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Effects of saprophytic microfungi application on soil fertility based on their decomposition properties

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Abstract

The objective of the research was to observe the effects of soil microfungi application on soil fertility. The soil fertility was determined based on nitrogen, phosphorus and potassium (NPK) content and plant (Lycopersicon esculentum Mill.) growth. Firstly, groups of microfungi were determined based on their decomposition properties on Coffea robusta. L leaf litters. The research covered several decomposition properties which were (1) substrate weight loss, (2) cellulase activity and (3) xylanase activity during the process of decomposition on the pure culture decomposition test. Secondly, the microfungi were grouped based on averages of all properties value paired in all possible ways. The result showed that there were 10 groups of fungi (P1 – P10) combined based on their decomposition ability. The groups of fungi (P1 – P10) were applied on sterile soils to determine the NPK content and on pots to observe L. esculentum Mill growth (plant height, leaf sizes, fresh and dry weight). The effects indicated majority groups of fungi were able to increase NPK content and growth parameters comparing to that of control. It concludes that the application of soil saprophytic microfungi on soils are possible to perform this process and able to increase soil fertility.

Keywords: weight loss, cellulase activity, xylanase activity, pure culture decomposition test

INTRODUCTION

Fungi and bacteria play a central role in litter decomposition processes in soil and will be very active in the present of plants and dead organic materials [1]. Fungi need organic nutrients as both an energy source and to provide carbon skeletons for biosynthesis like animals and most bacteria. An enormous range of organic compounds can be utilized by fungi, but this does not imply that all fungi can use all of the compounds. In general, fungi can only absorb small soluble nutrients such as monosaccharide and amino acids, or peptides composed of two or three amino acids [2]. Even disaccharides such as sucrose may need to be degraded to monosaccharide before they are taken up by most fungi. So, the nutrition of most fungi is strongly dependent on the release of degradative enzymes. One of the most important enzymes is cellulase because it can decompose cellulose, the most abundant natural polymer, representing about 40% of plant wall material. The other materials which are also plentiful in nature are lipid and xylan. Xylan is a homopolysaccharides consisting D-xylosa residues linked by ß-1,4 bond [3]. The hydrolyses of xylan requires the action of ß-1,4 xylanase and ß xylosidase enzymes. Fungi play the pre-eminent role in their breakdown.

Due to their characteristic digestion and nutrition properties, fungi are very important for materials supplies in nature required by plants. The decomposer activities of fungi in natural ecosystems are complex. Therefore it is almost impossible to define a niche of soil fungi in precise terms because it involves a combination of many factors. Consequently, this research was conducted in the laboratory condition in the pure culture decomposition test method [4] to give a limitation to the certain species, substrates, and environmental condition. Additionally, interactions between organisms are also under controlled.

In general, decomposers break down the proteins, starches, and other complex organic molecules. They convert compounds containing nitrogen, phosphorous, calcium, and sulfur into forms that can be utilized by plants. There was also evidence that the growth of fungi in soils has a very important role in soil carbon and nitrogen n turnover [5].

Although this theoretical aspects of decomposition are well known, little is known about the ability of a certain group of fungi in decomposing organic materials in sterile soils. Since the decomposition in nature is a complex process and involves many organisms, the role of a fungus in that process is not definitely understood. Therefore this research had been limited on sterile soil in order to understand the activities of fungi in term of their weight loss value, cellulase and xylanase activity.

MATERIAL and METHODS

The moist chamber technique modification of Cannon and Sutton [6] was used for isolating fungi from soil. Soils were collected from 10-20 cm soil depth from three points under Coffea robusta tree canopies. The soils were rinsed in sterile water and incubated in Petri dishes (9 cm dia) containing three layers of filter papers moistened with sterile water. Each Petri dish had 1 g soils and the petri dishes were sealed using parafilm and incubated in a light chamber with a 12 hr light: 12 hr dark cycle at 26+ 1 oC for 30 d. The soils were observed under a microscope daily for the presence of fungal spores from 3 d of incubation onwards up to 30 d. The soils were comminuted using sterile water and a scalpel, placed on a glass slide, stained with lactophenol and observed under a bright field microscope for the presence of fungal spores [7]. The isolated fungi were cultured and tested their decomposition abilities based on enzyme activities, weight loss of substrates and growth effect on plants. The data of all measurements were paired in all possible ways to get the average number. The grouping of pairing isolates was then used as

fungal group treatment applied in soil. Statistical analysis was applied in comparing the growth respond of plants due to fungal activity and designed within Completely Randomized Design (CRD). The differences among the treatments were tested using least significance difference test at 5%.

Cellulase activty measurenment

The study of cellulase activity was done by calculating reduction sugar from DNS method [8]. Firstly, the isolates were grown on the substrate of 10 g of Casia alata sterilized dry leaves for 7 days. Then, the samples were added with 10 ml citrate buffer at the pH of 6.0, vortexed for 15 minutes and centrifuged at 4-5oC for 5 minutes at 3500 rpm. Enzyme supernatants of 0.5 ml were mixed with 0.5 ml 0.1% carboxymethyl cellulose (CMC) in citrate buffers pH 6.0. The samples were incubated at room temperature for 30 minutes and the final reaction was added 2 ml 3.5-dinitrosalicylic acid. The samples were then warmed in boiling water for 5 minutes and freeze up at cold water for 20 minutes. The extracts were read on λ 575 using spectrophotometer. Sugar solution standard used was selobiosa sugar reduction in the interval of 0.1-0.5 mg. One unit of cellulose activity was defined as number of enzyme which released 1 µmol cellobiose within a minute at tested condition [9].

Xylanase activity measurenment

The study of cellulose activity was done by calculating reduction sugar from DNS method [8]. Firstly, the isolates were grown on the substrate of 10 g of sterilized dry leaves at room temperature for 7 days. Then, the samples were added with 10 ml citrate buffer at the pH of 6.0, vortexed for 15 minutes and centrifuged at 4-5oC for 5 minutes at 3500 rpm. Enzyme supernatants of 0.5 ml were mixed with 0.5 ml 0.1% oat spelt xylan in citrate buffers pH 6.0. The samples were incubated at room temperature for 30 minutes and the final reaction was added 2 ml 3.5-dinitrosalicylic acid. The samples were then warmed in boiling water for 5 minutes and freeze up at cold water for 20 minutes. The extracts were read on λ 575 using spectrophotometer. Sugar solution standard used was xylosa in the interval of 0.1-0.5 mg. One unit of xylanase activity was defined as a number of enzymes which released 1 µmol xylosa within a minute at tested condition [9].

Pure culture decomposition test (PCDT)

This method is modification of Osono and Takeda's method [4]. Firstly, microfungi isolates were grown on PDA. Secondly, C. alata dry leaves used for substrates on PCDT were cut on disc using cork borer. The disc size was 20 mm in diameter and prepared 7 discs as sub samples. The leaf discs were air dry at 40oC for 4 days and weighed to measure initial weight of leaf discs. The leaf discs were placed on top of moist filter paper on the base of plate and autoclaved at 120oC for 20 minutes. The sterilized leaf discs were then ready to be used as tested substrates. Inoculum which were tested their decomposition ability were cut on their growth side on growing media with a sterile cork borer (diameter 5.5 mm). The cut colony of agar were put on other plates and 7 sub samples of leaf discs were put on surrounding the agar. The plates were incubated in the dark at 20oC for 10, 20 and 30 days. Finally, the leaf discs were taken out, air dry at 40oC for 4 days and weighed. The weight loss value of leaf disc was based on percentage of its initial weight.

Nitrogen analysis

Sample of 10 g of dry soil were transfered to an 800-ml Kjeldahl flask. Materials should pass through a 20 –mesh sieve and added with 50 ml of the sulfuric-salicylic acid and mixed so as to bring the dry sample quickly into intimate contact with the reagent. The samples were allowed to stand overnight. The samples were added with 5 g of sodium thiosulfat and heated gently for about 5 minutes, and taken care to avoid frothing. After cooling, the samples were added with 10 g of the sulfate mixture, and digested in Kjeldahl apparatus at full heat. With soils added , the digestion is continued for 1 hour after the solution has cleared. When digestion is completed, the samples were cooled and added with 300 ml of distiled water and 100 ml of concentrated sodium hydroxide. The samples were added a large piece of mossy zinc and, with soils, 2 large teaspoons of glass beads. The samples were connected to distillation head, agitated, and distill off 150 ml into 50 ml of 2 % boric acid solution. Ten drops of the brom cresol green were added to methyl red indicator and titrate to the first faint pink with standard sulfuric acid. Blanks were run and the titration carried to the same end point [10].

Phosporus analysis

Determination of phosphorus in soils was firstly done by extracting soil. Two g air dry soils were put into a 100 ml erlenmeyer flask. The soils were then added 20 ml soil extraction solution which was prepared previously. The solution was then put on shaker for 10 minutes, filtered and collected the filtrates in a 100 ml erlenmeyer flask. Five ml filtrates were taken and put into a test tube and added 10 ml working solution. After 20 minutes the solution was transferred into a cuvet. After 30 minutes (no more than 1 hour) the value of % transmittance could be read on spectrophotometer on 800 nm. We need to use blanks to make a 100% T standard [10].

Potassium analysis

Potassium analysis was done as following: 0.25 g sample of finely ground soil was transferred to a platinum crucible. The samples were added 2 ml of water, 2 ml of concentrated sulfuric acid, and 4 ml of hydrofluoric acid. The samples were mixed and heated at low temperature on a hot plate until the volume was reduced to about half the original and then increased the temperature until fumes of sulfuric acid are given off. The sides of the crucibles was cooled and washed down with 2 ml of dilute hydrochloric acid. The samples were evaporatedd again to strong fumes of sulfuric acid but should not be taken to complete dryness. The samples were cooled slightly and added several ml of water. The samples were filtered into a 100-ml volumetric flask and washed with several portions of approximately 0.1 N hydrochloric acid. The samples were diluted to volume and potassium and sodium were determined on the flame photometer [10].

RESULTS

Cellulase activity

The results of cellulase activity test showed that all isolates which were able to decompose leaf litter were not all produce cellulase enzyme. The test indicated that there were 6 among 11 isolates releasing cellulase enzymes. The results were analyzed after 7 days of incubation and based on pure culture decomposition test. The isolates which were able to

show cellulase activity were Fusarium sp. 1, Fusarium sp. 2, Fusarium sp. 3, Humicola sp., Arthrinium sp. and Fusarium sp. 4 (0.05, 0.035, 0.034, 0.017, 0.01, and 0.005 unit/ml, respectively) (Table 1).

Table 1. Activity of cellulase and xylanase on pure culture decomposition test

No	Isolates	Enzyme activity (units/ ml)	
		Cellulase	Xylanase
1	*Synchepalastrum* sp.	0	0
2	*Fusarium* sp. 1	0.050	0
3	*Fusarium* sp. 2	0.035	0.109
4	*Fusarium* sp. 3	0.034	0.036
5	*Fusarium* sp. 4	0.005	0
6	*Rhizopus* sp. 1	0	0
7	*Rhizopus* sp. 2	0	0.012
8	*Absidia* sp.	0	0.002
9	*Arthrinium* sp.	0.010	0
10	*Aspergillus* sp.	0	0
11	*Humicola* sp.	0.017	0.003

Xylanase activity

The results of xylanase activity test showed that there were only 5 isolates indicating xylanase activity which were Fusarium sp. 2, Fusarium sp. 3, Rhizopus sp. 2, Absidia sp. and 0.003. Isolate Fusarium sp. 2 showed the highest activity of enzyme (0.109 units / ml) and were followed by Fusarium sp. 3, Rhizopus sp. 2, Humicola sp., and Absidia sp. with 0.036, 0.012, 0.003, and 0.002 units/ ml, respectively (Table 1).

Weight loss value

In general, all isolates demonstrated their decomposing ability during the time of incubation. All isolates indicated that they were able to decompose the leaves by decreasing the final weight and the initial weight of substrates. The rates of decomposing ability of all isolates were likely to increase as the time of incubation increased (Table 2). The percentage of weight loss of leaves per day ranged from 0.693% to 1.337%. Decomposing ability of Fusarium sp. was found to be the highest among others which was 1.337% per day, while Absidia sp. was the lowest with 0.693% per day.

Table 2. Weight loss of coffee leaves on pure culture decomposition test

No	Isolates	Weight loss (mg)						Averages/day (%)
		10 Days	%	20 days	%	30 days	%	
1	Synchepalastrum sp.	6.100	10.7	8.300	18.7	12.733	25.4	0.951
2	Fusarium sp. 1	5.867	10.3	7.800	17.4	14.033	30.4	0.971
3	Fusarium sp. 2	5.367	11.9	10.040	7.44	14.533	23.1	0.777
4	Fusarium sp. 3	8.967	17.7	13.900	27.5	11.833	26	1.337
5	Fusarium sp. 4	5.733	11.8	6.733	17.4	14.567	26.4	0.977
6	Rhizopus sp. 1	5.233	10.8	15.867	21.0	12.367	17.7	0.907
7	Rhizopus sp. 2	8.367	17.8	9.367	20.0	12.400	21.1	1.161
8	Absidia sp.	6.400	10.4	5.467	10.7	7.267	15.1	0.693
9	Arthrinium sp.	5.700	13.7	12.733	21.2	13.900	25.3	1.091
10	Aspergillus sp.	6.733	14.8	12.733	17.2	12.100	21.8	1.022
11	Humicola sp.	8.267	13.4	8.300	19.6	6.233	13.1	0.919

Grouping of microfungi based on decomposition properties showed the result as follow (Table 3).

Table 3. Microfungi grouping

No	Groups	Isolates
1	P0	No isolates
2	P1	Fusarium sp. 2. Fusarium sp. 3
3	P2	Fusarium sp. 2. Fusarium sp. 3. Rhizopus sp. 2
4	P3	Fusarium sp. 2. Fusarium sp. 3. Fusarium sp. 3. Arthrinium sp.
5	P4	Fusarium sp. 2. Fusarium sp. 3. Aspergillus sp.. Humicola sp.
6	P5	Fusarium sp. 2. Fusarium sp. 3. Fusarium sp. 4. Rhizopus sp.
7	P6	Fusarium sp. 2. Fusarium sp. 3. Fusarium sp. 1. Arthrinium sp.
8	P7	Fusarium sp. 2. Fusarium sp. 3. Fusarium sp. 4 Fusarium sp. 1
9	P8	Fusarium sp. 2. Fusarium sp. 3. Rhizopus sp. 1. Absidia sp.
10	P9	Fusarium sp. 2. Fusarium sp. 3. Rhizopus sp. 1.
11	P10	Fusarium sp. 2. Fusarium sp. 3. Synchepalastrum sp.. Absidia sp.

Major nutrients of soil

Effect of application of fungi spore inoculums on sterile soils after 7 days on incubation showed that there was an indication of several variation changes of nitrogen, phosporus and potassium total. Also, the data indicated a positive respond about the application of inoculums toward the total value of NPK (Table 4).

Table 4. Nitrogen. Phosporus and Potassium total on sterile soil following fungal inoculums application after 7 days

Treatment	Total		
	N (%)	P (mg kg^{-1})	K (mg kg^{-1})
P0	0.41	8.36	575.31
P1	0.50	20.90	638.43
P2	0.36	16.29	564.68
P3	0.43	18.99	623.10
P4	0.42	62.43	1574.60
P5	0.51	61.70	1226.08
P6	0.40	63.19	1717.46
P7	0.45	17.82	655.78
P8	0.45	63.30	1733.33
P9	0.36	63.05	1574.59
P10	0.49	16.46	688.46
LSD 5%	0.147	7.176	99.345

Table 4 shows 90% of groups of fungi gave a positive impact to the total NPK and groups of P4, P6, P8 and P9 showed a relatively high effect to the total NPK. This phenomenon indicated that most groups of fungi were able to decompose organic materials in sterile soils. Moreover, it has given an idea that applying fungi spore inoculums on soil will open a prospective study about fungi effects on soil fertility. It has also described an understanding that the process of decomposition could take place naturally in sterile soils and is possible to occur in nature.

Growth parameters

The result shows that the effect of saprophytic microfungi application on plant growth was positive (Figure 1). The values of plant height, leaf length and width were higher than that of control. The height is increasing until the sixtieth day and slowly goes down afterward.

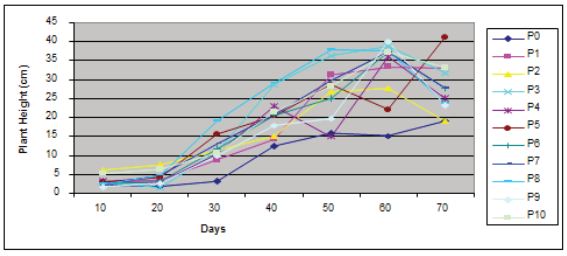

Figure 1. Plant height of L. esculentum Mill. as a result of microfungi application on soil.

The same phenomenon happens on length and width of the leaves after they are treated with the groups of microfungi (Figure 2). All groups give better effect on all parameters comparing to the control.

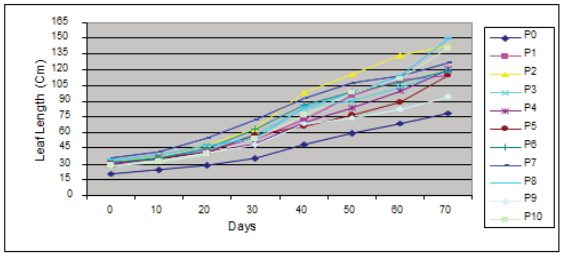

Figure 2. Leaf length of L. esculentum Mill. as a result of microfungi application on soil.

The leaf length is still increasing until the sixtieth day and all treatments give better effects comparing to the control and P8 give the best leaf length (Figure 3).

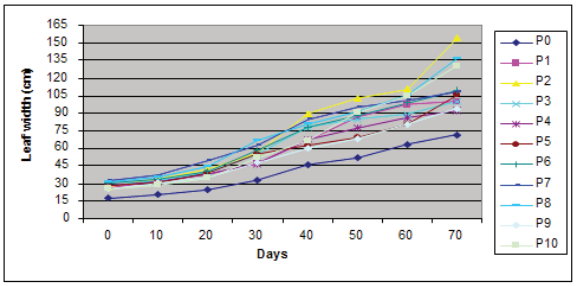

Figure 3. Leaf width of L. esculentum Mill. as a result of microfungi application on soil.

Moreover, the activity of groups of fungi also increases the fresh weight of the plants. There are 8 among 10 groups of fungi able to increase the fresh weight comparing to the control (Figure 4). P8 is the group which causes increasing the fresh weight optimally.

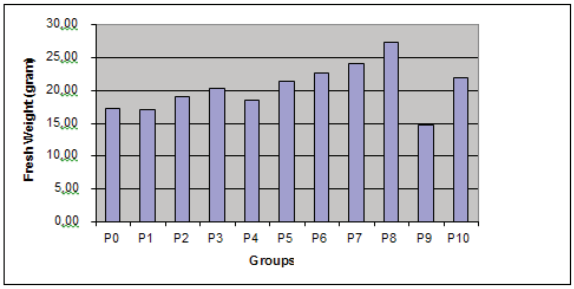

Figure 4. Fresh weight of L. esculentum Mill. as a result of microfungi application on soil.

In the observation on plant dry weight, it indicates that there are 7 among ten groups of fungi are able to increase the plant dry weight comparing to that of control (Figure 5). It implies that applications of soil fungi on media of the plant growth are possibly to increase the fresh weight.

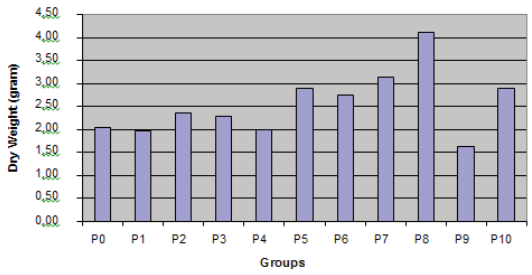

Figure 5. Dry weight of L. esculentum Mill. as a result of microfungi application on soil.

DISCUSSION

The result suggests that application of saprophytic microfungi on sterile soils indicates that the role of fungi as a decomposer agent in soil is clearly proven. It was also indicated by the fact that the NPK content of soils was significantly increasing when the soils were applied by fungal biomasses and the plant growth abruptly increased. Theoretically, all minerals or chemical substances in soils were derived from breaking down of complex organic compounds conducted by soil fungi. Soil fungi are able to release extracellular enzymes which degrade organic dead materials such as leaf litters into simple inorganic substances.

The presence of inorganic substances in soil due to fungal activity indicates that the condition of soil is good because cycle nutrient of soil can occur. The research shows a promising method which needs further investigation to get a significant impact in the field. We believe that this method is still a preliminary study and needs more extensive explorations. However, we assume that increasing NPK content in soil and the growth of plants is due to soil microfungi activity.

In the condition where most agricultural systems use chemical fertilizer, the application of groups of saprophytic microfungi as a decomposition agent on soil will be an alternative choice to increase soil fertility. The uses of artificial inputs in the agricultural systems have possibly reduced the total productivity of agriculture net. Mader et al. reported that agriculture product of organic agriculture systems and conventional in center Europe has decreased to 20% even though fertilizers input have been reduced 34 – 53% and pesticide up to 97%. From that research was also reported that external input influences less biodiversity and soil fertility [11]. This report implies that internal input or intrinsic input of soil would be more beneficial in term of soil fertility. Fungi are one of soil microorganisms taking part in maintaining nutrient cycle in soil.

CONCLUSION

It is concluded that soil microfungi showed specific decomposition properties which include cellulase and xylanase activity and weight loss value of substrates. Application of selective saprophytic microfungi based on decomposition properties on soil was able to increase soil fertility in the term of NPK soil content and plant growth of Lycopersicum esculentum Mill.

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