Seaweed extract effect on arbuscular mycorrhizae spore in soil engineered by earthworm, and the soil effect on upland rice growth

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Abstract. Seaweed extract is known to contain nutrients and growth-regulating substances that affect soil biota, and a source of protection against pests and diseases. Earthworm, which is an example of a soil biota and playing the role of ecosystem engineer, has the ability to produce suitable land biostructures, for the inhabitation of arbuscular mycorrhizal fungi (AMF), which has an impact on upland rice growth. Therefore, this study aims to determine, (i) the effect of seaweed extract on the population of earthworms and spores of arbuscular mycorrhizal fungi, and (ii) the impact of the engineered soil on the growth of local upland rice varieties. Furthermore, the extract of seaweed, such as Kappapychus alvarezi, was divided into five concentration levels, namely 0%, 20%, 40%, 60%, and 80%. Each treatment was drenched into the soil from the cogongrass vegetated area, mixed with 20 Pheretima sp., and maintained for 49 days in the greenhouse. The result showed that the total difference in the earthworms' concentration treatments was not significant. It also showed that the total AMF spores in the engineered soil products of 20% concentration was the highest. Based on treatment with the earthworm engineered soil products, the highest and lowest vegetative growth and yield components of upland rice were observed at the concentrations of 80% and 0%, respectively. In conclusion, the application of seaweed extract to the soil did not significantly reduce the earthworm population. The extract concentration of 20% also increased the total AMF spore in the engineered soil. Moreover, highly treated engineered soil products increased the growth and yield components of upland Kambowa rice on cogongrass soils.

1. Introduction
Variety of local upland rice is cultivated in the tropics by small-scale farmers, due to being a source of staple food [1]. The cultivation is majorly carried out in Southeast Asia, including Indonesia, in various forms, such as red, yellow, green, black, purple, brown, and white rices, which have been

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prepared through a variety of cultural and spiritual traditions [2]. Varieties of local upland rices which have adapted to agro-ecological conditions are mostly cultivated by small-scale farmers [3]. They are also planted through the sweeden system on poor soils [4], however, low biotic and high abiotic stresses greatly contribute to the decrease in productivity [5]. Furthermore, the sources of abiotic stress that inhibits growth performance of upland rice are generally related to organic carbon content, fertility, and low soil pH [6;7]. The implication is that the water holding capacity, biological activity, and fertility of the soil are low, which in turn has an impact on achieving optimal growth and yields of upland rice.

The improvements to soil quality and biological fertility is carried out by increasing the activity of biota, including earthworms, which have the abilities to play a collaborative dual role, both as decomposers and engineers in soil ecosystems [8]. These engineering roles have been widely applied in the process of mining and agro-ecosystems, as well as restoring the physical, chemical, and biological qualities of soil in degraded areas [9;10;11;12]. Furthermore, engineered soils are known to have stable structure and organic matter fraction, available nutrients, growth hormone-like substances, and plant health, due to the activities of earthworm [13]. Deposited AMF spores and other beneficial microbes are also capable of promoting growth, based on the soil biostructure (cast) created by earthworm activities [14;15].

Based on the use of plant or animal biomass, agricultural ecological intensification is a key source of organic matter, nutrients, and beneficial microbes, to improve the production and quality of yields from food crops [16;17]. The application of liquid organic fertilizers has also received considerable focus for media-based cultivation systems, water, and soil media [18;19]. This is because the fertilization process increases soil organic matter, as well as the efficiency of macro and micronutrient uptakes, compared to mineral fertilizers [20]. Furthermore, seaweed extract is continuously promoted as a source of liquid organic fertilizer containing hormone-like substances, as well as macro and micronutrients. These are used to increase the adaptive capacity of plants to biotic, abiotic, and environmental stresses, respectively [21]. The method of applying liquid seaweed extract was carried out through foliar, root, and soil, or a combination of all [22]. This extract application was also able to increase the dominance of fungi groups and saprotrophic bacteria to the soil, using an irrigation system [23;24]. Based on these descriptions, the aims of this study were to examine (i) the effect of seaweed extract concentration on the population of earthworms and arbuscular mycorrhizal fungi in engineered soil; and (ii) the effect of engineered soil on the growth and yield of local upland rice varieties.

2. Materials and methods

2.1. First experiment

Five levels of seaweed (*K. alvarezi*) extract were tested in this experimental, namely 0%, 20%, 40%, 60%, and 80% (symbolized C0, 20, 40, 60, and 80) concentrations. Each concentration was repeated six times following a simple randomized block design. The experiment was carried out between March to November 2019, at the Worm House and Greenhouse within the Department of Agrotechnology, Faculty of Agriculture, Halu Oleo University.

Fresh *K. alvarezi* was obtained from seaweed farmers in North Buton Regency. These seaweeds were thoroughly dried under the hot sun, as a total of 5 kg was placed into a bucket containing 10 L of tap water for 24 hours. Based on this condition, 200 g of seaweed were obtained and cut into sizes of 1-2 cm, which were further crushed by using a kitchen blender. Furthermore, finely ground seaweed of 100 g was placed into a 1000 ml Erlenmeyer and filled with water. This continued until the required volume was attained, as the mixture was heated on a hot plate of 60°C and stirred till a slurry form is achieved. The cooled slurry was further placed in a plastic bottle, which was stored in a refrigerated condition of 4-5°C, until its usage as a stock solution. Based on this solution, the treatments of five concentration levels were applied to the soil, which was placed in a triplex reactor and measured at 25, 21, and 21 cm in length, width, and height, respectively.
The topsoil of the cogon grass vegetated land within the Halu Oleo University Botanical Gardens was obtained to a depth of 10 cm from the surface. This soil was separated from plant debris and other objects, before being air-dried in a greenhouse. Furthermore, 2 kg of wind-dried soil was transferred through a 2 mm sieve per hole into each reactor, with the remaining soil being stored for subsequent use as upland rice growing media. A total of 100 ml from each seaweed concentration was further mixed with the reactor soils until manual homogeneity is achieved. Based on the mixture being abandoned for 2 days, approximately 20 adult earthworms (*Pheretima* sp.) collected from the Experimental Garden of the Faculty of Agriculture, Halu Oleo University, were released into each reactor. The entire surface of the reactor was covered using a plastic net of 2 mm per hole, after the whole-body parts of the earthworms had submerged into the soil mixture [25]. The soil moisture in the vermireactor was also maintained by spraying 100 ml of tap water on the surface, once every two days.

The soil mixture was further removed from the vermireactor, after seven weeks of engineering. Furthermore, the earthworms were removed from the mixture through a hand sorting technique and were individually counted according to their growth stage group. Each of these soil mixtures was also wind-dried in the worm house, as a total of 50 g was used to calculate the entire AMF spores. The remainder were then placed into a zipper pack, which were added to the growing medium of the Kambowa local upland rice variety.

Based on the extraction procedure operated by [25], sub-samples of each engineered soil were also obtained, in order to determine the total contents of the AMF spore. In fact, approximately 50 g of each soil mixture was placed into a container, which was filled with 400 ml of water, stirred until homogeneous, and filtered using stratified sieves of 212 and 38 m from top to bottom. The remaining retained particles on the 38 m sieve were then poured into tubes and centrifuged at 200 rpm in different gradients of 20 and 60% sugar solutions for 5 mins. This suspension was then poured into a 38 m sieve, as the sugar solution completely disappeared using a slow tap water flow. Furthermore, the retained spores were transferred into a plastic petri dish equipped with a grid, and the total AMF contents were counted under a stereomicroscope, based on the procedure of [26].

### 2.2. Second experiment

The treatments in these experiments were soil products derived from the first experimental series, namely C0, C20, C40, C60, and C80 (SE-C0, SE-C20, SE-C40, SE-C60, and SE-C80). Furthermore, 5 kg of each soil from the previous growing media stock was placed into a different polybag of 30 pieces. A total of 200 g derived from each engineered product was then added to the polybag contents. Based on the randomized block design procedure, each polybag was randomly placed in the Greenhouse. Two seedlings obtained from Kambowa upland rice were further transplanted into each polybag and maintained until harvest. Additionally, the growing media was diary with 100 ml of tap water per polybag daily.

Plant height, number of leaves, and leaf area were also measured at 14, 28, 42, 56, and 70 days after planting (DAP), respectively. Leaf area was estimated using the formula [27]: \( LA = (L \times W) \times 0.73 \), where \( LA = \) leaf area (cm\(^2\)), \( L = \) leaf length (cm), \( W = \) leaf width (cm), and 0.73 is a constant. Dry and fresh weights, number of tillers, panicle length, as well as total and percentage of grains were measured at the final stage of the experiment, at 120 DAP.

### 2.3. Statistical analysis

All data were collected from the first and second experiments, as subjects for the analysis of variance. Based on the use of the LSD test at a level of \( p < 0.05 \), the difference between the treatments in both experimental series was tested when the \( F_{\text{calculate}} \) value > \( F_{\text{tab}} \) at a significance < 0.05.
3. Results and discussion

3.1. Earthworm abundance and total of AMF spore

The abundance of earthworms (*Pheretima* sp.) in the soil mixture was calculated after seven weeks of engineering processes, as the results showed that they totally ranged from 20 – 23 individuals per vermireactor (Table 1). Furthermore, the analysis of variance showed that the effect of the seaweed extract concentrations had no significance on the abundance of earthworms, based on total individuals (F = 0.326; Sig. = 0.857), cocoons (F = 1.438; Sig. = 0.258), immature (F = 0.644; Sig. = 0.637), and adults (F = 1.598; Sig. = 0.214). Although it showed no significant effect, there was a tendency in the total individuals and adults within the concentration of 0% (C0), which was higher than those of 20%, 40%, 60%, and 80%. Meanwhile, the tendency of cocoons and immature occurred in the concentration of 60% (C60) (Table 1). These results re-emphasized that the application of *K. alvarezii* extract through the soil did not have a significant effect on reducing microbe populations [28], especially from the *Phertima* sp.

| Abundance of earthworm (individual) | Growth stage | Total |
|-------------------------------------|--------------|-------|
|                                      | Cocoons      | Immature | Mature |       |
| C0 3±2a                              | 1±2a         | 19±1a    | 23±5a  |
| C20 2±2a                             | 1±1a         | 18±2a    | 20±4a  |
| C40 2±3a                             | 1±1a         | 18±2a    | 21±4a  |
| C60 4±5a                             | 3±5a         | 15±6a    | 22±13a |
| C80 1±2a                             | 1±2a         | 17±4a    | 20±4a  |

Note: Numbers followed by the same letter in the same column showed no significant difference according to the LSD test at the p < 0.05 level.

The survival of *Pheretima* sp was caused by the application of seaweed extracts to the soil, which further triggered their activities in modulating bacteria and fungi that were positively correlated with plant productivity [28]. Based on the soil fungi group, AMF played a functional role in facilitating plant access to nutrients and water, as well as stimulating root growth [29]. However, the population of infective AMF spores resided in the engineered soil [15; 25; 30]. According to this study, the analysis of variance showed that the concentration of seaweed extract had a significant effect on the total AMF spores in the engineered soil (F = 3.872, Sig. = 0.017). This indicated that the concentration was a factor that needed consideration regarding the application of seaweed extracts, in order to improve soil biological quality. Furthermore, Figure 1 showed that the highest content of AMF spores was observed in the concentration of 20%, which was also significantly different (LSD at p < 0.05) than others. However, the difference between other treatments was not significant (LSD at p level). > 0.05). This indicated that the application of *K. alvarezii* extract with high concentrations negatively affected the increase in AMF spores. High concentration of *K. alvarezii* extracts also contained large amounts of organic bioactive molecules (biostimulants) and inorganic constituents, including Na, K, Ca, Mg, Zn, Mn, Fe, Cr, Cu, Ni, and P [31]. Additionally, biostimulants in high and low concentrations also inhibited and triggered AMF spore germinations, respectively [32].

The studies by [33], found that the addition of mineral salts in the media did not reduce AMF spore germination. However, organic substrates such as glucose, fructose, sucrose, L-arabinose, as well as malic, pyruvic, succinic, and aspartic acids suppressed the germination of AMF spore. Furthermore, *K. alvarezii* extract also contained glucose, galactose, organic acids, proteins, lipids, and phenolics [34; 35; 36]. This provided an overview for a more in-depth investigation, based on the relationship between abiotic and biotic factors that mostly contributed to the reduction of total AMF spores, with increasing concentrations of *K. alvarezii* extract.
Figure 1. Total of AMF spores (mean±sd, n = 6) in the soil with the addition of seaweed extract (K. alvarezii) that has been engineered by earthworms (Pheretima sp.) during seven weeks. The different letter above bars shown a significant different according to the LSD test at the p < 0.05 level.

3.2. Height, leaf number, leaf area, tillering number of upland rice
The engineered soil provided services as a suitable growing medium for plant growth [9;10]. Similarly, the addition of vermicast (soil biostructure) to the growing media also improved plant growth performance [37]. The effect of the engineered product to the growth media (leaf height, number, and area, as well as the rate of upland rice tillers), is shown in Figure 2.

Figure 2a showed that the plant height increased with time, as its relationship with the effect of engineered products application was significantly shown in 56 (F = 4.149; Sig. = 0.013) and 70(F = 3.416; Sig. = 0.028) DAP. The highest plants at both times occurred in the concentration of 80% (SE-C4) and was also significantly different (LSD test at level p < 0.05) than other treatments. However, the difference that occurred in the plant height of the other four products was not significant (LSD test at level p > 0.05). Furthermore, Figure 2b showed that the number of leaves relatively increased rapidly and slowly after 42 and 56 DAP, respectively. The analysis of variance also showed that the application of engineered products had a significant effect on the number of upland rice leaves, at 28 (F = 3.636; Sig. = 0.022), 42 (F = 4.361; Sig. = 0.011), 56 (F = 4.149; Sig. = 0.013), and 70(F = 3.416; Sig. = 0.028) DAP, respectively. Additionally, the highest number of leaves occurred in the concentration of 80% (SE-C4) and was significantly different (LSD test at level p < 0.05) from the other treatments. However, the difference between the other four treatments was insignificant (LSD test at level p > 0.05).
Figure 2c showed that leaf area increased after 14 DAP, as a relatively rapid increment also occurred in SE-C80 treatment. The analysis of variance further showed that the application of engineered products had a significant effect on the leaf area of upland rice, at 28 (F = 7.025; Sig. = 0.001), 42 (F = 5.080; Sig. = 0.005), 56 (F = 5.084; Sig. = 0.005), and 70 (F = 5.662; Sig. = 0.003) DAP. Furthermore, the widest leaf also occurred in the 80% concentration (SE-C4), which was significantly different (LSD test at level p < 0.05) than the other four treatments. However, the difference between the other four treatments was not significant (LSD test at level p > 0.05). The highest development performance of upland rice at a concentration of 80% indicated a greater availability of sufficient nutrients, due to their level of substances such as growth hormones, being more suitable than other treatments [38].

3.3. Dry weight, tillers number, panicle length, spikelet total, and spikelet filled

The analysis of variance showed that the application of engineered soil products produced in the first experimental series, had a significant effect on dry weight (F = 7.716; Sig. = 0.004), panicle length (F = 3.082; Sig. = 0.040), total grain (F = 7.824; Sig. = 0.001), and percentage of upland rice spikelet (F = 7.737; Sig. = 0.001). Table 2 below showed the differences in dry weight, number of tillers, panicle length, as well as grain total and percentage of upland rice plants. The dry weight treated with SE-CS80 (application at 80% concentration) was observed to be the highest and was significantly different (LSD test at level p < 0.05) than other concentrations. Most tillers also occurred in plants with SE-CS80 treatment, which was not significantly different (LSD test at level p < 0.05) than other concentrations. Furthermore, the longest panicles occurred in the SE-CS80 treatment, although were not significantly different (LSD test at level p > 0.05) than SE-CS0, SE-CS20, and SE-CS60. Meanwhile, the shortest panicles occurred in the SE-CS40 treatment, which was not significantly different (LSD test at level p > 0.05) than the SE-CS0 and SE-CS60.

Table 2. Dry weight, tillers number, panicle length, spikelet total and spikelet filled of upland rice of Kambowa local variety with soil product treatment applied with different concentrations of seaweed extract and engineered by earthworms (Pheretima sp.).

| Soil product treatments | Plant dry weight (g) | Tillers number (tiller) | Panicle length (cm) | Total of spikelet (spikelet) | Spikelet filled (%) |
|------------------------|----------------------|------------------------|---------------------|-----------------------------|---------------------|
| SE-CS0                 | 3.56±0.82a           | 0.33±0.52a             | 17.60±2.47ab        | 100.83±32.63a               | 11.54±1.30a         |
| SE-CS20                | 2.42±0.78a           | 0.67±1.03a             | 18.48±1.93b         | 79.84±41.60a                | 11.58±2.23b         |
| SE-CS40                | 2.92±1.51a           | 0.33±0.52a             | 14.85±4.24a         | 91.67±26.69a                | 17.93±4.81c         |
| SE-CS60                | 4.55±1.87a           | 0.50±1.22a             | 18.05±2.66ab        | 110.33±43.56a               | 17.67±1.41c         |
| SE-CS80                | 8.05±1.64b           | 1.33±1.03a             | 20.65±2.30b         | 172.67±16.79b               | 19.69±4.11c         |

Note: Numbers followed by the same letter in the same column showed no significant difference according to the LSD test at the p < 0.05 level.

Table 2 showed that the total spikelet produced by upland rice ranged from 79 – 110 per plant. The highest and lowest total spikelets also occurred in the SE-CS80 and SE-CS20 treatments, which was significantly and insignificantly different (LSD test at p < /> 0.05) from the others, respectively. Furthermore, the percentage of filled spikelets ranged from 11-19%, as the highest occurred in the SE-CS80 treatment, which was not significantly different (LSD test at p > 0.05) than the SE-CS40 and SE-CS60. However, the lowest filled spikelet occurred in the SE-CS0 treatment, which was significantly different from the SE-CS20. Based on the three parameters presented in Figure 2, the value of plant height, as well as leaf number and area, had the highest occurrence at concentrations ranging from 40% - 80%. This higher value correlated with the available nutrient content (N and P) and growth-regulating hormones carried by the engineered soil product [24;38]. All engineered soil product also stored AMF spores, as the total content at 20% concentration were observed to be the highest (Figure 1). Based on this condition, the performance of the spores at a concentration of 40% - 80% was more
effective in facilitating the root uptake nutrients and water from the growing media. This was due to improving the growth of vegetative and reproductive components that were associated with the yields of Kambowa local upland rice variety [39;40].

4. Conclusions
The application of seaweed extract (K. alvarezzi) to the soil had no significant effect on the reduction of the total number of earthworms (Pheretima sp.). Furthermore, the concentration of 20% extract increased the total spores of the arbuscular mycorrhizal fungus in the soil. The addition of engineered soil product with high extract concentrations also increased the growth and reproductive components associated with the yields of Kambowa local upland rice varieties. Future studies are also needed in increasing the soil ecosystem services, towards the increment of a sustainable upland rice productivity.

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