Modified *Ganoderma* selective medium to meet Indonesia’s government regulation

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Abstract. *Ganoderma boninense*, the causal agent of basal stem rot in oil palm, has caused great losses on oil palm industry. Isolation method of the fungus from the field for further study is considered problematic due to high contaminations. To tackle this, *Ganoderma* selective medium (GSM) has been developed by scientists from Malaysia. The problem occurred when the media is going to be used in Indonesia due to the prohibition of one of its ingredients by Indonesia’s government regulation No. 85/1995 on the management of hazardous wastes and toxic. This study aims to test whether the selective medium is still effective even without Pentachloronitrobenzene (PCNB) and Ridomil. The former is the prohibited ingredients and the latter is not well distributed in Indonesia thus considered as a rare item. The study showed that contamination can be kept to minimal and the distinguished feature of GSM, formation of a brown halo around the colony, was seen. However, the growth of *Ganoderma*’s mycelium seems hampered. We hypothesize that besides being a fungicide, PCNB may play a role as a carbon source that supports growth for *Ganoderma*. The find for substitute ingredient of PCNB in GSM is on its way.

1. Introduction

*Ganoderma boninense*, the causal agent of basal stem rot in oil palm, has caused great losses as much as IDR 3.6 billion on oil palm industry in Indonesia [1]. This disease has become ‘cancer’ in the oil palm industry due to its persistence and sometimes asymptomatic. The fungus can live in the soil for a long period of time, as long as its substrates, such as root debris or decayed root, are still present in soil [2]. Based on field observation on an area which has been undergone crop rotation from oil palm to sugarcane for 5 years, young oil palm seedling that grows from left-over oil palm fruit during harvesting period shows symptoms that indicate *Ganoderma boninense* is still present. This observation supports Hasan and Turner [3] findings that the inoculum was still viable in the ex-oil palm-planted-soil even though fallow period occurred for many years.

Isolation of this fungus from the field is also problematic due to high contaminations during the process. Ariffin, Idris and Singh [4] came up with a solution of formulating a selective media that can distinguish *Ganoderma* among others. However, *Ganoderma* Selective Medium (GSM) may not be used in Indonesia due to the restriction of one from its ingredients, pentachloronitrobenzene, which considered hazardous and toxic as stated by Indonesia’s government regulation No. 85/1999 on Toxic...
and Hazardous Waste Management [5]. According to a review from Arora and Bae [6], PCNB may cause liver damage, renal agenesis, and hepatomas on tested animals and thus PCNB is considered as carcinogenic by the United States Environmental Protection Agency (EPA). Moreover, another GSM’s ingredient namely Ridomil has not distributed anymore in Indonesia. Therefore, this study aims to test whether the selective medium was still effective even without Pentachloronitrobenzene (PCNB) and Ridomil and to check alternative ingredient that can substitute PCNB.

2. Materials and Methods

2.1 GSM Preparation

GSM consists of two parts: part A and part B. The original composition of GSM is showed in Table 1.

Table 1. GSM composition [4].

| Ingredients                  | Amount |
|------------------------------|--------|
| Part A                       |        |
| Bacto-Peptone                | 5 g    |
| Agar                         | 20 g   |
| K\(_2\)HPO\(_4\)             | 0.5 g  |
| Distilled water, pH 5.5      | 900 ml |
| Part B                       |        |
| Streptomycin Sulphate        | 300 mg |
| Chloramphenicol              | 100 mg |
| PCNB, pure                   | 285 mg |
| Ridomil (25% WP)             | 130 mg |
| Benlate – T20                | 150 mg |
| Ethanol, 95%                 | 20 ml  |
| Lactic acid, 50%             | 2 ml   |
| Tannic acid                  | 1.25 g |
| Distilled water, pH 5.5      | 80 ml  |

Part A was homogenized by stirring it on a hot plate until dissolved with a temperature of 100 °C followed by autoclaved for 15 minutes. Meanwhile, part B was stirred for two hours at room temperature. Part B is aseptically poured on Part A when the temperature of part A has decreased to around 45 °C.

2.2 Selectivity test of GSM without PCNB and Ridomil towards Ganoderma

Isolate *Ganoderma boninense* AR77 and B23 originated from oil palm field in Riau and Lampung, respectively, were used in this study. To check GSM selectivity, other fungi were used, namely *Fusarium* sp., *Omphalina* sp., *Pleurotus* sp., and *Trichoderma* sp. The isolates were rejuvenated by growing it on Potato Dextrose Agar (PDA) and was let sit in the dark for 7 days. The edge of the freshly growing hyphae was then cut and aseptically transferred to GSM without PCNB and Ridomil and put in the dark for another 7 days. At the end of incubation, the formation of the brown halo was checked.

2.3 Selectivity test of GSM added with guaiacol towards Ganoderma.

Guaiacol was used to test whether it can function as a substitute ingredient for PCNB. The method for preparation of GSM+guaiacol as well as fungi inoculation, incubation and the observation of brown halo were not different from GSM without PCNB and Ridomil. The only difference was the addition of 1.2 ml Guaiacol to Part B of GSM.
3. Results and Discussion

3.1 Selectivity test of GSM without PCNB and Ridomil

As can be seen from Figure 1, brown halo was formed around Ganoderma colony due to its specific reaction with tannic acid, but not on the other ligninolytic fungi [7]. Tannic acid is also functioned as an inhibited growth compound for contaminant due to its antibacterial activities [8]. The hyphae of Ganoderma seemed did not grow well on modified GSM (without PCNB).

![Figure 1](image1.png)

**Figure 1.** The morphology of the fungi that were grown on GSM without PCNB and Ridomil. Noticed that only Ganoderma that gave the formation of brown halo around the agar.

GSM selectivity is based on the formation of brown halo when Ganoderma grow upon the medium [4]. Moreover, it would inhibit other bacteria or fungi growth because there are abundant of antibacteria and anti-fungi that are mixed in the media. These characteristics were still can be seen on modified GSM. However, Ganoderma seemed cannot grow normally on modified GSM. Its hyphae are not growing even when the isolate was kept in dark chamber for 30 days. When Ganoderma from modified GSM was transferred to PDA, the hyphae could grow normally (Figure 2). Therefore, it is suggested that PCNB might act as a carbon source in GSM.

![Figure 2](image2.png)

**Figure 2.** Ganoderma can grow normal when transferred from modified GSM to PDA.
Although PCNB is widely used as a fungicide, several microorganisms, including fungi, can degrade PCNB [6]. They can degrade PCNB into its derivatives, namely pentachloroaniline, pentachlorothioanisole, pentachlorothiophenol, and pentachlorophenol (Figure 3). More likely, *Ganoderma* can also be one of the microorganisms that can degrade PCNB. This needs further studies.

**Figure 3.** PCNB degradation into its derivatives via metabolism of microorganisms. Taken from Arora and Bae [6].

### 3.2 Guaiacol performance as substitute ingredients for PCNB
*Ganoderma* that grow on GSM+Guaiacol was somehow not showed a formation of brown halo which gives GSM its unique characteristic that can separate *Ganoderma* colony from others. Moreover, *Ganoderma* hyphae were not growing. Other fungi were also seen to be not growing either (Figure 4). Guaiacol is thought to be a substitute ingredient for PCNB due to its similarity in the chemical structure. Both of them are aromatic compound and *Ganoderma* has the enzyme that can utilize such aromatic compound [4]. However, the hypothesis was proved wrong based on the result of this study.

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Guaiacol has been used as an ingredient to distinguish whether the tested isolate has the ability to produce laccase, an extracellular enzyme which is grouped to oxidase enzymes and a part of ligninolytic system enzyme, by changing the colour of agar media into dark-brown [9]. Its mechanism is the same with tannic acid because both of guaiacol and tannic acid react to the presence of phenoloxidase [10] [9]. However, when they were combined, they did not change the colour of the media in this study. Guaiacol is thought to be a substitute ingredient for PCNB due to its similarity in the chemical structure. Both of them are aromatic compound and *Ganoderma* has the enzyme that can utilize such aromatic compound [11]. However, the hypothesis was proved wrong based on the result of this study.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** The morphology of the fungi that were grown on GSM added with guaiacol as substitute ingredients for PCNB. Noticed that GSM lose its unique characteristic and *Ganoderma* hyphae cannot grow either.

### 4. Conclusions

GSM could still form a brown halo when *Ganoderma* was inoculated on it even with the absence of PCNB and Ridomil. The brown halo was not observed when other fungi were grown on modified GSM. PCNB is thought to be the carbon source because the growth of *Ganoderma* is restricted without the presence of the compound in the medium. In spite of that, modified GSM can still be used as a medium that can selectively isolate *Ganoderma* from others when one’s try to isolate *Ganoderma* whether from soil or oil palm tissue.

### 5. References

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