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Biostimulasi Perkecambahan Padi Lokal Yang Mengalami Dormansi Melalui Optimalisasi Lama Perendaman Ekstrak Keong Mas Diperkaya Bioaktivator PGPR

Biostimulation of Dormant Local Rice Germination Through Optimization of Soaking Time of Gold Snail Extract Enriched With PGPR Bioactivator

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ABSTRACT

Seed dormancy poses a serious challenge when aiming for precise and uniform sowing times in crop cultivation activities. Dormancy can reduce seed viability thus inhibiting germination and early plant growth. The purpose of this study was to test the effectiveness of gold snail extract enriched with PGPR (Plant Growth Promotion Rhizobacteria) bioactivator by optimizing the length of soaking in local rice seeds that experience dormancy. The research was conducted using a Completely Randomized Design (CRD) with 5 treatments of control soaking duration (P0), 12 hours soaking duration (P1), 24 hours (P2), 36 hours (P3) and 48 hours (P4) with 4 replications so that a total of 20 experimental units were obtained. Each experimental unit consisted of 25 local Kamba rice seeds by observing the percentage of germination, vigor index, growth speed, fresh weight, dry weight, root length and pH changes during the fermentation process. The results showed that various treatments of soaking time in gold snail extract enriched with PGPR bioactivator did not provide statistically significant differences in the germination of local rice seeds that experienced dormancy. However, there was an increasing trend in all observation parameters indicating that the viability and vigor of seeds soaked in gold snail extract for 36 hours (P3) were better than the control. pH and temperature observed before and after fermentation, showed conditions that remained optimal. The combination of gold snail extract added with PGPR as a bioactivator is an alternative solution that can accelerate the breaking of dormancy, increase seed viability and vigor as an effort to support more optimal and sustainable early growth of rice plants.

Keywords: *Dormancy, germination, gold snail extract, local rice, PGPR*

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INTRODUCTION

Rice has a strategic role as the main source of rice and staple food in meeting the food needs of most of the population. Currently, the Government of Indonesia places food availability as a top priority that plays a major role in national economic growth and has become one of the strategic work programs of President Prabowo Subianto.

The rice harvest area in Indonesia based on 2023 data reached ± 10.21 million hectares with a production of 53.98 million tons of milled dry grain (MDG). This has decreased compared to the previous year (2022) of 54.75 million tons of GKG with a harvest area of 10.45 hectares (BPS, 2024). In addition to superior rice varieties, local rice varieties continue to be cultivated by most farmers. Local rice still occupies a special position because it has high genetic diversity, is able to adapt to diverse environmental conditions and climate change (Wijayanto *et al.,* 2024; Marlina *et al.,* 2023; Yulina *et al.,* 2021). Local varieties have unique characteristics such as better flavor, fluffier rice texture, and high adaptability to certain environmental conditions (Septaria *et al.,* 2023; Abas *et al.,* 2019).

Local rice has evolved through natural selection and a long process of adaptation, so that it is able to survive in soil conditions, climate, and agricultural practices that are specific to its area of origin (Chaniago, 2019; Hafiz *et al.,* 2020). In addition, local rice has a high cultural and economic value to the community, as it is often used in various traditional ceremonies and local traditions (Saharudin, 2021; Sudewi *et al.,* 2020; Najamuddin, 2023). Despite its advantages in terms of adaptation and cultural value, the availability of local rice seeds that are ready for planting at any time is absolutely necessary. One of the factors that hamper the availability of seeds is the nature of the seeds themselves, which easily experience dormancy. A condition that causes newly harvested rice seeds to not germinate quickly or simultaneously despite optimum environmental conditions (Nurhaliza *et al.,* 2023; Rumahorbo *et al.,* 2020). Dormancy can inhibit the early growth process, decrease seed viability, and even reduce productivity, especially when a precise and uniform planting time is desired (Klupczyńska & Pawłowski, 2021; Wijayanti, 2023). Various methods have been developed to overcome these problems, including the use of natural ingredients that can stimulate germination. One of the promising materials to be applied is the utilization of gold snail extract. This extract is known to have activity as a bio-ZPT (Growth Regulator) that plays a role in stimulating plant growth (Mulyanti *et al.,* 2023). Gold snail has been known as a major pest in rice fields that has a relatively fast breeding period (C. Zhang *et al.,* 2023). Hutasoit et al. (2016) reported that this pest can damage ≥ 80% of rice fields. Furthermore, Saputra et al. (2018) stated that the meat and shells of gold snails have high protein, fat, carbohydrates, Na, K, riboflavin, Niacin, Mn, C, Cu, Zn and Ca content. In addition to meat and shells, gold snail eggs contain the microbe Aspergillus niger which can produce growth hormones such as auxin and gibberellin which act as ZPT (Situmeang *et al.,* 2022; Andriani, 2019; Abdullah *et al.,* 2017). Various types of amino acids are found in the gold snail including the amino acid tryptophan which is a precursor compound for the formation of the ZPT Indole Acetic Acid (IAA) so that it can be utilized as a growth regulator (Ghosh *et al.,* 2025; Asmono *et al.,* 2024; Asmono *et al.,* 2024). (Madusari *et al.,* 2021; Firmansyah *et al.,* 2022) reported that coffee seed germination increased after being treated with soaking extract of gold snail egg solution for 36 hours.

The utilization of PGPR (Plant Growth-Promoting Rhizobacteria) as a bioactivator is reported to have great potential in increasing seed germination and plant growth (Hussain *et al.,* 2022; Nadia *et al.,* 2023). The addition of PGPR and pineapple 60% as a bioactivator in the manufacture of seaweed POC was able to increase the content, C, P, K respectively 7.02%, 3.44%, and 3.41% (Indriyanti *et al.,* 2017). Rahma et al. (2023) stated that PGPR has an active role as a bioactivator in making natural compost. The bioactivator that will be used in this study is sourced from PGPR from previous research with its potential ability to produce IAA, namely isolates KBU9 (10.48 mg L^{-1}), KBU22 (10.92 mg L^{-1}) and KLE25 (11.43 mg L^{-1}), the potential to dissolve phosphates, and siderophore producers (Sudewi, 2020; Sudewi *et al.,* 2021; Sudewi *et al.,* 2022).

The utilization of PGPR will enrich the microorganism components in the process of making gold snail extract.

Gold snail extract is rich in phytohormones and amino acids that play a role in the process of seed germination and plant development (Asmono *et al.*, 2024). The use of this extract in seed soaking is expected to break dormancy and increase the germination of local rice. In addition, PGPR can contribute in stimulating germination and early growth of a plant. This study aims to determine the effectiveness of soaking rice seeds that experience dormancy by utilizing gold snail extract as a bio-ZPT enriched with PGPR as a bioactivator on the germination of local Kamba rice seeds. The combination of the two approaches is expected to obtain a more effective method to increase the germination of local rice seeds, overcome dormancy, and contribute to the development of sustainable agricultural technology. Therefore, this research needs to be conducted in order to provide innovative and sustainable solutions in overcoming dormancy of local rice seeds, increasing crop productivity and thus strengthening food security in Indonesia.

RESEARCH MATERIALS AND METHODS

The research was conducted from November 2024 to February 2025 at the Microbiology Laboratory and Green House of the Faculty of Agriculture, Alkhairaat University, Palu. The materials used were local Kamba rice seeds that had experienced dormancy for ± 2 years, 1 kg of snail meat (*Pomacea caniculata* L.) and its eggs, PGPR microbes as bioactivators (isolate formulations KBU22, KBU9, KLE25), coconut water, molasses, soil, manure, husk charcoal. The tools used were germination tubs, glass bottles (for soaking seeds), jars, pans, stoves, blenders, sieves, sprayers, 10 L buckets + lids, 10 L jerry cans, clear hoses, wooden stirrers, knives, funnels, cloth filters, pH meters, thermometers, rulers, analytical scales, envelopes, ovens to remove plant moisture content, labels, and stationery to record research results.

Experiments to determine the effect of the effectiveness of golden apple snail extract enriched with PGPR bioactivator with optimization of soaking time on local rice seeds experiencing dormancy were carried out on kamba rice seeds under laboratory conditions. The study was arranged in a completely randomized design (CRD) with soaking time as a single treatment consisting of four levels and one control. Rice seeds were soaked in a formulation of golden apple snail extract and distilled water (a ratio of 50 ml bio ZPT: 500 ml distilled water) for 12, 24, 36, and 48 hours, respectively, and one level only used distilled water with a soaking time of 24 hours.

Research Procedure. Kamba rice seeds were obtained from the harvest of Lembah Bada farmers in Poso Regency which had undergone a storage period of ± 2 years. The making of gold snail extract as a natural ZPT begins with soaking 1 kg of gold snail with 1.5 L of water that has been brought to a boil, so that the meat and shell are separated. Next, the meat and eggs are blended until smooth. Add molasses as much as coconut water/rice washing water and PGPR bioactivator, mix all ingredients in a bucket and stir until homogeneous. The extract was then transferred to jerry cans and fermented for 15 days in a place protected from sunlight. The acidity level of the extract was measured using a pH meter before and after the fermentation process was complete. The process of making gold snail extract until fermentation is presented in Figure 1.

Kamba local rice seeds are soaked according to the treatment. After the soaking process is complete, the seeds are then air dried for ± 15 minutes and are ready to be germinated in a nursery with a composition of 1:1:1 mixing between soil, manure, and rice husk media. The germination media was previously allowed to stand for 1 week and then moisturized to field capacity. A total of 25 rice seeds were germinated according to the treatment. Maintenance is done by watering once a day until moist using a hand sprayer.

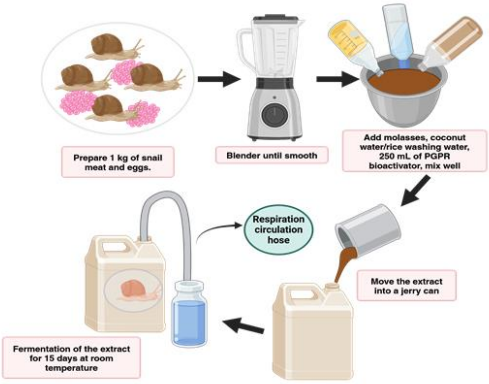

Figure 1. Procedure for Making Gold Snail Extract Enriched with PGPR Bioactivator with a Fermentation Duration of 15 Days.

Observation Parameters. Observations on pH and temperature values were made on the solution before immersion was applied. Seed observations were carried out up to 14 days with the observed variables being germination power (%), vigor index (%), maximum growth potential (%). Parameters for seed growth rate (%/etm), fresh weight (g), dry weight (g), and root length (cm) were used, using five seeds each..

Data Analysis. The research data were analyzed using non-factorial ANOVA test at the 5% significance level and to determine whether there is a real difference between treatments, the smallest real difference (BNT) further test was conducted ($p < 0.05$).

RESULTS AND DISCUSSION

pH and Fermentation Temperature. Parameters that affect the success of microbes in breaking down organic compounds include pH and temperature. pH determines microbial activity while the production of organic compounds (enzymes and organic acids) and the rate of microbial metabolism in the fermentation process are influenced by temperature (Dewi *et al.*, 2022). The effect of pH and temperature before and after fermentation of gold snail extract is presented in Table 1 as follows:

Table 1. Observation Results of pH and Temperature Values of Goldfish Extract Enriched with PGPR Bioactivator at Fermentation Duration for 15 Days.

Observation Parameter	Before Fermentation	After Fermentation	Optimal During Fermentation*
pH	7.3	4.2	5.0 – 6.5
Temperature	28^0C	27^0C	30 – 37^0C

Source: Personal date analysis

Table 1 shows that the initial pH of the extract before fermentation shows 7.3, which means that the resulting pH is in the ideal range, while the pH after fermentation is in the range of 4. This indicates that the fermentation process carried out is running optimally. A less effective fermentation process is characterized by the pH of the solution between before and after treatment is still relatively high, the pungent smell of rot, the color and texture do not change, there is no gas formed, there is a foul-smelling sediment (Mohamad *et al.*, 2021; Sarah *et al.*, 2023; Sudewi, Jaya, & Saleh, 2024; Babich *et al.*, 2024).

One effective solution that can be done is to add more molasses as an energy source for microbes for growth. (N. Zhang *et al.*, 2024) reported that the addition of molasses to the fermentation of a mixture of leftover pumpkin skins and sunflower stalks was able to improve the quality of fermented materials and microbial communities. The optimal pH after the fermentation process for soaking application to seeds

should be around 4.0 - 5.5. If the resulting pH is too low (<3.5), then the gold snail extract must be diluted again before use.

Temperature measurements of the gold snail extract carried out before fermentation were in the range of 28^0C while after fermentation, the temperature obtained was 27^0C (Table 1). The temperature will increase along with the ongoing fermentation process. The increase in temperature during the fermentation process is due to the increasing microbial activity (Brugnini *et al.*, 2024). The temperature during the fermentation process that increases in the range of 30 - 37^0C can be categorized as optimal (Sun *et al.*, 2022).

Percentage of Germination, Vigor Index, and Maximum Growth Potential of Local Rice Seeds. Viability and vigor of a seed can be seen from indicators such as germination, vigor index and maximum growth potential. The results of data analysis on these components are presented in Figure 2.

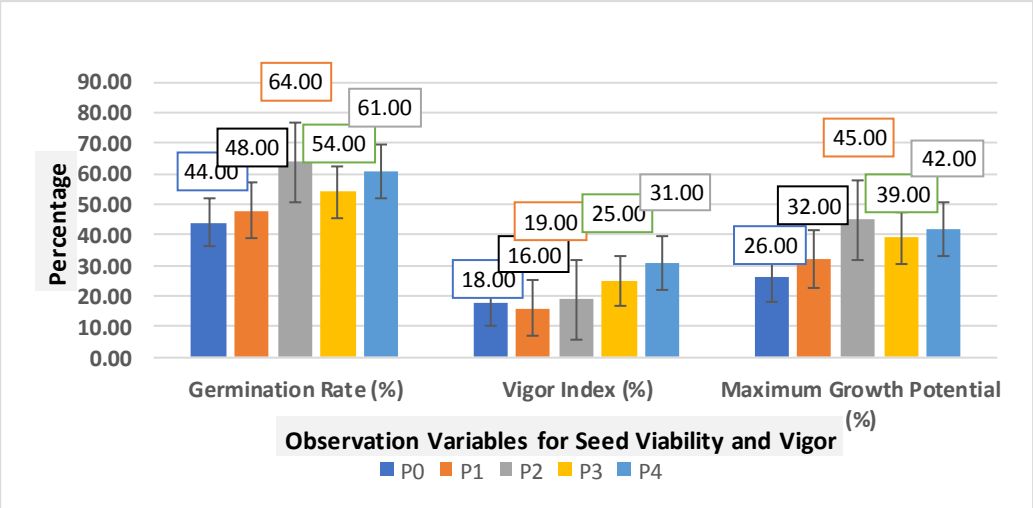

Figure 2. Viability and Vigor Performance of Local Rice Seeds (Germination Rate, Vigor Index,

Maximum Growth Potential) After Treatment of Soaking Time of Goldfish Extract (P0= Control, P1= 12 Hours Soaking; P2= 24 Hours Soaking; P3= 36 Hours Soaking; P4= 48 Hours Soaking)

The results showed that the soaking time treatment of gold snail extract had a significant impact on seed viability and vigor with indicators of germination, vigor index and maximum growth potential although statistically not significantly different. Soaking seeds for 36 hours gave the highest germination rate, followed by soaking for 48 hours. Germination decreased with soaking for 24 and 12 hours, and the lowest germination rate was in the control, with values ranging from 64 to 40% (Fig. 2). These results indicate that soaking dormant seeds in gold snail extract has a positive effect on germination. The germination parameter shows the ability of seeds to start the growth process after being given certain treatments (Leddy *et al.*, 2024).

The gold snail extract contains active compounds that can break seed dormancy (Schweizer *et al.*, 2019) by increasing membrane permeability and allowing the absorption of water and nutrients compared to the control (P0In the vigor index variable, 48 hours of immersion gave results of 31% to 25% in 36 hours of immersion, while the shorter immersion time of the two gave a vigor index below 20%, but statistically it was not significant (Fig. 2).

Gold snail extract contains proteins and lipids (Visca & Palla, 2018; Pertiwi & Saputri, 2020) which are needed during the germination phase so that they contribute to providing energy for the early stages of

growth. In line with Yanti & Kaimuddin (2023) who revealed that priming chili seeds using liquid organic fertilizer keong mas, was able to spur germination and increase the fruit weight of chili plants. The P4 treatment based on Figure 2 was able to increase the vigor index by 13% compared to the control. This increase is significant enough to indicate that the gold snail extract is able to improve the quality of local rice seeds that experience dormancy. The highest growth potential observation variable was recorded at 36 hours of immersion at 45%, but decreased to 42% at longer immersion at 42%, while 12 and 24 hours of immersion gave results of less than 40% and the lowest was in the control at only 29% (Fig. 20.The percentage increase in the maximum growth potential of various treatments is better than the control, indicating that the gold snail extract and PGPR bioactivator have the potential to increase the growth rate of seeds that have decreased viability.

Fresh Weight, Dry Weight, and Root Length of Kamba Local Rice. The results of observations on fresh weight, dry weight and root length of Kamba local rice after being treated with the soaking time of gold snail extract are presented in Figure 3 as follows:

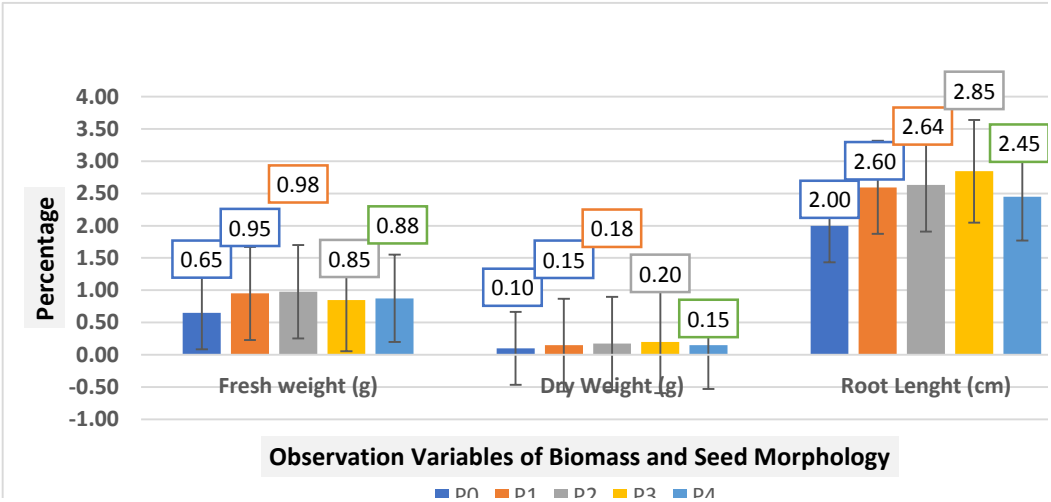

Figure 3. Biomass Performance and Morphology of Kamba Local Rice (Fresh Weight, Dry Weight, and Root Length) After Treatment of Soaking Time of Goldfish Extract (P0= Control, P1= 12 Hours Soaking; P2= 24 Hours Soaking; P3= 36 Hours Soaking; P4= 48 Hours Soaking)

Based on the graph in Figure 3, it can be seen that the three parameters observed showed different effects from the various soaking time treatments given. The results of the study showed an average fresh weight of between 0.85 - 0.98 g/seed, which was far above the control which gave a result of 0.65 g (Fig 3).. Observations of the dry weight of local rice plants showed the best results in the 24-hour soaking treatment with a yield of 0.20 g/seed, higher but not significant compared to other treatment levels of 0.15 g/seed and control or control of 0.10 g/seed (Fig. 3). Meanwhile, in the root length parameter, soaking for 36 hours produced an average root length of 2.85 cm, the highest but not significant compared to other treatment levels with lengths ranging from 2.60-2.64 cm (Fig. 3).

The longer roots from these observations indicate that the seeds soaked in gold snail extract are able to stimulate better root growth, so that nutrient absorption becomes more efficient. It is known that gold snail meat contains tritophan amino acid compounds which are precursors to the formation of the hormone IAA. In line with (Nurshanti *et al.*, 2021) reported that gold snail extract acts as a biostimulant in increasing the fresh weight and dry weight of okra plant roots. The duration of soaking that gives the best results in all parameters is generally found in the P3 treatment (36 hours soaking time). Longer soaking time is able to

activate the germination mechanism of seeds that were previously stored for a long time and have even experienced dormancy. According to Chatchavanthatri et al. (2020) that soaking and longer germination time can increase the germination rate of rice seeds. Widnyana et al. (2024) suggested the same thing based on the results of his research that rice seeds soaked longer with PGPR were able to have a significant effect on the length of the roots of longer rice sprouts. This result is inseparable from the ingredients used in the gold snail extract that also influence. PGPR bioactivator also plays a role in increasing microbial activity in the extract used. The active compound components contained in the gold snail extract act as a source of biostimulants that support germination and early seed growth.

CONCLUSION

This study showed that the soaking time of local Kamba rice seeds did not statistically significantly affect seed viability and vigor. However, there was a trend towards an increase in several parameters, such as germination percentage, vigor index, fresh weight, dry weight, and root length, particularly after 36 hours of soaking. The 36-hour soaking time tended to yield better results than other soaking times, thus providing a basis for further testing. Furthermore, follow-up observations through the generative phase are crucial to determine any significant impact on yield.

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