Effect of *Trichoderma* Addition on *Sargassum* Organic Fertilizer

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**Abstract.** *Trichoderma* is a fungus that can function as decomposers, biological agents, and stimulators of plant growth, bio fungicides so that agricultural plants can avoid diseases caused by poisonous fungi. This fungus can live in media containing seaweed by breaking down the seaweed's carbohydrate macromolecules into simple sugars. *Sargassum* seaweed is now starting to be used as raw material for organic fertilizers and *Trichoderma* can be added to a certain amount of organic fertilizer formulations to produce biological fertilizers. For this reason, this study aims to find the best time to obtain the highest density of *Trichoderma* in organic fertilizer *Sargassum* and its characteristics. The highest density of *Trichoderma* in *Sargassum* SLF which received additional silage was obtained on the 21st day namely 1.3x10^6 cfu/mL, whereas in *Sargassum* SLF without silage on the 7th day namely 46x10^6 cfu/mL. Based on the comparison of chemical parameters between *Sargassum* SLF without silage and commercial SLF, it was known that *Sargassum* SLF was better in terms of c-organic content, macronutrients (P and K), and minerals (Na, Mg, S, Fe, Mn, Cu, Zn, B, Al, Cd, and Mo).

1. Introduction
Seaweed can be used directly as a soil conditioner or fertilizer [1], seaweed extract has also been widely marketed as fertilizer [2], because it contains minerals and growth-promoting hormones needed by plants, for growth and crop yields [3]. Type of fertilizer preparation can be in the form of liquid seaweed fertilizer (LSF), seaweed liquid fertilizer (SLF), liquid fertilizer (LF), and chopped powdered algal manure [2]. The raw material used for some commercial organic fertilizers *Sargassum* made from brown seaweed *Sargassum* sp. which is now widely recognized in the international market are Maxicrop (United States), Kelpar (South Africa), Algifor (UK), Boost (AS), Seaweed Extract, and others. While the raw materials are used for commercial organic fertilizers of which the type *Sargassum* polycystum, Ascophyllum nodosum, Macrocystis pyrifera, Ecklonia maxima, Durvillaea potatorum, and *Durvillaea antarctica* [4].

Chemical fertilizers that have been used for a long time and are often excessive have led to the emergence of various kinds of side effects such as serious crop diseases, deterioration of soil quality, and poor seed growth [5]. Utilization of *Trichoderma* spp. to help deal with environmentally friendly plant health needs to be supported. *Trichoderma* spp. is a species of cellulose-decomposing fungi that is also antagonistic and easily found in organic soil. This mushroom is frequently used for biological
control against rhizosphere pathogens and the phyllosphere. Biological control agencies are currently a concern because they are cheap, easily obtained, and safe for the environment. *Trichoderma* spp. including types of parasitic fungi can attack and take nutrients from other fungi [6], [7]. The commercial *Trichoderma* bioinoculant is now readily available.

The various mechanisms that *Trichoderma* can carry out on plant growth can support increased nutrient uptake, increase carbohydrate metabolism, photosynthesis, and phytohormone synthesis. *Trichoderma* can stimulate plant growth by affecting the balance of hormones such as IAA, gibberellin acid, and ethylene [8]. The volatile organic compound produced by *Trichoderma* is able to actively encourage plant growth in its absence of physical contact between fungi and plants. This non-contact induction may be caused by several different compounds and mechanisms. There have been reports that 6-pentyl-2H-pyran-2-one (6-PP) from the *Trichoderma* strain played an important role in growth promotion, however, other reports stated there was no correlation [9]. Enzymatic hydrolysis of Sargassum sp. by Tricodherma was reported to be able to produce simple sugars at a temperature of 28°C and pH of 5.77 for 4 days and resulted in a total sugar of 3.01 g/L and total reducing sugar of 4.26 mg/L [10]. Based on the regulation of the Minister of Agriculture of the Republic of Indonesia Permentan 70/2011, the maximum density of fungi in biofertilizer 1x10⁴ to 1x10⁵ propagul/g [11]. To get the best period for using Sargassum SLF containing Trichoderma, this research aimed to find the best incubation time for Tricodherma in Sargassum SLF.

2. Material and method

2.1. Raw Material

The material used was seaweed *Sargassum* sp. obtained from Binuangeun beach, Banten, microorganism used in research *Trichoderma* sp. molasses and silage used for fertilizer formulation, and some chemicals for analysis.

2.2. Method

2.2.1. Sap Sargassum preparation

As much as 1 kg of fresh *Sargassum* that had been washed soaked in 0.3% KOH with a ratio of 1:10 (w/v) for 5 nights. It was boiled for 1 hour at 80-90°C then filtered to separate the *Sargassum* sap after reaching room temperature. The resulting sap was stored in a plastic bottle at cold temperatures.

2.2.2. *Trichoderma* isolate preparation

*Trichoderma* isolates were propagated on Oxoid PDA (Potato Dextrose Agar) medium, incubated for five days, then the isolates were transferred aseptically into Oxoid PDL (Potato Dextrose Liquid) in an Erlenmeyer flask and homogenized with a speed shaker of 150 rpm for 4 days at room temperature [6].

2.2.3. Sargassum fertilizer preparation as *Trichoderma* isolate media

The treatment in this study was *Trichoderma* media using *Sargassum* fertilizer with the addition of silage (SS) and without the addition of silage (TS). The SS formulation was prepared by mixing homogeneously, respectively, 71.6% of the sap of *Sargassum*, 21.4% of silage, and 7% of the molasses. The TS formulation was prepared by mixing homogeneously 93% of the sap of *Sargassum* and 7% of the molasses, respectively.

2.2.4. Application of *Trichoderma* on *Sargassum* fertilizer

Application of *Trichoderma* sp. on *Sargassum* fertilizer was carried out as much as possible 5% of the weight of the fertilizer used. *Trichoderma* isolate was poured into each beaker with an aerobic circulation and the fungi density was observed on days 0, 7, 14, 21, and 28. In the highest observation results, the parameters of growth hormone levels (cytokinins, gibberellins, and auxin) were measured, pH, C-organic levels, macronutrients (N, P, K) and micronutrients (Na, Ca, Mg, S, Fe, Mn, Cu, Zn, B, Al, Cd, and Mo).
3. Result and discussion
Research on the growth of *Trichoderma* spp. in different media has been reported. PDA and wheat bran were stated the best medium for *Trichoderma*, whereas in cornflour and carrot broth it did not grow well [12]. The life cycle of *Trichoderma* is quite short and achieves 2 cm/day growth under optimal conditions. The best temperature for growing sprouts is between 20-30°C and pH 5.5-7.5. The nutritional intake for *Trichoderma* is a dead organic matter or other fungi [9], [10].

3.1. Sargassum LSF containing silage
Many nutrients in the soil are slightly soluble or even insoluble, so that circulation nutrients in the soil have limitations. *Trichoderma* can excrete organics acid to dissolve minerals and release nutrients thereof into the soil rhizosphere. The extracellular enzymes which are the secretions of *Trichoderma* are sucrase, urease, and phosphatase. *Trichoderma* can decompose nitrogen compounds into simple nitrogen elements including NO2. Even the presence of this fungus would allow a reduction in the use of nitrogen-based fertilizers [5].

Figure 1 shows the density of *Trichoderma* grown in Sargassum SLF media with added silage. It appears that on the 21st day the highest density is reached, slowly since the 0th day of inoculation there is an increase in *Trichoderma* density and it is estimated that it will increase rapidly in the range of days 14 and 21. Then there was a rapid decrease in density between the 21st and 28th days. This shows the need for planting on the 14th and 28th day ranges to observe the length of time *Trichoderma* is in solid condition in the media. This will be useful to provide live information from the Sargassum SLF added silage.

![Figure 1. The density of Trichoderma in Sargassum SLF containing silage](image)

3.2. Sargassum LSF without silage
Genus *Trichoderma* is also characterized as hyperparasitism by releasing cell wall breakdown enzymes such as chitinase, cellulase, xylanase, glucanase, and proteinase. *Trichoderma* species absorbed nutrients through soil microbial degradation cells, causing changes in the structure of the soil microbial community [5].

Figure 2 shows the density of *Trichoderma* grown in Sargassum SLF media without silage added. It appears that on the 7th day the highest density is reached, it is estimated that it will increase rapidly in the range of the 1st and 7th days. Then there is a rapid decrease in density between the 7th and 14th days. This shows the need for planting on the 1st and 14th day ranges to observe the length of time *Trichoderma*
is in solid condition in the media. It will be useful to provide live information from the Sargassum SLF without silage added.

**Figure 2.** The density of *Trichoderma* in Sargassum SLF without silage

### 3.3. Comparison with commercial SLF

Many *Trichoderma* species are capable of producing auxins phytohormone indole-3-acetic acid (IAA) and its presence affects root growth. Production of *Trichoderma* from IAA is strain-dependent and has various external stimuli. In fungi, environmental factors, including pH and temperature impact the biosynthesis of IAA. Volatile organic compounds become growth stimulants in bacteria and fungi. However, IAA production is not the main determinant of the initiation of plant growth. The reverse mechanism, that microbial-induced plant growth may occur, may involve IAA but not as a key determinant [9].

**Table 1.** Chemical characteristics of Sargassum SLF with the highest *Trichoderma* density

| Parameter     | Sargassum SLF | SLF Commercial |
|---------------|---------------|----------------|
| Cytokinin (%) | 0.003 ± 0.0001 | 0.003          |
| Gibberellin (%) | 0.002 ± 0.0001 | 0.002          |
| Auxin (%)     | 0.002 ± 0.0001 | 0.002          |
| pH            | 6.20 ± 0.00   | 8.00           |
| C-organic (%) | **1.11 ± 0.12** | **0.25**       |
| Total N (%)   | 0.21 ± 0.01   | 0.78           |
| P2O5 (%)      | **0.15 ± 0.06** | **0.04**       |
| K2O (%)       | **1.13 ± 0.01** | **0.41**       |
| Na (%)        | 0.15 ± 0.00   | 0.04           |
| Ca (%)        | 0.14 ± 0.10   | 0.37           |
| Mg (%)        | 0.09 ± 0.01   | Nd             |
| S (%)         | 0.09 ± 0.01   | 0.01           |
| Fe (ppm)      | 24.50 ± 26.16 | Nd             |
| Mn (ppm)      | 2.50 ± 0.71   | 0.40           |
Based on the comparison of chemical parameters between Sargassum SLF without silage and commercial SLF, it was known that Sargassum SLF is better in terms of c-organic content, macronutrients (P and K), and minerals (Na, Mg, S, Fe, Mn, Cu, Zn, B, Al, Cd, and Mo). According to the Regulation of the Minister of Agriculture of the Republic of Indonesia number 70/Permentan/SR.140/10/2011, the maximum incubation time of Trichoderma in the SLF Sargassum can be a sign of the period of its use for cultivated plants. For Sargassum SLF media that contain silage, biofertilizers should be used between 7 and 21 days after inoculation. As for Sargassum SLF media without silage, it should be used before the 7th day of inoculation.

4. Conclusion
The highest density of Trichoderma in Sargassum SLF which received additional silage was obtained on the 21st day namely 1.3x10^6 cfu/mL, whereas in Sargassum SLF without silage on the 7th day namely 46.5x10^6 cfu/mL. Based on the comparison of chemical parameters between Sargassum SLF without silage and commercial SLF, it was known that Sargassum SLF was better in terms of c-organic content, macronutrients (P and K), and minerals (Na, Mg, S, Fe, Mn, Cu, Zn, B, Al, Cd, and Mo).

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