Alternative medium for the growth of endophytic fungi

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Abstract. Medium for the growth of endophytic fungi generally uses Potato Dextrose Agar media, but because the price is expensive, it is necessary to find materials for alternative media from organic materials that are easy to obtain and inexpensive. The legume group was one of the alternative ingredients as the source of protein, corn, and rice as the source of carbohydrates for the growth medium. This study aimed to determine the potential of organic matter such as rice, corn, and legumes as a medium for the growth of endophytic fungi. The research methods included: rejuvenation of endophytic fungus isolation, preparation of organic medium from rice, corn, legumes, and potatoes, growth test of endophytic fungus on 4 types of organic media.

1. Introduction
Medium is a material consisting of a mixture of nutrients that serves as a place to grow microorganisms [1]. Microorganisms, including fungi require certain nutrients are available in the medium for their growth [2,3]. Fungi need carbon, nitrogen, vitamins, minerals, and enzymes [4]. Potato Dextrose Agar medium (PDA) is a medium that is often used for fungal growth in the laboratory. according to Devi et al., (2018), PDA medium has a simple formulation and is able to support the sporulation of several fungi [5]. However, the problem with using PDA medium is costly.

Alternative materials that can be used as an energy source for the growth of microorganisms can be sourced from carbohydrates. Sources of carbohydrates can be from tubers such as arrowroot, gayong, gembili, sweet potato and taro, cassava [1,4,6]. Types of nuts, according to Garraway and Evans (1984), the medium must contain protein for apical hyphal spore formation [7,8].

Source of protein from local plants, vegetable waste, bran, sorghum, corn and millet [6,9–12]. Based on the description above, this study aimed to obtain an alternative medium from corn, beans, greens and rice for the growth of endophytic fungi.
2. Research methods

2.1. Endophyte fungi

Pure cultures of six endophytic fungal isolation used in this study were grown on PDA medium. Each fungus was taken one plate using a cork borer, placed on the surface of the PDA medium. All isolates were incubated at room temperature for 7 days.

2.2. Culture medium preparation

2.2.1. PDA medium. Potatoes were peeled and cut into small pieces, then rinsed and drained. 200 grams of potatoes were put in Erlenmeyer added 500 grams of equates then cooked in a pot that already contained water until it boiled. The boiled potatoes were filtered and put in an Erlenmeyer and added 20 g of agar, 20 g of sucrose and added with distilled water to a volume of 500 ml. The dough was stirred until homogeneous and cooked until boiled. The solution was sterilized by autoclaving, then poured into a sterile petri dish in a laminar airflow. After cooling the cup was wrapped in plastic wrap. Medium was ready to be used as a control in this study.

2.2.2. Alternative medium. Materials for the manufacture of alternative mediums such as mung bean, corn and rice were purchased at the local market. Each material was washed under running water and drained. Mung bean as much as 200 grams and put in an Erlenmeyer then add 500 ml of distilled water and cook it until it boiled over a pot filled with water. The mung bean decoction was filtered using filter paper and put in an Erlenmeyer until the filtrate was obtained. Into the filtrate was added 20 g of agar, 20 g of sucrose, and distilled water to a volume of 1000 mL. The solution was stirred until homogeneous and cooked again. After boiling and homogeneous, Erlenmeyer covered with aluminium foil. The medium was sterilized by autoclaving at 121ª C for 15 minutes at a pressure of 2 atm. The medium was poured into a sterile petri dish in laminar airflow.

2.3. Growth of endophyte fungi

Endophytic fungi isolation E1, E2, E3, E4, E5 and E6, which had grown on PDA medium, were taken one plate each using a cork borer and placed on three types of alternative medium, namely green beans, corn, rice and PDA medium as controls. Each medium was done repeatedly three times. The growth of endophytic fungi in each medium was observed and documented.

3. Results and discussion

The three types of alternative media were able to support the growth of the six isolates of endophytic fungi. Mung bean and rice medium showed maximum growth almost the same as growth on PDA medium (control). The alternative medium used for fungal growth medium had different carbohydrate content. Corn had the highest carbohydrate content but the lowest protein content (Table 1). According to Sharma (2010), colony diameter, surface texture, zonation and fungal sporulation were influenced by the type of medium [6].

| Medium     | Protein (%) | Carbohydrate (%) | Fat | Source |
|------------|-------------|------------------|-----|--------|
| Mung Bean  | 21.04       | 63.55            | 1.64| [13]   |
| Corn       | 1.99        | 72.81            | 4.9 | [14]   |
| Rice       | 4.10        | 49.6             | 0.20| [15]   |
| Potato     | 2.4         | 18               | 0.1 | [16]   |

Table 1. Nutrients of alternative materials for fungal growth medium

The characteristics of the alternative medium of mung bean were brown, corn and rice were transparent white (Table 2). The growth of endophytic fungi on 3 types of alternative media, namely mung bean, corn, and rice, showed that all endophytic fungi isolates could grow on the three types of
alternative media. Mycelia growth, colony character and sporulation pattern of the 6 isolates of endophytic fungi varied (Table 3).

Table 2. Characteristics of medium alternative

| Medium Alternative | Color     |
|--------------------|-----------|
| Mung Bean          | light brown|
| Corn               | White     |
| Rice               | White     |

Endophytic fungi isolate E1 and E3, and E5 showed different textures in the three types of medium. The growth rate of isolates E1, E3, and E5 on the three types of the medium was the same as on PDA medium, but sporulation on corn medium was very less than on mung bean and rice media (Figure 1). This showed that the nutrients contained in the mung bean and rice medium were able to support the growth of the fungus. According to Shareef (2019), the composition of the culture medium was an important factor for the growth of microorganisms [7].

Table 3. Mycelia growth, colony character and growth type and sporulation of endophytic fungus isolate on alternative medium.

| Isolate | Medium | Texture | Top surface color | Color Surface Lower | Growth | Sporulation |
|---------|--------|---------|-------------------|---------------------|--------|-------------|
| E1      | PDA    | Thick   | White             | Beige               | Concentric | Tall        |
|         | Mung Bean | Thick   | White             | White               | Concentric | Currently   |
|         | Corn   | Thin    | White             | White               | Concentric | Less        |
|         | Rice   | Thin    | White             | White               | Concentric | Less        |
| E2      | PDA    | Thick dense | White         | White               | Concentric | Tall        |
|         | Mung Bean | Thick dense | White       | White               | Concentric | Currently   |
|         | Corn   | Grow thin | Yellow white-ish | Yellow white-ish    | Concentric | Less        |
|         | Rice   | Grow thin | Yellow white-ish | White               | Concentric | Less        |
| E3      | PDA    | Thick dense | Black and white | Black and white     | Concentric | Tall        |
|         | Mung Bean | Grow thin | White           | White               | Concentric | Currently   |
|         | Corn   | Fine cotton growth | Black and white | white middle black | Concentric | Less        |
|         | Rice   | Grow thin | White mixed with black | White mix black | Concentric | Currently   |
| E4      | PDA    | Thick dense | Green and yellow | Yellow             | Spread    | Tall        |
|         | Mung Bean | Thick dense | Gray white, green bordered | White | Spread | Currently |
|         | Corn   | Thick dense | Green Edge White | Yellow             | Spread    | Low         |
|         | Rice   | Thick dense | Whiteside chocolate | Black          | Spread    | Tall        |
|         | PDA    | Thick dense | White           | White               | Spread    | Currently   |
|         | Rice   | Fine cotton growth | Chocolate | Black               | Spread    | Low         |
| E5      | PDA    | Fine cotton growth | Whiteside chocolate | Black            | Spread    | Currently   |
|         | Mung Bean | Fine cotton growth | Gray and white | yellowish         | Spread    | Tall        |
|         | Corn   | Fine cotton growth | Gray and white | Gray               | Spread    | Currently   |
|         | Rice   | Fine cotton growth | Gray and white | Gray               | Spread    | Tall        |

Endophytic fungi isolate E1 showed the same growth rate of isolates in the three types of alternative medium (mung bean, corn, and rice) and control (PDA). The color of the upper surface of the colonies was white and the same in all alternative and control media. The surface color on the
Mung Bean medium was not very clear because the medium colors were red, while on the Corn and Rice medium, the color was the same as the PDA medium, which was white. The texture on medium mung bean thick was almost the same as the control (PDA), but on medium corn and rice, the texture was slightly thin (Figure 1).

![Image of medium samples](image1)

**Figure 1.** Top surface (top), a bottom surface (bottom) of isolate E1 on PDA medium (control) and mung bean, corn, rice medium (alternative medium)

The fungal isolate E2 showed different growth in the three types of alternative media, on medium bean, the surface characteristics of thick hairy colonies with white color were almost the same as growth on PDA (Control) medium, the growth type of isolate E2 was slower than isolate E1. The growth of isolate E2 on corn and rice medium, the surface of the colony, was white with yellow pigment at the edges, but the pigment was wider in corn medium than in rice medium. The color of the lower surface of the medium corn and rice is yellow, the color of the mung bean and PDA is cream (Figure 2). According to Daly et al, (1984), the formation of pigments depends on the balance of metabolism, which was supported by the availability of peptone, minerals and iron in the medium [7].

![Image of medium samples](image2)

**Figure 2.** Top surface (top), a bottom surface (bottom) of isolate E2 on PDA medium (control) and mung bean, corn, rice medium (alternative medium)

E3 endophytic fungus isolates showed various growth on the three types of alternative media, including beans, corn and beans and PDA (control). The growth rate of isolates was almost the same in all types of alternative and control medium. Colony color was white on medium bung mean, grey
on medium corn, black mixed with yellow on medium rice. Colony texture was thin and dense on medium mung bean, hairy and thick texture on medium corn, thin and hairy on medium rice (Figure 3).

![Figure 3. Top surface (top), a bottom surface (bottom) of isolate E3 on PDA medium (control) and mung bean, corn, rice medium (alternative medium)](image)

E4 endophytic fungus isolates showed different colony growth rates in each medium. Isolate growth was faster on rice medium and the same as growth on PDA medium (control). The growth type of isolates was almost the same in all alternative and control media. Colony color on the mung bean medium was predominantly white with grey in the middle. On corn and rice medium the colour was the same dominant grey with white edges (Figure 4).

![Figure 4. Top surface (top), bottom surface (bottom) of isolate E4 on PDA medium (control) and mung bean, corn, rice medium (alternative medium)](image)

Endophytic fungi isolate E5 showed the same growth rate in all alternative and control media. The surface texture of the colonies on all mediums was different. Texture on thick-haired mung bean medium was almost the same as growth on PDA medium (control), on corn and thin-haired rice medium. Colony colour on mung bean medium were white, on corn medium blackish brown, the lower surface of the colony on all alternative medium was white (Figure 5)
The growth of endophytic fungus E6 isolate on mung bean medium was faster than the growth of corn, rice and PDA medium (control). The colour of the colonies on all types of medium was the same, namely grey, the surface texture of the colonies on mung bean and PDA medium was thickly hairy like velvet, while on corn and rice medium it was like fine and thin grains (Figure 6).

4. Conclusions
Alternative growth media that can be used to support the growth of endophytic fungal isolates can be taken from food crops such as green beans, corn, and rice. The best alternative medium for the growth of the six isolates of endophytic fungi are mung bean and rice. Mung beans and rice can be used as sources of protein and carbohydrates in the manufacture of growth medium for endophytic fungi isolates.
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