Effect of Cogongrass (*Imperata cylindrica* L.) root extract on earthworms, arbuscular mycorrhizae fungi spore, and growth of upland rice (*Oryza sativa* L.) for local Kambowa variety

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**Abstract.** Earthworms have the ability to create new conditions in the soil environment and modulate the growth of beneficial soil microbial populations. *Imperata* releases exudate which creates less suitable soil conditions for growing food crops and horticulture. This study aims to: (i) study the effect of the concentration of cogongrass root extract on the abundance of earthworms and arbuscular mycorrhizal fungi spores- (ii) to determine the effect of the soil-extract mixture engineered using earthworms on the upland rice growth for local Kambowa variety. The first experiment, extracts of cogongrass roots were made into five concentration levels, namely 0%, 20%, 40%, 60%, and 80%. Each concentration was mixed with soil from cogongrass land in a different vermireactor. In reactor, earthworms were released that had emptied their stomach contents, and were allowed to manipulate the mixture until the vemicast covered part of the soil surface. As a result, the total earthworms in all reactors were relatively the same, and the total spores of arbuscular mycorrhizal fungi in the soil treated with a concentration of 60% was the most. The second experiment, the upland rice seedlings were grown on engineered soils. The differences in height, leaf area, number of tillers, panicle length, and total spikelet’s per panicle were significantly, except for leaves number, wet weight, dry weight, and percentage of spikelet filled. In conclusion, earthworms as potential ecosystem engineers are utilized in ecological engineering of soil quality in land dominated by reeds vegetation for the development of upland rice cultivation areas, particularly the local varieties of Kambowa.

1. **Introduction**

The growth of harvested areas and production of upland rice has increased by more than 10%; meanwhile, lowland rice is around 2% in Indonesia for the 2016-2017 years [1]. Although it’s small contribution, upland rice is a source of calories and household income for millions of people living on dry land [2]. Various local varieties of upland rice are cultivated by small farmers on dry land scattered in various regions in Indonesia, including North Buton Regency, Southeast Sulawesi. In this area, local farmers have traditionally cultivated upland rice of the local Kambowa variety, where the rice smells good and the rice tastes fluffier [3].
Indonesia has the most extensive land for cogongrass in tropical Asia [4]. Utilization of this land as a new area for cultivation of a local variety (including Kambowa local variety) has abiotic and biotic stress components that must be managed in such a way that maximum yields can be achieved [5]. A number of abiotic stressors to the soil environment include water availability and low essential nutrients, salinity, and heavy metal pollutants [6], while biotic stressors can include soil pathogens, parasitic nematodes, and weeds via an allelopathic mechanism to suppress the growth of neighboring plants [7].

Rhizome roots of cogongrass release exudates which contain a number of allelochemical compounds that can inhibit seed germination and root elongation [8]. In addition, these compounds can also have antimicrobial properties that can inhibit the symbiotic efficacy of food plant roots with beneficial microbes, such as arbuscular mycorrhizal fungi [9]. The approach to the restoration of soil ecosystems that have been exposed to allelochemical and antimicrobial compounds can be carried out through the biodegradation of the phytotoxic compounds in the soil [10]. Earthworms have the ability to promote the biodegradation of soil organic matter [11]. The activity of earthworms mixes organic matter with soil particles to produce vermicasts which can provide microhabitat of soil biota such as arbuscular mycorrhizal fungi, bacteria, protozoa, and nematodes [12; 13]. The synergistic benefit of vermicast against beneficial microbial agents, so that currently vermicast has been utilized as beneficial microbial bioinoculant carrier materials, such as bacteria and vesicular-arbuscular mycorrhiza fungi for improved plant growth [14; 15]. Arbuscular mycorrhizal fungi have the ability to facilitate increased plant growth under conditions of allelochemical stressors [16]. Objectives of the study are (i) to study the effect of the concentration of cogongrass root extract on the abundance of earthworms and arbuscular mycorrhizal fungal spores, and (ii) to determine the effect of the soil-extract mixture engineered using earthworms on the upland rice growth for local Kambowa variety.

2. Material and methods

2.1. First experiment

The first series of experiments aimed to examine the effect of the concentration of cogongrass root extracts on the abundance of earthworms and arbuscular mycorrhizal fungal spores in a vermireactor. This experiment was carried out at the Biodiversity Unit Laboratory, Faculty of Agriculture, Halu Oleo University, Southeast Sulawesi, from March to November 2019. The five levels of extract concentration from reeds roots that were tested were 0% (labeled A0), 20% (labeled A1), 40% (labeled A2), 60% (labeled A3), and 80% (labeled A4). Each concentration was repeated six times following a randomized block design procedure, for a total of 30 vermireactors.

Cogongrass roots were collected from Experimental Station II of the Agriculture Faculty, located in the area of the Bumi Tridharma Campus, Halu Oleo University. The roots are cleaned using tap water, and cut into 1 cm in sizes. A total of 200 g of root pieces and 600 ml of water are put into a container and mashed using a kitchen blender. The fine roots solution is poured into an Erlenmeyer 1000 ml, and heated using a hot plate at a temperature of 60°C while stirring using a stirrer which is set to a speed of 650 rpm for 5 minutes. The extract which had been cooled for 10 minutes was separated from the remains of the roots using a filter cloth, and the extract is referred to as the 100% concentration. From this concentration, it is diluted using water to make different concentration levels, respectively is 80%, 60%, 40%, 20%, and 0%. Each extract was put in a different plastic bottle, and stored in a cooling room at a temperature of ± 4°C until applied.

A total of 2 kg of soil taken from the Imperata rhizosphere which passed the 4 mm sieve was put into a beam-shaped vermireactor with a size of 25 cm x 21 cm x 21 cm. In different vermireactors, the concentration of cogongrass root extract was applied. A total of 100 ml of each concentration is mixed with soil in the vermireactor by hand and left to stand for two days. Furthermore, as many as 20 adult earthworms (Pheretima sp.) whose stomach contents had emptied were released into each vermireactor. After all parts of the earthworm body entered the soil, then each vermireactor surface is covered using plastic gauze measuring 2 mm per hole. Each vermireactor was left for 42 days up to more than 50% of the soil surface covered by the vermicast. During the process, the soil moisture is maintained using tap water.
water. The earthworm removed from the vermicast by hand sorting technique. Adult, immature, and eggs numbers of earthworm from each vermireactor were counted, while the soil was dried for one day, mashed, and sieved using a 0.2 mm filter per hole. A total of 50 grams of the vermicast sub-samples were taken to determine the total spores of arbuscular mycorrhizal fungi, and the remaining vermicast was put into a zipper pack measuring 25 x 30 cm for application purposes on the soil for upland rice growing.

Sub-sample of vermicast for determination of the number of spores is put into a container filled with 400 ml of tap water and stirred. The suspension is filtered with a stratified sieve, in which a 212 µm sieve is placed on the surface of the 38µm sieve. The remaining suspension that is accommodated on the surface of the 38 µm sieves is flowed using a sugar solution 20% in concentration into the test tube containing sugar solution 60% and centrifuged at 200 rpm for 5 minutes. The solution is poured over the 38µm filter surface, using tap water flow on the surface filter the sugar solution completely washed off. All the spores that are stuck on the filter surface are slowly sprayed using a bottle filled with water, the water flow from the filter was collected into a petri dish size 5 cm in diameter. Next, to the spores of the arbuscular mycorrhizal fungi counted under a dissecting microscope, and the total spore lived in each sub-sample was estimated following the instructions from INVAM [17].

2.2. Second experiment

This second series of experiments aims to determine the effect of the vermicast on the growth and yield component of the upland rice. The experiment was carried out in a glasshouse at the Department of Agrotechnology, Faculty of Agriculture, Halu Oleo University from May to November 2019. The air temperature in the house range is 30-32°C. Soil for growing media of the upland rice was taken to a depth up to 10 cm from the same in the first series experiment. The soil has been win-dried for three days, sieved using sieving 2 mm per hole. A total of 5 kg of soil passed the sieving was put into a polybag, saturated with tap water, and left overnight. An amount of 200 g of every vermicast was sown on the soil surface in each polybag.

The Kambowa local varieties of upland rice seeds are obtained from local farmers in Kambowa District, North Buton Regency. The seeds that pass the sorting sown on the surface of a cloth carpet that has been moistened, and covered with another cloth carpet, then remoisten using tap water, and germinated up to seedlings old 14 days. Every two seedlings with uniform in height and its root completely developed transplanted into each polybag that had been applied with the vermicast treatment. Next, everyone was placed in a greenhouse following a randomized block design procedure. The moisture of the growing medium is retained by watering it with tap water once per day.

Plant height, leaves number, leaf area, and tillers number were measured at the 14, 28, 42, 56, 70, and 84 days after planting (DAP). Wet weight (g), dry weight (g), panicle length (cm), total spikelets per panicle, and percentage of spikelets filled (%) was measured at the 120 DAP. Leaf area is determined by measuring the length and width of the upland rice leaves. Measurement located at the bottom, middle, and top of the leaf part. The leaf area was estimated using the formula from [18]: \( LA = l \times w \times 0.77 \), where \( LA \) is the area of the leaf (cm²), \( l \) is the length (cm) and \( w \) is the width (cm). For the dry weight determination, each plant was dried at an initial temperature of 50°C for 24 hours, and then the temperature was increased up to 70°C for 24 hours in oven.

2.3. Statistical analysis

The analysis of variance at the level of \( p < 0.05 \) was used to detect the effect of vermicast application on plant height, the number of leaves, leaf area, wet weight, dry weight, panicle length, total spikelet per panicle, percentage of spikelet filled. To detect significant differences between treatments were used the comparison test from Student-Newman-Keuls (SNK) at the \( p < 0.05 \) level.
3. Results and Discussion

3.1. Results

3.1.1. Abundance of earthworms and total spore of AMF. A total of 20 adults of *Pheretima* sp were released to manipulate soil treated with cogongrass root extract in a vermireactor. After 42 days of the engineering process, earthworms in vermireactor with A0 treatment were found in 19 adults, one immature, and one egg. A total of 19 adults and one egg in the vermireactor with A1 treatment. In vermireactor with treatment A2 was found 19 adults and two eggs. In the vermireactor with treatment A3 was found 19 adults, one immature and one egg. In the vermireactor with treatment A4 was found 18 adults, one immature, and two eggs (table 1). Analysis of variance on the number of adults, immature, and eggs showed that the effect of cogongrass root extract concentration on abundance of *Pheretima* sp was not significant (at p> 0.05 level).

Table 1. Number of earthworm (mean±s.d, n = 6) at 42 days after rearing in vermireactor containing soil treated with different concentrations of cogongrass (*Imperata cylindrica* L.) root extract.

| Treatments | Mature | Immature | Eggs |
|------------|--------|----------|------|
| A0         | 19±1.9a| 1±0.8a   | 1±0.8a|
| A1         | 19±1.5a| 1±1.8a   | 0±0.4a|
| A2         | 19±1.8a| 2±2.3a   | 0±0.5a|
| A3         | 19±0.8a| 1±1.4a   | 1±1.2a|
| A4         | 18±1.4a| 2±1.4a   | 1±1.2a|

Note: Numbers followed by the same letter in the same column are not significantly different at the p> 0.05 level.

The analysis of variance showed that the root extract concentration had no significant effect (at the p > 0.05 level) on the total spores of AMF in vermicasts. Nevertheless, the total spores in the vermicast yield from soil mixed with cogongrass root extract tended to be higher (figure 1).

![Figure 1](image-url)  
*Figure 1. Total spore of AMF (mean ± s.d) in vermicast produced from soil treated with cogongrass root extract in different concentrations. The same letter above bars shown the difference is not significant at the level of p > 0.05.*
3.1.2. Vegetative component. The analysis of variance listed in table 2 shows that the vermicast application produced from each concentration of cogongrass root extract has a significant effect (p = 0.020 level) on plant height 28 DAP, while time others were not significant (at the p > 0.05 level). The significant effect of the vermicast was shown on leaf area at the 28 (p = 0.004), 42 (p = 0.005), and 56 (p = 0.040) DAP, while other times were not significant (at the level of p> 0.05). No significant effect (at the level of p> 0.05) was also shown on the number of leaves and tillers at all observation times, wet weight and dry weight of plants.

Table 2. Analysis of variance of the vermicast applied on the vegetative component of upland rice local variety Kambowa.

| Component of vegetative | Vermicast from concentration level root extract of cogongrass | ANOVA (df1 = 4; df2 = 25) |
|-------------------------|-------------------------------------------------------------|---------------------------|
|                         | A0              | A1              | A2              | A3              | A4              | F    | p level |
| Plant height (cm):      |                 |                 |                 |                 |                 |      |         |
| 14 DAP                  | 26.72±2.98a     | 28.63±1.42a     | 27.05±3.06a     | 26.07±4.04a     | 25.63±1.80a     | 1.004 | 0.424   |
| 28 DAP                  | 35.17±2.38a     | 36.92±3.81a     | 35.96±0.94a     | 34.17±3.20a     | 33.90±3.52a     | 1.078 | 0.389   |
| 42 DAP                  | 40.28±2.70a     | 45.88±1.93b     | 41.08±4.02a     | 40.93±4.04a     | 40.73±1.34a     | 3.529 | 0.020   |
| 56 DAP                  | 50.07±3.32a     | 55.17±1.72a     | 53.18±6.63a     | 51.28±2.46a     | 50.93±3.35a     | 1.992 | 0.126   |
| 70 DAP                  | 55.50±1.10a     | 59.87±5.43a     | 58.15±2.67a     | 56.50±2.81a     | 55.92±0.76a     | 2.112 | 0.109   |
| 84 DAP                  | 56.40±3.05a     | 61.30±8.59a     | 58.93±6.10a     | 57.68±2.67a     | 57.33±5.92a     | 0.538 | 0.709   |
| Leaf number             |                 |                 |                 |                 |                 |      |         |
| 14 DAP                  | 3.67±0.52a      | 3.50±0.55a      | 3.67±0.52a      | 3.50±0.55a      | 3.67±0.52a      | 0.179 | 0.947   |
| 28 DAP                  | 3.83±0.75a      | 4.50±0.55a      | 4.33±0.52a      | 4.17±0.41a      | 4.17±0.41a      | 1.250 | 0.316   |
| 42 DAP                  | 4.50±0.84a      | 5.33±0.52a      | 4.83±0.98a      | 4.50±0.55a      | 4.50±0.55a      | 1.579 | 0.211   |
| 56 DAP                  | 5.00±0.63a      | 5.83±0.41a      | 5.83±0.75a      | 5.50±0.55a      | 5.33±0.52a      | 2.206 | 0.097   |
| 70 DAP                  | 5.67±0.52a      | 6.33±0.52a      | 6.33±0.52a      | 5.83±0.41a      | 5.83±0.41a      | 2.574 | 0.062   |
| 84 DAP                  | 6.17±0.98a      | 6.83±0.41a      | 6.67±0.52a      | 6.50±0.84a      | 6.50±0.84a      | 0.764 | 0.559   |
| Leaf area (cm²):        |                 |                 |                 |                 |                 |      |         |
| 14 DAP                  | 11.61±2.40a     | 11.42±4.24a     | 11.20±2.57a     | 9.35±3.92a      | 10.43±2.32a     | 0.504 | 0.733   |
| 28 DAP                  | 30.84±6.34a     | 38.52±7.2b      | 31.52±3.3a      | 30.95±3.20a     | 27.20±2.15a     | 5.174 | 0.004   |
| 42 DAP                  | 4.50±0.84a      | 5.33±0.52b      | 4.83±0.98a      | 4.50±0.55a      | 4.50±0.55a      | 4.774 | 0.005   |
| 56 DAP                  | 5.00±0.63a      | 5.83±0.41b      | 5.83±0.75a      | 5.50±0.55b      | 5.33±0.52a      | 2.942 | 0.040   |
| 70 DAP                  | 104.60±17.96a   | 117.35±34.28a   | 113.27±8.16a    | 107.53±17.30a   | 107.33±8.29a    | 0.415 | 0.796   |
| 84 DAP                  | 129.16±22.83    | 152.75±47.00    | 140.45±18.21    | 131.88±20.99    | 129.26±9.65     | 0.849 | 0.508   |
| Number of tillers:      |                 |                 |                 |                 |                 |      |         |
| 70 DAP                  | 0.33±0.52a      | 1.00±0.63a      | 0.33±0.52a      | 0.33±0.52a      | 0.33±0.52a      | 1.679 | 0.186   |
| 84 DAP                  | 0.33±0.73a      | 1.00±1.85a      | 0.50±1.00a      | 0.50±1.00a      | 0.50±1.00a      | 1.102 | 0.378   |
| Wet weight (g)          | 4.64±0.58a      | 7.13±0.64a      | 7.09±0.32a      | 5.05±0.50a      | 4.98±0.62a      | 0.636 | 0.545   |
| Dry weight (g)          | 2.49±0.75a      | 3.64±0.68a      | 3.54±0.35a      | 2.71±0.29a      | 2.56±0.22a      | 0.580 | 0.575   |

Note: Numbers followed by different letters in the same row indicate significant differences according to the Student-Newman-Keuls test at the p <0.05 level.

3.1.3. Yields component associated with grain yields. Analysis of the variance of the three yield components of Kambowa local upland rice showed that the application of vermicast produced by soil mixed with cogongrass root extract had a significant effect (at the p <0.05 level) on the number of spikelet per panicle and panicle length, while the percentage of spikelet filled were not significant (at the p > 0.05 level).
Figure 2. (A) Panicle length (cm); (B) Spikelet number per panicle; and (C) Spikelets filled (%). Note: the different letters that are located above the bar in each image show significant differences according to the Student-Newman-Keuls test at the p < 0.05 level.

In figure 2 shows that the total spikelets of plant applied with vermicast produced from treatment A1 was the highest, while applied with vermicast from treatment A0 was the lowest. The difference between vermicast from treatment A1 compared to treatments from A0, A3, and A4 was significant (at the p < 0.05 level), except that the vermicast from treatment A2 was not significant (at level p < 0.05). The differences between the vermicasts from treatment A0, A3, and A4 are not significant (at the p < 0.05 level), also vermicast from treatment A1 to A2 were not significant (at the p < 0.05 level).

3.2. Discussion

In root extract of cogongrass contains chemical substances of phenolic acid which have inhibitory activity against germination and root growth of plant [19]. A study conducted by [20] found extracts obtained from cogongrass root using aqueous as solvent showed inhibiting activity to plant roots and beneficial microflora in the soil [21]. This inhibition has created less chance of root colonization by the arbuscular mycorrhizal fungi [9]. Phenolic acid compounds can be degraded biologically using decomposer agents [22]. Functionally, earthworms can play two different roles simultaneously in the soil, namely as ecosystem engineers and decomposers [13]. Earthworms as soil ecosystem engineers are related to their ability to create new microhabitats modulate microbial communities that are beneficial for the degradation of phytotoxic organic compounds, and pathogens suppressed [23]. Earthworms can utilize phenolic acid compounds as a source of carbon and energy through the decomposition process for the growth and maintenance of their population [24]. In this study was found that the adult population had decreased in all root extract concentrations level. Although there are deaths of adults earthworm, on the other in each treatment found immature and eggs of earthworm. So that the final total of earthworms was more than 20 individuals, except at a concentration of 20% which was 20 individuals (Table 1). These results provide an overview of the growth in the earthworm population. Finally, this fact indicates that earthworms (Pheretima sp.) can survive under soil conditions which heavily exposed by root exudates of cogongrass.

Pheretima sp mixing organic matter with soil particles, releasing vermicast through anus into soil [12;13]. In the vermicast of Pontoscolex corethrurus and Diploptrema heteropora containing morphologically intact spores of vesicular mycorrhizae arbuscular fungi [25]. The results of this study found that the spores of arbuscular mycorrhizal fungi in each vermicast. Although the spore density was similar in each vermicast, there was a trend of higher density in the soil mixture treated with than without root extract of cogongrass (Figure 1). This fact indicates the opportunity to use Pheretima sp as an ecosystem engineer in creating new conditions for the soil environment on land dominated by cogongrass vegetation.
Soil that has been engineered by earthworms always contains a concentration of organic carbon; total nitrogen, P-available, K, Ca, and Mg can be exchanged higher than without being disturbed by earthworm’s activity [26]. Based on the nutrient and beneficial microbe's contents, vermicast often used as a biofertilizer and carrier material beneficial bacteria for improving the performance of soil quality and plant growth [27; 28]. In this study (Table 2) it was found that vermicast applied had a significant effect on height, leaf area, panicle length, and the number of grains per panicle of the plant. These results illustrate that the agronomic characters of the local Kambowa variety give different responses to changes in the modified soil environmental conditions through the application of vermicast produced by *Pheretima* sp activities. A significant influence on plant height occurred at 42 DAP, while on leaf area occurred at 28, 42, and 56 DAP. This fact reaffirms that the effect of the application of organic fertilizers (vermicast) on the vegetative growth of local varieties of upland rice is different in time [29].

The wet and dry weight of plants applied with vermicast from soil treated with cogongrass root extract with a concentration of 20-40% showed a higher tendency than others. This result may be related to the leaf area that occurred in the application of the vermicast which was higher than the others (Table 2). The higher plant fresh weight indicated that more water was stored in the plant tissue [30; 31] which was applied with the two vermicasts. The wider leaves and the water status stored in the plant tissue relatively much allows the photosynthesis rate to be higher, the implication is that the plant dry weight is also higher [32; 33].

The vermicast applied in the experiment carried arbuscular mycorrhizal fungal spores (Figure 1). Arbuscular mycorrhizal spores carried by soil are able to sporulate and infect the roots of plant seedlings [34]. Plant roots infected with arbuscular mycorrhizae have the ability to access water and nutrients under the low fertility of tropical soils [35]. The high biomass of upland rice that occurs in the application of vermicast produced from soil treated with cogongrass root extract with a concentration of 20% - 40% is likely to contribute to the performance of the arbuscular mycorrhizal fungi spores it carries. This possibility provides an opportunity to evaluate the composition, colonization, and infectivity of the mycorrhizal spore communities carried by the vermicast, and their relationship to phenolic acid and other soil environmental factors needs to be studied for future research.

Dry weight correlates positively with yield components associated with grain yields of upland rice [36]. This study shows that panicle length and spikelet number per panicle also occur in the same vermicast with fresh weight and dry weight of plants, and the value of each of these agronomic characters in vermicast without extract is always lower. The percentage of spikelet filled achieved from this study was around 10% which occurred for all vermicasts (Figure 2). This percentage is lower than the percentage of spikelet filled (mean 78%) of the upland rice local Kambowa variety when measured under its original agroecological conditions in the North Buton District [3]. The low value achieved in this study is probably related to the low fertility factor of the soil that has been used as a growing medium, without the addition of chemical fertilizers, and temperatures ranging from 30° - 32°C in the glass-house during the experiment [37; 38]. Up to now, studies related to agroecological factors that contribute to the growth and productivity of upland rice of the local Kambowa variety in their natural habitat has been neglected. Therefore, future research is needed to analyze the interrelationship of ecological factors in above- and belowground that affected yield components associated with grain yields of upland rice Kambowa local varieties.

In conclusion, earthworms as ecosystem engineers are most potentially utilized in ecological engineering on soil quality for the development of upland rice local varieties of Kambowa at a land dominated by reeds vegetation. Therefore, further studies are needed to investigate other agroecological factors that support the maximum performance of soil quality that has been ecologically engineered by earthworms for the development of upland rice local Kambowa variety outside its natural habitat, especially at a land dominated by cogongrass vegetation.

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