials. Of ten tested strains, the ratio of cellulose decomposing bacteria made up for more than 40%. The ratio of bacteria decomposing insoluble phosphate reached at 44% in the treatment T1, much higher than in the control (non-mulching) and in the treatment T3 (used tea tree wastes as mulching material). Ratio of actinomycetes which have bioactivity in the treatment T2 was higher than that in other treatments.

Table 10. The bioactivity of some microorganism groups (after 180 days).

Treatments	Microorganisms	Decomposition activity (%) per Total of strains isolate							
		Cellulose		***Insoluble phosphates***		***Starch***		***Mucous membrane***	
		Good	***Relatively good***	***Good***	***Relatively good***	***Good***	***Relatively good***	***Good***	***Relatively good***
T0 (Control)	Bacteria	1	20	12	16	0	0	0	0
	Actinomycetes	0	0	0	0	0	0	8	11
	Filamentous fungi	0	6	3	14	0	7	0	0
T1(mulching by Guot - 30 tons/ha/year)	Bacteria	44	32	5	44	2	6	0	0
	Actinomycetes	16	7	24	11	0	9	5	8
	Filamentous fungi	19	45	23	12	0	0	0	0
T2 (mulching by tea tree wastes - 30 tons/ha/year)	Bacteria	45	16	16	38	13	5	0	0
	Actinomycetes	12	24	38	16	0	34	38	14
	Filamentous fungi	2	0	23	11	2	6	0	0

Effect of mulching on the diversity of microorganisms in the soil

Analysis of the diversity and distribution of microorganisms in the soil which was mulched by distinct materials was performed.

Results in Table 11 show that 22 species were identified in samples. Of 22 species, twelve species belong to bacteria group, eight species are in the group of filamentous fungi, one is in *Actinomycetes* and one is in *Lipomyces*. The frequency of occurrence of strains was higher in the treatment T2 (used tea tree wastes to mulch) than in the treatment T1 which used Guot as mulching material. Notably, *Actinomyces* producing antibiotics strongly developed in the treatment T2. In contrast, *Lipomyces* producing polysacharide was more abundant in the treatment T1 than in the treatment T2.

Table 11. The diversity and distribution of microorganisms in the soil mulched by different materials (after 180 days).

No.	Group	Strains	The frequency of occurrence of strains*		
			T0 - Control (Non-mulching)	**T1 -** mulching by Guot *(30 tons/ha/year*)	**T2 -** mulching by tea tree wastes *(30 tons/ha/year*)
1	Cellulose-and insoluble phosphates-decomposing bacteria, and nitrogen-fixing bacteria	*Pseudomonas*	+	+++	+++
2		*Bacillus*	+	+++	+++
3		*Cellulomonas*	-	++	+++
4		*Enterobacter*	+	-	++
5		*Agrobacterium*	-	-	+
6		*Enterobacter*	+	++	+++
7		*Nitrosomonas*	-	-	++++
8		*Nitrobacter*	-	++	++++
9		*Achromobacter*	-	-	+++
10		*Azotobacter*	+	+	+
11		*Mycobacterium*	-	-	++
12		*Rhizobium*	+	++	++
13	Filamentous fungi decompose cellulose and insoluble phosphates	*Aspergillus*	-	++	+++
14		*Penicillium*	-	++	+++
15		*Trichoderma*	++	++	+++
16		*Fusarium*	+	+++	+++
17		*Mucor*	-	++	+++
18		*Rhizopus*	-	-	++
19		*Beauveria*	+	+	+++
20		*Mertahzium*	-	+	+++
21	*Actinomycetes*	*Actinomyces producing antibiotics*	-	+	++++
22	*Lipomyces*	*Lipomyces producing polysacharide*	-	+++	+

* so many: (++++), many: (+++), relative: (++), less: (+) and none: (-).

The positive effects of different mulches on soil physical properties such as maintaining greater soil moisture and moderating soil temperatures (Maggard *et al.*, 2012), improving soil organic matter (Ni *et al.*, 2016), increasing soil phosphorus content (Yang *et al.*, 2017) have been reported. In our study, mulch had a positive effect on the population of bacteria, fungi and actinomycetes as compared to unmulched plots. All the treatments with mulches favoured the population of fungi and actinomycetes. These results agree with previous studies, in which the microorganism population increased in the organic mulch treatments as compared to non-mulching (Muhammed *et al.*, 2015; Fehmi and Kong, 2012). Mulches create an environment favorable for the development of microorganisms in the underlying soil, that is moderate moisture and temperature (Harris, 1992). This suggested that increased bacteria population under the Guot mulch may be related to its faster decomposition. In contrast, an increase in the population of actinomycetes and fungi under the mulch of tea tree wastes after 270 days may be due to slow decay of this material.

Conclusions

In this study, the effect of microbial organic fertilizer on beneficial microorganisms have been analysed. The microbial organic fertilizer has a significant effect on the variation in the amount and composition of the microorganisms in the soil. Organic fertilizer at 3.0 tons per ha has significantly increased the number of bacteria, the number of actinomycetes and the diversity of soil microorganisms. The bacterial population has a dramatically increased in the treatment T3 (3.0 tons of organic fertilizers per ha), up to $14.32*10^6$CFU/g (soil). Of ten strains tested bioactivity in the treatment T3, 46% of the strains were cellulose decomposition, while the number of the strains decomposing insoluble phosphates, starch and mucous membrane was lower, 32%, 22% and 15%, respectively.

The effect of mulching material on the microorganisms in the soils have been also studied. The population of bacteria has thrived and stabilized early as guot was used to mulch. The bacterial population in the treatment T1 mulching by Guot at 30 tons/ha/year is higher ($11.3*10^6$CFU/g) than in the experiment mulching by tea tree wastes at 30 tons/ha/year ($10.86*10^6$CFU/g) after 270 days. The population of actinomycetes increased after 90 days and reached the highest level of $11{,}05*10^6$CFU/g (soil) after 270 days in the treatment T2 (mulched by tea tree wastes). Mulching by tea tree wastes most affected to the population of actinomycetes, in comparison with the number of bacteria and the number of fungi. Fungi thrived after 90 days and they still developed the days after.

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