Rice Waste Hydrolyzation at Subcritical Temperature to Produce Bioherbicide to Control Terrestrial Weeds

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Abstract. Thermal anaerobic hydrolyzation process on rice husk with higher lignin content produced organic complexes constituents including phenolic groups. Phenolic groups expose herbicide effects on growing vegetations, where the process in nature mainly require anaerobic condition. The degradation rate in nature is very slow with very low concentration availability. Thermal hydrolyzation is an appropriate choice to produce phenolics herbicide for sustainable pesticide management and application. This study aimed to identify the potency of rice husk extract by thermal hydrolyzation process as an organic herbicide to control terrestrial weeds. The hydrolyzation was processed in 60-90 bars and 280±20 ºC for 30±10 minutes. The existence of the phenolic groups in the hydrolysate was considered for generic active ingredients to control weed in the terrestrial ecosystem. Its damage impact was tested on Borrella alata, Eleusine indica, Cyperus kyllingia, Paspalum conjugatum, Asystasia intrusa, and Axonopus compressus.

The assay covered the growth at pre-emergence, early post-emergence, and post-emergence growing stages. The rice waste hydrolysate was capable to suppress the growth of the weed growth especially at the pre-emergence stage at 30-64%, and at early post-emergence stages at the suppressing rate of 77-100%. However, the suppressing affectivity is lower at the post-emergence stage which only 17-25%.

1. Introduction
Lignocellulosic waste from agriculture and forestry mostly comprising wooden materials, straw, barks, and the rest of other biomass are available in an enormous quantity. Its application was broadly known such as for organic fertilizer or incorporation into the soil, for soil conservation purposes, soil amelioration, and bioenergy resources as well.

Another challenging alternative for application in agriculture and silviculture fields as an organic herbicide is to degrade it to produce biologically active substances such as phenolic acids, which groups have potency as herbicide effects. Its similar effect was reported previously, that fermented sago waste inhibited the growth of special weeds on dwarf pepper [1]. Extracted active substances mostly purposing for allelopathic substances from various kinds of plants were tested on weeds such as Imperata sp.[2].

Zea mays [3], Gysophyla sp. [4], Haplophyllum sp.[5], Vicia cracca[6], Medicago sativa [6] and Echinochloa colonum [7]. Recent extracts of various plant species and types such as citrus[8], cardoon[9], essential oils[10], and aerial spontaneous plants[11] were also exploited for bioherbicide application. The main active ingredients of bioactive substances from plants extraction are supported by phenolic compounds [12,13]. Eubiotic active ingredients of herbicide application promise more sustainability to minimize the negative impacts of xenobiotics chemicals formula found in synthetic herbicide [14].

Rice husks as the feedstock of the initiated bioherbicide provide more security in resource availability. Due to its higher content of lignin of rice husk ranging 13.3-19.9% [15,16], the biomass products would offer more various degraded products complexes. Since the cellulose degradation products are less considered bioactive substances, the lignin degradation of these two shells promises more potential resources for the feedstock.

A natural process to produce active biochemical substances from lignocellulosic material having herbicide effects occurs only in supported anaerobic conditions e.g. in peat soil, where it requires a long time duration [17]. However, to produce the proposed ingredient(s) in a relatively short time, the feedstock can be thermally hydrolyzed for degrading the biomass resource. Conversely, each specific bioactive substances or formula from the produced hydrolysate in this experiment were not further exactly identified. It was described only in a generical description or group of chemical compositions.

The purpose of the experiment was to identify the potency of rice husk extract by thermal hydrolyzation process as organic herbicide to control terrestrial weeds Eleusine indica as grass weed and Cyperus kyllingia as sedge group. Moreover, Bossiaea alata, Asystasia intrusa, and Axonopus compressus were also included in the tested weeds as the broadleaf weed group.

2. Methodology
Greenhouse and laboratory experiments were conducted at the Campus of IPB University, Bogor, Indonesia in 2014/2015. The thermal hydrolyzation process was conducted in a 250 ml mini reactor at the temperature level of 280±20°C, with the pressure ranging 80±20 bars in 30±10 minutes. Finely granulated rice husk in a measurement of approximately 30 mesh was stirred in water in a concentration of 150g l⁻¹. The filtrate furtherly as Rice Husk Hydrolysate (RHH) was spread to the tested weeds at a dose of 4 ml hydrolysate per mini cylinder pot with 7.5 cm diameter.

The testing was conducted at early post-emergence and post-emergence growing stages. The application at a pot diameter of 5.5 cm with a dosage of 5 ml per pot. Single-factor of treatment in Randomized Completely Block design was arranged in 4 replications. The tested weed species as treatment in the pre-emergence stage consisted of the emergence of A. compressus, B. alata, P. conjugatum, A. intrusa, C. Kyllingia, and E. indica. Duncan’s multiple new range test was adapted for the mean comparison of tested weed observed parameters. The tested weed species as treatment in the early-post emergence stage consisted of A. compressus, B. alata, C. Kyllingia, and E. indica. The tested weed species as treatment in the post-emergence stage consisted of the emergence of A. compressus, B. alata, C. Kyllingia, and A. intrusa. Duncan’s multiple new range test was adapted for mean comparison of tested weed observed parameters. The testing included the pre-emergence stage, early post-emergence stage, and post-emergence stage of bioherbicide application [18].

The observation covered parameters of leaf normality and damage and plant height. At the early-post emergence stage, plant damage level was calculated as a ratio of damage leaf number and total leaf number per plant. At the post-emergence stage, growth pressure level was approached as the ratio of damage leaf number and total leaf number per plant. The active ingredient of bioherbicide substances was determined with Gas Chromatography Mass Spectrometry (GCMS GC Agilent 7890 with 5760 MS detector).
3. Result and discussion

3.1. Active ingredients yields of the organic herbicides

After the running of the hydrolyzation process, the produced hydrolysate extracts comprised at least 6 carboxylic acid groups, 10 ketone groups, 19 phenolic groups, and 6 other chemical compounds. Other chemical groups in smaller proportions including alcohol and sugar were found. The filtrate of hydrolysate as a solution was applied as a bioherbicide. Remained solid and insoluble substances were separated. In this experiment, there was no testing conducted for every single chemical formula as an active ingredient or interactive effect of combined chemical formulas, but only total combined compounds in the hydrolysate were considered as generic substances for bioherbicide, in which the chemical composition should be considered as not xenobiotics chemical substances.

After the hydrolyzation process in a relatively short duration time, mostly less than 5% of the lignocellulosic materials were degrade into shorter molecular compounds, and around 95% of the rice husk feedstock remained as solid matter. The yield of degraded chemical complexes was generally considered very low. Carboxylic acid yield exhibited a low measurement at an average of 6.70 mg kg\(^{-1}\) rice husk. The produced phenolic compounds reached only 0.79 mg kg\(^{-1}\) rice husk. However, the process to produce the generic active ingredients took only a short time in approximately half an hour. The generic active substances of hydrolysate and their yield is presented in Table 1. Some phenolic compounds suppressing vegetation growth as organic herbicide due to the toxicity characteristics are coumaric acids, p-hydroxybenzoic acids, vanillic acids, ferulic acid, and cinnamic acid are considered toxic [19].

| Groups                | Concentration in the hydrolysate (ml l\(^{-1}\)) | Hydrolysate Yield (mg kg\(^{-1}\)) |
|-----------------------|-------------------------------------------------|-----------------------------------|
| Carboxylic acids group| 0.89                                            | 6.70                              |
| Phenolic group        | 0.10                                            | 0.79                              |
| Ketone group          | 0.41                                            | 3.09                              |
| Other complex groups  | 0.11                                            | 0.86                              |
| Total                 | 1.52                                            | 11.44                             |

3.2. Herbicide effects on tested weeds

The three groups of terrestrial weeds were tested at the pre-emergence stage, early- post-emergence stage, and post-emergence stage. At the pre-emergence stage, the RHH succeeded in reducing emerged individu number per pot. At pot surface, A. Compressus dominated the emerged weed species, where its seedling rate was significantly inhibited by 60% in the first week after application and still showed an effective pressure by 25% in the third week after application. The pressure capacity of the RHH to inhibit the weed seedling exposed 64% in the first week and remained only 30% in the third week. Other emerged species were in a very small quantity, furtherly it was not justified, whether the RHH had a significant effect on the tested weeds or not. The effect of the RHH application on the performance of tested weed species at the pre-emergence stage is presented in Table 2.

The RHH application of hydrolysate was applied to damage leaf of tested weeds. The plant height of three types of terrestrial weeds was repressed by 52-85%. After 7 days of RHH applications, where RHH was applied in 1 week of growing time, the normal leaf number of C. Kyllingia sedge weed was lowered by 58%, and the normal leaf number of E. indica as grass weed was successfully reduced by 100 %. The plant damage level of A. compressus, and B. Alata reached 84% and 77%, respectively. The test weed performance due to the RHH application at the early post-emergence stage is presented in Table 3.
Table 2. The herbicide effect of the RHH application on tested weed species at pre-emergence stage*

| Treatment  | Weed Species | Emerged individu number per pot*** | Rate of seedling pressure (%) |
|------------|--------------|-------------------------------------|-----------------------------|
|            |              | 1 | 2 | 3 | 1 | 2 | 3 |
| Control    | Total weeds  | 12.3a | 15.3a | 15.3a | 0a | 0a | 0a |
| Rice husk  | Total weeds  | 4.3b | 10.3b | 10.5b | 64b | 32b | 30b |
| Control    | A. compressus| 10.0a | 12.0a | 12.0a | 0a | 0a | 0a |
| Rice husk  | A. compressus| 4.0b | 9.0b | 9.0b | 60b | 25a | 25a |
| Rice husk  | B. alata     | 0.3 | 0.3 | 0.3 | ** | ** | ** |
| Rice husk  | P. conjugatum| 0.0 | 1.0 | 1.2 | ** | ** | ** |
| Rice husk  | A. intrusa   | 0.0 | 0.0 | 0.0 | ** | ** | ** |
| Rice husk  | C. kyllingia | 0.0 | 0.0 | 0.0 | ** | ** | ** |
| Rice husk  | E. indica    | 0.0 | 0.0 | 0.0 | ** | ** | ** |

*Unequal indexed letters (a,b) at the same column and weed species show significant difference level at p=0.05

**Not analyzed, emerged individu was dominated by A. compressus

***WAA = Weeks After Application of the RHH

Table 3. The herbicide effect of shell hydrolysates application on the tested weeds at early post-emergence stage*

| Weed Species | Treatment | Plant height (cm) | Normal leaves number | Damage leaves number | Plant Damage level (%) |
|--------------|-----------|-------------------|----------------------|----------------------|------------------------|
| A. compressus| Control   | 0.4a              | 2.9a                 | 0.0a                 | 0.0a                   |
| A. compressus| RHH       | 0.1b              | 0.5b                 | 2.5b                 | 84b                    |
| E. indica    | Control   | 0.6a              | 1.0a                 | 0.0a                 | 0.0a                   |
| E. indica    | RHH       | 0.2b              | 0.0b                 | 1.0b                 | 100b                   |
| C. kyllingia | Control   | 1.9a              | 3.1a                 | 0.0a                 | 0.0a                   |
| C. kyllingia | RHH       | 0.8a              | 0.7b                 | 2.7b                 | 81b                    |
| B. alata     | Control   | 0.7a              | 4.0a                 | 0.0a                 | 0.0a                   |
| B. alata     | RHH       | 0.1b              | 0.9b                 | 3.1a                 | 77b                    |

* Unequal indexed letter (a,b) at the same column and weed species shows significant difference level at p=0.05

**DAA = Days After Application of the RHH

Plant height and normal leaves number per individu at the post-emergence stage were not affected by the application of RHH (table 4). Only leaf growth of A. compressus from all of the tested weed plants was inhibited so that the plant leaves number was reduced by 59%, which significantly underwent the damaged leaves. The application of RHH was also effective to cause damage to the leaf of C. Kyllingia, A. intrusa, and B. Alata.

Growth pressure parameters represented the pressure of weed growth due to the application of RHH compared to the control treatment. It showed, that only A. intrusa was repressed by the RHH application by 25% of growth pressure (table 4). Although it is significantly different, the application at the post-emergence stage is not appropriate enough to be recommended, because the effect was too weak at this stage. Moreover, this point of view was supported by the fact, that the affectivity of the RHH application had no significant suppression on all of the other growth of tested weeds.
Table 4. The herbicide effect of shell hydrolysates application on the tested weeds at post-emergence stage*

| Weed Species | Treatment | Plant height (cm) | Normal leaves number | Damage leaves number | Growth pressure (%) at 7 DAA** |
|--------------|-----------|-------------------|----------------------|----------------------|-------------------------------|
| *A. compressus* | Control | 3.0 | 7.3a | 0.0a | 0 |
| *A. compressus* | RHH | 2.7 | 3.0b | 3.0b | 23 |
| *C. kyllingia* | Control | 3.1 | 8.0 | 0.0a | 0 |
| *C. kyllingia* | RHH | 1.8 | 4.3 | 3.3b | 10 |
| *A. intrusa*  | Control | 1.0 | 6.0 | 0.0a | 0a |
| *A. intrusa*  | RHH | 0.6 | 3.7 | 2.3a | 25b |
| *B. alata*   | Control | 1.9 | 8.0 | 0.0a | 0 |
| *B. alata*   | RHH | 1.4 | 4.7 | 3.3a | 17 |

*Unequal indexed letter (a,b) at the same column and weed species shows significant difference level at p=0.05

**DAA = Days After Application of hydrolysate

4. Conclusion

Thermal anaerobic hydrolyzation process on rice husk with higher lignin content produced organic complexes constituents including phenolic groups. The hydrolyzation was processed in middle pressure 60-90 bars and temperature 280±20 °C for a relatively short time of 30±10 minutes. The existence of the phenolic groups in the hydrolysate was considered for generic active ingredients to control weed in the terrestrial ecosystem. Its damage impact was tested on *Borreria alata*, *Eleusine indica*, *Cyperus kyllingia*, *Asystasia intrusa*, and *Axonopus compressus*. The assay covered the growth at pre-emergence, early post-emergence, and post-emergence growing stages. The testing was conducted in a greenhouse and on a model experiment scale. The rice waste hydrolysate was capable to suppress the growth of the weed growth especially at the pre-emergence stage at the rate of 30-64%, and at early post-emergence stages at the suppressing rate of 77-100%. However, the suppressing affectivity is lower at the post-emergence stage at the rate of only 17-25%.

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