Effect of indigenous cellulolytic fungi enhancement on organic carbon and soybean production on peat soil

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Abstract. The two main issues of any application of agricultural technologies in empowering peatlands for crop cultivation are their impacts on carbon storage and crop production. Indigenous cellulolytic fungi enhancement in peat is a technology to increase crop production on peat. The cellulolytic fungi decay lignocellulosic complex to release humic acids and improve soil fertility. We conducted a greenhouse (GH) experiment to study the organic C and soybean yield on enhanced peat soils with indigenous cellulolytic fungi. The experimental design to test soybean performance on peat was a completely randomized design (CRD) 3 treatments, i.e., fibric, hemic, and sapric of peat. There were six pots of each peat that were enriched by consortia of cellulolytic fungi Penicillium singorense, Aspergillus aculeatus, and Trichoderma sp. On average, CO₂ flux increased from 0.39 mg CO₂/kg/day to 0.72 mg CO₂/kg/day, and the organic carbon and fiber content reduced 13.3% and 26.2%, respectively of all peat soils enriched by indigenous cellulolytic fungi. The highest beans yield of soybean was 3.56 g/population, equivalent to 1.25 tons/ha, was on sapric peat enriched by the cellulolytic fungi.

Keywords: peat soils, cellulolytic fungi, Organic C, CO₂ flux, soybean

1. Introduction

Peat soil cultivation for agriculture will face the obstacle of physical, chemical, and biological characteristics, such as subsidence and irreversible shrinkage, excess water volume, low pH, low nutrients content, high iron (Fe²⁺), and high salinity (1); (2). For the peatlands to become productive agricultural land, agricultural experts continue to strive to find appropriate and sustainable management technologies. Nowadays, microbial fertilizer technology could be one of the most promising technology to improve crop productivity in an eco-friendly manner (3).

Cellulolytic fungi are one of the microorganisms having the potential to become active ingredients for microbial fertilizers. They produce cellulase enzymes (4); (5) to break down lignocellulosic materials consist of cellulose, hemicellulose, and lignin. The lignocellulosic complex decomposing will release many essential nutrients and humic acid, which can increase the fertility of peat soil (6); (7). The lignocellulosic complex is the main ingredient of peat soils, especially in tropical peat soils 8).

Enrichment of indigenous cellulolytic fungi will increase their activity in the peat soil. It is well known the microbial activity is a source of CO₂ (9). The higher the microbe activity in peat soil, the higher the CO₂ emissions to the atmosphere. Due to that, behind improving peat soil fertility, the utilization of microorganisms like fungi as microbial fertilizer is likely to increase CO₂ flux from peat
(10). Solikhah et al. (10) reported cellulolytic microorganisms could decompose organic matter in peat soils up to 86% within six weeks.

About 10 million ha peat soils are the hope of the future to be developed as productive agricultural land in Indonesia (12). Soybean is one of the commodities targeted to be self-sufficient by the Indonesian Ministry of Agriculture, needs to be tested for its growth and production on peat soils. Some research results showed that soybean planting, supported by bio-fertilizers and water management was able to produce well on peat soils (11); (12).

Therefore it is necessary to study the impact of enhancement of peat indigenous cellulolytic microorganisms both on peat as agricultural land in the hope of the future and peat as a carbon sink. We conducted a greenhouse experiment to study the organic C and soybean yield on enhanced peat soils by indigenous cellulolytic fungi.

2. Materials and Methods

2.1. Preparation of peat samples

The peat samples for greenhouse experiments (GH) was derived from the Batara sub-district, Tanjung Jabung Barat District, Jambi Province. It was carried with a waterproof plastic bag and sent by an expedition. Three types of sampled peats were sapric, hemic, and fibric. The determination of peat kinds was by the squeeze method (15). When the peat was squeezed, ± 1/3 peat came out between the fingers was fibric, ± 1/2 of the peat came out between the finger was hemic, and ± 2/3 of the peat came out between the fingers was sapric. The characteristics of the peats are presented in Table 1.

Table 1. Some characteristics of peat types of Tanjung Jabung Barat, Jambi Province

| Peats     | Ash (%) | pH     | Organic C (%) | Fiber (%) |
|-----------|---------|--------|---------------|-----------|
| Fibric    | 9.13    | 3.73   | 52.70         | 66.64     |
| Hemic     | 15.32   | 3.49   | 49.11         | 51.82     |
| Sapric    | 16.73   | 4.15   | 48.29         | 10.72     |

2.2. Cellulolytic fungi

Cellulolytic fungi in the experiment were the collection of the Indonesian Industrial and Beverage Crop Research Institute. They were *Penicillium singorense*, *Aspergillus aculeatus*, and *Trichoderma* sp. The last one was still in the process of the analysis of molecular to identify the species. They were indigenous cellulolytic fungi of the peats that were used for GH testing. To treat peat soils, the consortia of the three cellulolytic fungi were used.

2.3. Greenhouse (GH) experiment

The experimental site was in the GH of Indonesian Industrial and Beverage Crops Research Institute, Sukabumi, which was started from March to November 2017. We treated three-peat soils, fibric, hemic, and sapric, by the consortia of the cellulolytic fungi, *Penicillium singorense*, *Aspergillus aculeatus*, and *Trichoderma* sp. There were two parts of the experiment. The first experiment was to study the cellulolytic fungi effect on organic C of peats, while the second experiment was on the soybean production. The separated experiment was due to the soybean could not grow on peat if there was no lime added.

The experimental design to test soybean performance on peat was a completely randomized design (CRD) with 3 treatments, i.e., fibric, hemic, and sapric of peat. There were six pots of each peat that were enriched by consortia of cellulolytic fungi, a concentration of $10^8$ CFU/mL, by adding 25 mL to 5 kg peat soil in each pot. The inoculated peat was stirred evenly and incubated in GH. Thirty days after fungi inoculation, the peat soil was sampled ± 100 g from each the pot to observe CO₂ gas flux.
Next, lime was added to all inoculated peat as much as 25 g /pot equivalent to 3 tons/ha (13) and re-incubated for two weeks. Then the soybean (Dana varieties) on peats was grown and the moisture content of peats was kept by maintaining a 5 cm water level at the bottom of the pot. The parameters observed were soybean growth and production.

2.4. Observation of flux of CO₂, organic C, and fiber content of peat soils

The measurement of the CO₂ gas flux from peat soils (Sampled 30 days after peat enrichment with cellulolytic fungi) was performed by the Verstraete method [Anas in (14)]. Into a bottle of 1 L, we put 100 g of peat soil together with two small beakers containing 5 ml of 0.2 N KOH and 10 ml of distilled water, respectively. The bottle was closed by a plastic sheet until it was airtight, then it was incubated at temperature 28 °C - 30 °C, in a dark place for seven days and fourteen days. At the end of the incubation, the titration method was used to measure the CO₂ gas amount. Two drops of phenolphthalein were added to the KOH beaker and then titrated with HCl until the red color disappeared and the required HCl volume was recorded. Two drops of methyl orange was added. It was titrated again with HCl carefully until the yellow turns pink. The amount of CO₂ absorbed by KOH was related to the amount of HCl used in the second stage of titration.

The CO₂ gas measured in the bottle containing peat minus by in the control bottle (empty bottle + beaker containing KOH), was the CO₂ flux from the peat soil. The CO₂ gas from a kilogram of moist peat soil per day (r) was calculated by the following formula:

\[
r = \frac{(a - b) \times t \times 120}{n} \quad \ldots(1)
\]

Where a is mL HCl for soil samples, b is mL HCl for control, t is normality HCL, and n is the number of incubation days.

The changes of organic C and fiber content of the peat soils were other indicators of the effect of indigenous cellulolytic fungi enhancement on C dynamic in peat soil. For those reasons, the organic C was analyzed by the LOI (the loss on ignition) method, while the fiber content was analyzed by the Hiroki and Watanabe method (15). For the analysis, the sampling of the peat soil was performed after harvesting soybean.

3. Results and Discussions

3.1. Flux of CO₂

Rising CO₂ emission from peat soil due to increasing microorganism activity by enhancement of cellulolytic fungi are difficult to avoid. The CO₂ flux measured after 30 days of fungi consortia inoculation showed that case. Within fourteen days of measurement, on average CO₂ gas from the peat soils increased 85% by enrichment of cellulolytic fungi consortia (Table 2). Sources of the peat CO₂ flux were microbial organic matter mineralization and microbial respiration (16); (17). Although microorganism activities tended to increase CO₂ emissions, the highest CO₂ gas emissions on tropical peat soils are due to plant root respiration (18).
Table 2. Total CO$_2$ flux/kg peat/14 days and average CO$_2$ flux/day from peats enriched with indigenous cellulolytic fungi

| Inoculated peats | Total mg CO$_2$ flux from a kg peat in 14 days | Avarage mg CO$_2$ flux/kg peat/day | CO$_2$ flux increase from N to I (%) |
|------------------|-----------------------------------------------|-----------------------------------|-------------------------------------|
|                  | No Fungi Inoculation (N)          | Fungi Inoculation (I)            | No Fungi Inoculation (N)          | Fungi Inoculation (I)            |                                      |
| Fibric           | 4.29 b                           | 8.03                             | 0.31 b                            | 0.57                             | 86.96                                |
| Hemic            | 4.99 ab                          | 9.01                             | 0.36 ab                           | 0.64                             | 80.37                                |
| Sapric           | 6.91 a                           | 12.97                            | 0.49 a                            | 0.93                             | 87.84                                |
| Average          | 5.40                            | 10.00                            | 0.39                              | 0.72                             | 85.06                                |

*Significantly different (at P 0.05 according to Tukey HSD) between peat types.

3.2. Organic C and fiber content

On average, the enhancement of indigenous cellulolytic fungi reduced the organic C (Figure 1) and fiber content (Figure 2) of the peat soils by 13.3% and 26.2%, respectively, with more reduction in fibric peat. Saffari et al. (5) reported that fungi produce more cellulose enzymes. That ability makes them more effective in degrading the lignocellulosic complex as the main element in tropical peat soils (8). Degradation of the lignocellulosic complex and increase of microbial respiration will release a lot of CO$_2$ to the atmosphere (19); (20). These are the cause the organic C and fiber of peats decrease.

![Figure 1. The change of the organic C content in peat soils due to indigenous cellulolytic fungi enhancement](image-url)
Figure 2. The change of fiber content in peat soils due to indigenous cellulolytic fungi enhancement

However, any changes in peat soil utilization will affect the carbon stores, and the greenhouse gas emissions of peat, as well as the use of bio-fertilizers, tended to accelerate the release of C into the atmosphere (21). For that reason, enhancement of indigenous peat soil microbes to improve peat soil fertility should be careful, although the use of microbes as a fertilizer is friendly environmental fertilizer technology. Further research is necessary to study more on how to cultivate the peat soil better because in the future, potential lands such as peat soil are needed to support Indonesian national food security.

3.3. Soybean growth and yield

The number of pods and bean production of soybean was higher on sapric and hemic than fibric peat soil enhanced by indigenous cellulolytic fungi (Table 3). There was no significant difference in the bean yield between the sapric and hemic enhanced by cellulolytic fungi.

Table 3. Growth and production of soybean on each peat enriched with indigenous cellulolytic fungi

| Peats    | Soybean growth | Soybean Production |
|----------|----------------|--------------------|
|          | Hight (cm)     | Branch             | Pods    | Beans/pot (g) | 100 beans (g) | Beans/ha* (ton) |
| Sapric   | 119.53         | 4.00               | 22.3 ab | 3.56 a        | 13.68         | 1.25           |
| Hemic    | 119.83         | 4.67               | 30.0 a  | 3.43 a        | 14.00         | 1.22           |
| Fibric   | 121.67         | 5.00               | 20.7 b  | 3.19 b        | 15.59         | 1.12           |
| Sig.     | Sig = Significantly different (at P 0.05 according to Tukey HSD) between peat types. | * assumed the soybean population was 350,000 / ha |

| Sig.     | 0.013 0.026 | |

These results showed that the soybean could grow and produce on fibric, hemic, and sapric soil enriched by indigenous cellulolytic fungi. However, the better result was soybeans on hemic and sapric than fibric. The use of fungi bio-fertilizer and lime has not yet shown an encouraging potential for soybean cultivation in peat soils because the yield of soybean on average was 3.4 g/pot, or only equivalent to 1.2 tons/ha. An experiment of Asie et al. (22) found the soybean planted on peat soil ameliorated by fish pond sludge could produce bean 7.3 g/pot.
4. Conclusions
Enhancement of indigenous cellulytic fungi increased CO₂ flux from peat soils from 0.39 mg CO₂/kg peat /day to 0.72 mg CO₂/kg peat /day, and they reduced the organic C and fiber content of peat soils on average 13.3% and 26.2%, respectively. The organic C was decreased in fibric 9.7%, hemic 15.7%, and sapric 14.5 %, and the fiber content was decreased 41.6% in fibric, 33.2% in hemic, and 2% in sapric. Enhancement of the indigenous cellulolytic fungi of peat had a better effect on the soybean yield on hemic and sapric peat than on fibric peat. The soybean yield on the two peats was 3.56 and 3.43 g/population, equivalent to 1.24 and 1.22 tons/ha, approximately.

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