Improving the growth of clove seedlings by the application of seaweed waste as organic fertilizers

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Abstract. One of the problems in providing quality clove seedlings is slow growth of clove seedlings and the unavailability of technology that can accelerate seedling growth for planting. Based on this, research has been carried out which aims to obtain the concentration and frequency of providing appropriate seaweed organic fertilizer to accelerate seedling growth. The experiment was arranged in a two-factor factorial design, four replications. The first factor were 7 concentrations of Seaweed Liquid Fertilizer (SLF): 0, 250, 500, 750, 1000, 1250, and 1500 ppm, and the second factor were frequency of application of 2 and 3 times every 4 weeks. The parameters observed were seedling growth (seedling height, leaf number, leaf length, leaf width, stem diameter, root length and root volume), chlorophyll content, wet and dry biomass weight (leaves, stems, and roots). The results showed that seaweed from Euchema cottonii can improve the growth of clove seedlings. The E. cottonii seaweed in 1250 ppm concentration and two times every 4 weeks can improve the growth of clove seedlings.

Keywords: Clove, seedlings, growth, seaweed waste, organic fertilizer

1. Introduction

The cloves (Syzygium aromaticum) are spice plants that can be used for the pharmaceutical and food industries. Currently Indonesia is the biggest producer and consumer of cloves in the world. Demand for clove plants is increasing, but it is constrained by the low national clove productivity. The productivity of Indonesian cloves is lower, which is 260 - 360 kg/ha¹, compared to other countries, although the production potential reaches 500 - 600 kg/ha¹. The highest cloves productivity during the period of 1980-2013 was 374.77 kg/ha² in 2003 [1].

The low productivity of cloves in Indonesia is caused by the limited used of superior plant material. The limited quality of clove seedlings caused by the slow growth of clove seedlings and technological innovations that can accelerate their growth have not been optimal. [2] reported that clove seedlings in nurseries can be transplanted into the field after 1 - 1.5 years old. Furthermore reported that the slow growth of clove seedlings was caused by low meristematic cell growth [3]. The low regeneration of meristem cells associated with the balance of endogenous hormones that play a role in controlling meristematic cell growth. One of the efforts to increase the growth of clove seedlings by giving seaweed waste fertilizer which in the form of solid and liquid Seaweed Solid Fertilizer (SSF) and Seaweed Liquid Fertilizer (SLF) were made of fermenting seaweed anaerobically. Those SSF and SLF contains macro nutrients (N, P, K, C-organic, Ca), micro nutrients (Cu, Fe, Mn, Mg, Mn, B, Cl, Na), humid acid and...
Plant Growth Regulator (PGR) such as auxin (IAA), cytokinin (kinetin & zeatin) and gibberellin. Microbial consortium such as N-fixing microbes, phosphorus, potassium degradation and soil fertilizing microbes are added to increase the effectiveness of the seaweed fertilizer [4]. The seaweed fertilizer might be used to substitute the synthetic fertilizers, and expected able to improve the growth of clove seedlings so that it can accelerate the distribution of clove seedlings with a good vigor. The advantage of using seaweed fertilizer is that it can reduce the pollution due to the seaweed processing industry waste and also the use of synthetic fertilizers continuously.

Sub tropical seaweed wastes such as Laminaria and Dictyota as organic fertilizers have been produced commercially from seaweed extract, it is called “sap”, while brown seaweed such as Euchema cottonii which are tropical seaweed have not been produced commercially. The identification and characterization results of E. cottonii and Gracilaria proved to contain macro nutrients (N, P, K, Corganik, Ca), micro nutrients (Cu, Fe, Mn, Mg, Mn, Boron, Cl, Na), humid acid, and Plant Growth Regulator (PGR) with higher levels of auxin, cytokinin (kinetin & zeatin) and gibberelin than PGR in commercial organic fertilizers. [4] [5]. The highest IAA content is found in E. cottonii with a value of 1128 ppm reported that seaweed extract commonly is called sap liquid E. cottonii from Madura (21 days old ) contained: IAA 159.87 ppm, GA3 128 ppm, GA7 110.03 ppm, zeatin 72.9 ppm, kinetin 117.01 ppm, while sap liquid from Pulau Panjang (35 days old) contains: IAA 2000 ppm, GA3 1500 ppm, zeatin 1200 ppm and kinetin 1000 ppm [4]. Mention that auxin plays a role in stimulating stem growth and coleoptile [7]. Supply of auxin in the sub apical area of the stem or coleoptile is required for the elongation process, rapidly increasing the extensibility of the cell wall, and cell extension. Furthermore, gibberellins play a role in stimulating elongation and division cell, enhancing cell wall extensibility. Cytokinins hormones play a role in the following: regulate cell division in shoots and roots, promote cell and expansion in leave, induce bud formation, promote movement of nutrients, promote chloroplast development, and regulate growth of stem and roots.

The use of seaweed waste as organic fertilizer in increasing the growth and yield of food crops and horticulture has been widely reported [4]. Previous research reported that the application of SLF in concentrations of 25-75% significantly increased the spinach growth [8]. It was also reported that the application of seaweed fertilizer can increase the growth of chili plants twice that of controls [9], and seaweed fertilizer from Sargassum can improve the germination, growth and production of Vignaradiate [10]. Meanwhile, the use of seaweed fertilizer in spice plants, according to our knowledge, has never been reported. The research was aimed to determine the proper concentration and frequency of application of SLF from seaweed of E. cottonii to increase the growth of clove seedlings.

2. Material and methods
Experiment was carried out at the Laboratory and Green House of the Ecophysiology Department, Indonesian Spice and Medicinal Crops Research Institute (ISMACRI), from January to December 2018.

The study used a two-factors factorial design with three replications. The first factor were: Concentration of SLF (C) : C1 = 0 ppm (control), C2 = 250 ppm, C3 = 500 ppm, C4 = 750 ppm, C5 = 1000 ppm, C6 = 1250 ppm, C7 = 1500 ppm. The second factor were frequency of application (F) : F1 = 2 times every 4 weeks and F2 = 3 times every 4 weeks. Each of the experimental unit consisted of 10 seedlings so the total number of clove seedling in this experiment was 420 plants.

The clove seeds that used in this study obtained from the mother tree in Garut Regency, West Java Province. The seeds were sown in the cocopeat media and and transplanted uniform seedling to the polybag. SLF made from sap of E. cottonii, and obtained from the Research Center for Marine Prosessing and Biotechnology, Jakarta. The macro and micro nutrient, and PGRs content in SLF can be seen in Table 1.

The first application of SLF was conducted when the seedling were 1 month old after transplanting in polybag and then repeated every 4 weeks. Seaweed liquid fertilizer were sprayed evenly on all leaves. The variables observed were the seedling growth (seedling height, leaf number, leaf length, leaf width, stem diameter, root length, and root volume), chlorophyll content, and fresh and dry weight of
biomass (leaves, stems, and roots). The data were analyzed using analysis of variance (ANOVA) and further analysis with Duncan Multiple Range Test (DMRT) of 5% level.

Table 1. The content of macro and micro nutrient, and PGRs in SLF

| Macro nutrient | Value | Micro nutrient | Value       | PGRs Value | Value |
|----------------|-------|----------------|-------------|------------|-------|
| N organic total (%) | 0.19  | Bo (ppm)       | 18          | Kinetin (ppm) | 58 ±18 |
| P2O5 total (%)    | 7.5 x 10^-5 | Fe (ppm)     | 3           | Zeatin (ppm)     | 65 ±10 |
| K2O total (%)     | 1.7x10^-5  | Mn (ppm)      | 0.05        | IAA (ppm)      | 1.128±199 |
| Ca (ppm)          | 660    | Zn (ppm)      | 0.3         | GA (ppm)        | 130±3  |
| Mg (ppm)          | 285    | Mo (ppm)      | <0.02       | Co (ppm)        | <0.01  |

Source: [4]

3. Results and discussion

3.1. Clove Seedling Growth

The growth of clove seedling (seed height, number of leaves, leaf length, leaf width, and stem diameter) were affected by the interaction between concentration and frequency application of SLF (Table 2).

Table 2. Interaction between concentration and application frequencies of SLF on the growth of clove seedlings

| Treatments | Frequency (x times every 4 weeks) | Seeding height (cm) | Number of leaves | Leaf length (cm) | Leaf width (cm) | Stem diameter (mm) |
|------------|-----------------------------------|---------------------|------------------|-----------------|----------------|-------------------|
| 0 (control 1) | -                                 | 12.95 e             | 21.00 cd         | 8.98 def       | 3.14 de        | 2.52 cde          |
| 250        | 2                                 | 12.83 e             | 13.38 ef         | 6.93 g         | 2.72 e         | 2.33 de           |
| 500        | 2                                 | 14.41 e             | 18.67 de         | 9.21 cde       | 3.40 cde       | 2.51 cde          |
| 750        | 2                                 | 20.52 d             | 26.93 ab         | 10.46 bcd      | 3.59 bcd       | 2.64 bcd          |
| 1000       | 2                                 | 24.05 bc            | 24.47 bc         | 10.20 bcde     | 3.57 bcde      | 2.61 cd           |
| 1250       | 2                                 | 23.85 bc            | 25.87 abc        | 11.61 ab       | 4.22 a         | 2.78 abc          |
| 1500       | 2                                 | 25.51 ab            | 27.64 ab         | 11.72 ab       | 4.02 ab        | 3.01 a            |
| 0 (control 2) | -                                | 12.02 e             | 12.25 f          | 7.60 fg        | 2.70 e         | 2.27 e            |
| 250        | 3                                 | 13.81 e             | 15.38 ef         | 10.86 b        | 3.73 abcd      | 2.40 de           |
| 500        | 3                                 | 12.93 e             | 14.23 ef         | 8.79 ef        | 3.15 de        | 2.28 e            |
| 750        | 3                                 | 21.46 cd            | 25.82 abc        | 10.96 b        | 3.97 abc       | 2.63 bcd          |
| 1000       | 3                                 | 23.76 bc            | 24.95 abc        | 10.68 bc       | 3.74 abcd      | 2.54 cde          |
| 1250       | 3                                 | 25.40 ab            | 30.47 a          | 11.30 b        | 4.03 ab        | 2.92 ab           |
| 1500       | 3                                 | 27.12 a             | 26.60 abc        | 12.97 a        | 4.11 ab        | 2.59 cd           |

Notes: The numbers followed by the same letter in the same columns are not significantly different at 5% DMRT test.

In general, the higher the SLF concentration (for all application time), the better the growth of clove seedlings. The concentration of 750 ppm SLF, with application frequency of 2 times (C4F1) significantly increasing the clove seedling height (20.52 cm) and leaves number (26.93) compared to control-1 (12.95 and 12.02 cm respectively) and control-2 (12.25 and 21.00 respectively). The concentration of 750 ppm SLF, with frequency of 2 times (C4F1) also increasing number of leaves significantly (26.93) compared to control. Leaf length, leaf width and stem diameter began to increase significantly at C6F1, with values of 11.61 cm, 4.22 cm, 2.78 mm, respectively, compared to control-1.
(8.9 cm, 3.14 cm, 2.52 mm respectively) and control-2 (7.60 cm, 2.70 cm, 2.27 mm respectively)) (Table 2).

The fresh weight of stem and leaves were influenced by the interaction between the concentration and frequency application of SLF. The weight of fresh stem clove seedlings (1.84 g) significantly increased at a concentration of 500 ppm SLF, with 2 times application (1.84 g) compared to controls by giving 2 or 3 times application (0.79 g and 0.31 g). The fresh leaf weight of clove seedlings also significantly increased at concentration of 500 ppm SLF, with 2 times frequency application 3.03 g) compared to controls by giving 2 or 3 times application (1.60 g and 0.71 g). While the best leaf fresh weight were obtained in the SLF concentration of 1250 ppm with 3 times application (6.08 g) (Table 3).

The stem dry weight of the clove seedlings increased significantly obtained at concentration of 1250 ppm SLF, with 2 times of frequency application (1.09 g) compared to the control in 2 or 3 times application (0.32 and 0.71 g). On the other hand, the leaves dry weight of clove seedlings could increase at lower concentrations in 250 ppm SLF, with frequency application 3 times (1.07 g) and at concentration of 500 ppm SLF, with the frequency application 2 times (1.15 g) compared to controls by giving 2 or 3 times application (0.54 and 0.22) (Table 3).

### Table 3. Interaction between concentration dan application frequencies of SLF on the fresh and dry weight of clove seedlings biomass

| Treatments | Concentration (ppm) | Frequency (x times every 4 weeks) | Fresh weight of stem (g) | Fresh weight of leaves (g) | Dry weight of stem (g) | Dry weight of leaves (g) |
|------------|---------------------|----------------------------------|--------------------------|---------------------------|-----------------------|--------------------------|
| 0 (control-1) | 0                   | -                                | 0.79 bcd                 | 1.60 e                    | 0.32 cd               | 0.54 cd                  |
|             | 250                 | 2                                | 0.54 cd                  | 0.88 e                    | 0.19 d                | 0.26 d                   |
|             | 500                 | 2                                | 1.84 ab                  | 3.03 d                    | 0.48 cd               | 1.15 abc                 |
|             | 750                 | 2                                | 1.63 abc                 | 4.87 c                    | 0.62 bc               | 1.62 ab                  |
|             | 1000                | 2                                | 1.78 ab                  | 5.12 bc                   | 0.60 bc               | 1.27 ab                  |
|             | 1250                | 2                                | 2.12 a                   | 6.08 ab                   | 1.09 a                | 1.53 ab                  |
|             | 1500                | 2                                | 1.82 ab                  | 5.12 bc                   | 0.71 bc               | 1.77 a                   |
| 0 (control-2) | 0                   | -                                | 0.31 d                   | 0.71 e                    | 0.17 d                | 0.22 d                   |
|             | 250                 | 3                                | 1.00 abc             | 3.13 d                   | 0.37 cd               | 1.07 abc                 |
|             | 500                 | 3                                | 0.80 bcd               | 2.96 d                   | 0.33 cd               | 0.49 cd                  |
|             | 750                 | 3                                | 2.18 a                   | 5.51 bc                   | 0.69 bc               | 1.52 ab                  |
|             | 1000                | 3                                | 1.64 abc                 | 5.50 bc                   | 0.42 cd               | 0.89 bcd                 |
|             | 1250                | 3                                | 1.89 a                   | 6.67 a                   | 0.87 ab               | 1.81 a                   |
|             | 1500                | 3                                | 1.76 ab                  | 5.29 bc                   | 0.67 bc               | 1.71 a                   |

Notes: The numbers followed by the same letter in the same columns are not significantly different at 5% DMRT test

From the results of this experiment it is proven that the application of SLF is effective to increasing the clove seedlings growth (plant height, leaf number, length and width of leaves, and fresh and dry weight of biomass). The application of concentration of 1500 ppm seaweed liquid fertilizer, by giving 3 times application gave the highest height clove seedling and were significantly different compared to all treatment, except for the concentration 1500 in 3 time application.

This is caused by liquid organic fertilizer from SAP seaweed containing auxin (IAA) which plays a role in inducing the elongation of stem cells. Cell elongation process is influenced by cell enlargement through: osmotic uptake which is controlled by the difference in water potential in the plasma membrane and the expansion of cell walls which are influenced by turgor pressure in the cell wall and triggered by increased cell wall acidity [7].

Auxins in plants are transported mainly from the apical to the basal end (basipetally), so that the providing of SLF by spraying onto the leaf surface is absorbed immediately by the leaves and transferred
to the roots. Suggested that 750 ppm seaweed extract was significantly increased the number of leaves per plant, average leaf area, leaf and stem fresh weight per plant, leaf and stem dry weight per plant on snap bean compared to control [11]. Reported that providing of extracts sargassum (seaweed) 50% was able to increase soybean height compared to controls [12].

The leaf number of clove seedlings increased significantly in the providing of 750 ppm SLF, by giving 2 times application. This is caused by SLF from SAP (seaweed extracts) also containing the hormone cytokinin (kinetin and zeatin), macro and micro nutrients needed for plant growth. Cytokinin plays a role as a regulator in stem cell division, thus affecting the number of leaves. The increase in the number of leaves also affects the overall growth of clove seedlings, which is associated with increased photosynthesis.

Increasing leaf length, leaf width and stem diameter requires higher SLF concentrations of seaweed, which is 1250 ppm, with application of 2 times. This increase is caused by SLF from seaweed containing cytokinin which plays a role in the enlargement of leaf cells. According to [7], cytokines in addition to playing a role in the initiation of stem cell division, also play a role in enlarging the leaf area/plant organs. Reported that the application of cytokines with auxin was able to increase the number of leaves and the length of orchid leaves [13]. Suggested that the increase in stem diameter, number of leaves, number of shoots and length of shoots of tea plants was influenced by the application of cytokines, both from coconut water and BAP [14]. Macro nutrient content in SLF, although very small in number compared to chemical fertilizers, was able to increase the growth of clove seedlings.

3.2. The Roots Growth
Increasing the root length of clove seedlings was influenced by the interaction between the concentration and frequency of the SLF application, it shows that the SLF can increase the root length of clove seedling. The application of 1250 ppm SLF in 2 times gave the highest results (27.86 cm) and was not significantly different from the application of SLF at concentrations of 750, 1000 and 1500 in 2 times, as well as 1 time application of 1250 ppm (Table 4). The cytokinin (zeatin and kinetin) content in SLF can increase root growth which affects root length. Cytokinin is a plant growth regulator that is involved in the development of plant processes, including rhzogenes to increase the root growth [15].

Table 4. Interaction between concentration and frequency of application SLF on the roots length of clove seedlings

| Treatments          | Frequency (x times every 4 weeks) | Root length (cm) |
|---------------------|-----------------------------------|------------------|
| Concentration (ppm) |                                   |                  |
| 0 (control-1)       | -                                 | 19.70 ef         |
| 250                 | 2                                 | 20.13 ef         |
| 500                 | 2                                 | 21.96 de         |
| 750                 | 2                                 | 23.26 de         |
| 1000                | 2                                 | 22.70 cde        |
| 1250                | 2                                 | 26.10 abc        |
| 1500                | 2                                 | 24.09 bcd        |
| 0 (control-2)       | -                                 | 18.00 f          |
| 250                 | 3                                 | 22.75 cde        |
| 500                 | 3                                 | 22.61 cde        |
| 750                 | 3                                 | 24.99 abcd       |
| 1000                | 3                                 | 27.10 ab         |
| 1250                | 3                                 | 27.86 a          |
| 1500                | 3                                 | 26.09 abc        |

Notes: The numbers followed by the same letter are not significantly different at 5% DMRT test
The root growth (volume, fresh weight, and dry weight) of clove seedlings were not influenced by the interaction between SLF concentrations and the frequency of application, and by the single factor of applications frequency as well. The root growth only influenced by the concentration of SLF. The root volume of clove seedling significantly increased at a concentration of 500 ppm SLF (1.96 ml) compared to control (1.25 ml). While the highest wet and dry weight at the treatment of 1250 ppm SLF with a value of 1.21 g and 0.53 g, respectively (Table 5). Increased root growth was influenced by the content of cytokinins in SLF stated that the role of cytokinins in root growth or shoots depends on the ratio of auxin to cytokinin in the medium. Whereas high auxin: cytokinin ratios stimulated the formation of roots, low auxin cytokinin ratios led to the formation of shoots and balance of auxin: cytokinin in orchid plants increases the number of roots, but cannot increase root length [13]. Cytokinins greatly reduce lateral root density at low concentrations, therefore lateral root development is more sensitive to cytokinin treatment than primary root growth [16]. It is assumed that with the SLF application the cytokinin content in plants increases so that the lateral roots grow more which results in increased volume, fresh weight and dry weight of roots.

### Table 5. Effect of single factor concentration and frequency of SFL application on volume, fresh and dry weight of root

| Treatments          | Root volume (ml) | Fresh weight of root (g) | Dry weight of root (g) |
|---------------------|------------------|--------------------------|------------------------|
| Concentration (ppm):|                  |                          |                        |
| 0                   | 1.25 c           | 0.38 e                   | 0.22 d                 |
| 250                 | 1.33 bc          | 0.47 de                  | 0.27 d                 |
| 500                 | 1.96 a           | 0.70 cd                  | 0.35 cd                |
| 750                 | 1.69 abbc        | 0.97 b                   | 0.45 bc                |
| 1000                | 1.88 a           | 0.78 eb                  | 0.39 c                 |
| 1250                | 1.79 ab          | 1.21 a                   | 0.53 ab                |
| 1500                | 1.87 a           | 1.26 a                   | 0.59 a                 |
| Frequency of applications: |              |                          |                        |
| 2 Times             | 1.68 a           | 0.88 a                   | 0.41 a                 |
| 3 Times             | 1.68 a           | 0.78 a                   | 0.38 a                 |

Notes: The numbers followed by the same letter in the same columns are not significantly different at 5% DMRT test.

### 3.3. Chlorophyll Content

The chlorophyll content of clove seedling leaves was influenced by the interaction between SLF concentration and frequency of application (Table 6). The chlorophyll content of clove seedlings increased significantly by the application of 750 ppm of SLF in 2 times application (44.33) compared to two controls (37.62 and 34.03 respectively). Reported that providing of 500 ppm seaweed extract increased the chlorophyll content in snap beans[11]. Were stated that cytokinins play a role in the differentiation and development of chloroplasts, chlorophyll biosynthesis and prevent chlorophyll degradation [7] [17]. Furthermore, reported that the main function of cytokinin in the formation of chloroplasts is as a modulator to harmonize the function of organelles in addition to protecting the function of chloroplasts under high light pressure [18]. The increase in chlorophyll content has an effect on increasing the overall growth of clove seedlings as described previously in Tables 2, 3, 4 and 5.
Table 6. The interaction between the concentration and frequency of application of SLF to the chlorophyll content of clove seedling the leaves

| Treatments | Chlorophyll content (ppm) | Frequency (x times every 4 weeks) |
|------------|--------------------------|-----------------------------------|
| 0 (control-1) | 37.62 f | - |
| 250 | 38.63 ef | 2 |
| 500 | 41.40 de | 2 |
| 750 | 44.33 abcd | 2 |
| 1000 | 47.02 a | 2 |
| 1250 | 44.52 abc | 2 |
| 1500 | 44.94 abc | 2 |
| 0 (control-2) | 34.03 g | - |
| 250 | 38.62 ef | 3 |
| 500 | 41.99 cd | 3 |
| 750 | 43.77 bcd | 3 |
| 1000 | 44.90 abc | 3 |
| 1250 | 44.01 abcd | 3 |
| 1500 | 45.34 ab | 3 |

Notes: The numbers followed by the same letter are not significantly different at 5% DMRT test

4. Conclusions
The E. cottonii seaweed in 1250 ppm concentration and two times every 4 weeks can improve the growth of clove seedlings

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