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Utilization of Green Beans Vima 1 and Local as an Alternative Media Substitute Sabouraud Dextrose Agar (SDA) in the Growth of Trichophyton Rubrum

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A B S T R A C T

Green beans are cultivated plants and crops with nutrients from vegetable protein. Varieties of mung beans vary, including Vima 1 and local. The fungus *Trichophyton rubrum* generates dermatophytosis. This research explored green beans and local Vima 1 as an alternative medium in the growth of *Trichophyton rubrum* fungus. The type of research used by laboratory experiments was carried out at the STIKes Hutama Abdi Husada Tulungagung Microbiology Laboratory in March - April 2022. SDA media and alternative media green beans Vima 1 and local variations of the mass of 6 grams and 7 grams inoculated *Trichophyton rubrum* fungus using a single dot method with repetition as much as five times. The results showed that *Trichophyton rubrum* fungus grows optimally on alternative media green beans Vima 1 variation of 7 grams of mass that grows colonies on the fifth day with an average final diameter of 14 mm. While alternative media green beans Vima 1 variation mass 6 grams 11 mm, SDA media 11.65 mm, alternative media local green beans variation mass 6 grams 7.5 mm, and variation mass 7 grams 11.5 mm. The result of independent statistical tests was a p-value < 0.00, which shows significant differences in each type of media. This study result concluded that green beans Vima 1 and local could be used as an alternative medium to replace the SDA media in the growth of *Trichophyton rubrum* fungus.

INTRODUCTION

Indonesia has a tropical climate, so it has relatively high humidity. These climatic conditions can cause the spread of dermatophytosis. Dermatophytosis is an infection caused by fungi of the dermatophyte group (Khusnul, 2017). Fungi belonging to the dermatophyte group are divided into three genus: *Trichophyton*, *Epidermophyton* and *Microsporum* (Karyadini et al., 2018).

The fungus that most often causes the infection is *Trichophyton rubrum*. This fungus usually infects the toenail area, especially in people who need to maintain the cleanliness of the foot area, such as not wearing footwear when outside the home (Farihatun, 2018). The infectious process of dermatophytosis fungi occurs by attacking tissues that contain horny substances, such as the stratum corneum found in the epidermis, hair, and nails. Fungi use the Horn substance as a nutrient in forming colonization (Karyadini et al., 2018).

Laboratory tests can be carried out on samples to diagnose dermatophytosis infection by preparing using 10-20% KOH reagent directly and by culture. Breeding is the gold standard in examining fungi with the media Sabouraud Dextrose Agar (SDA). SDA Media belongs to the class of artificial media for the culture of dermatophyte fungi. Regarding economic Media, SDA includes media with a relatively high

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price, which becomes an obstacle in procuring media in the laboratory. In addition, SDA media has hygroscopic properties, which is easy to absorb water (Nur Aeni & Kurniawan, 2018).

Green bean plants are one of the cultivated crops widely known by people living in the tropics. This plant belongs to the tribe of legumes (*Fabaceae*) with benefits in everyday life as a food source containing high vegetable protein (Rajab, 2016). Green beans have wide varieties. These varieties include Vima 1, Vima 2, Vima 3, Vima 4, Vimil 2; Magpie, and local (Hijria & Syarni, 2019). The varieties used in this study are Vima 1 and local varieties.

Green beans have a relatively high nutritional content, mainly the content of carbohydrates and proteins. In comparison, 100 grams of green beans of the Vima 1 variety contains 67.22 grams of carbohydrates and 27.1 grams of protein. At the same time, the local varieties of green beans contain carbohydrates 67.62 grams and protein 28.02 grams (Balitkabi, 2016). Therefore, this study was conducted to determine whether Vima 1 and local green bean varieties can be used as an alternative medium for SDA in *Trichophyton rubrum*.

Previous research on green beans, showed that green bean media could be used as a growth medium for *Aspergillus flavus* fungi. The diameter of *Aspergillus flavus* fungal colonies that grow on mung bean media is 6.7 cm. It mentions that mung bean media can be an alternative to SDA media (Nuryati & Sujono, 2017).

This study was conducted to determine the ability of media made from green beans and whether the medium can be used to grow fungi, particularly the fungus Trichophyton rubrum. The gold standard media used to grow mushrooms is SDA media. Limitations that are overcome are natural resources media that have hygroscopic properties so that it is easy to absorb water and will cause damage to the media. The resulting contribution is to provide insight into where natural materials can be used as an alternative medium for the growth of fungi, so it is expected that research can be done on other natural materials to be used as an alternative medium.

METHOD

Fungal Isolates

Trichophyton rubrum fungal culture was prepared by rejuvenating pure isolates of Trichophyton rubrum fungi on SDA media that have been added with Chloramphenicol antibiotics. Fungi rejuvenated and incubated at room temperature for 14 days can then be used for research (Wantini & Octavia, 2018).

Experimental Design

Research conducted in this study is true-experimental laboratories conducted in vitro to determine the ability of green bean varieties Vima 1 and local as an alternative medium in the growth of the fungus

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Trichophyton rubrum. The trial was a post-test-only control to find the growth of *Trichophyton rubrum* fungus on mung bean media varieties Vima 1 and local mass variations of 6 grams and 7 grams.

Culture Media

1. Sabouraud Dextrose Agar

   SDA is a common medium that is often used for fungal growth. SDA Media contains nutrients needed by fungi, such as carbohydrates and proteins. The SDA media's content will regulate fungi's needs as in their natural habitat (Sophia & Yogica, 2021).

2. Green Beans Vima 1 and Local Media

   Green beans are cultivated plants and crops widely known by people living in the tropics. This plant belongs to the tribe of legumes (*Fabaceae*) with benefits in everyday life, namely as a food source containing high vegetable protein (Rajab, 2016).

   In SDA media containing carbohydrates as much as 40 grams with Vima 1 varieties of green beans containing carbohydrates as much as 67.62 grams and local varieties as much as 67.22 grams, while the protein contained in SDA media as much as 10 grams with Vima 1 varieties of green beans containing protein as much as 28.02 g and local varieties as much as 27.1 grams (Nuryati & Sujono, 2017). The content of carbohydrates and proteins found in green beans is expected to replace carbohydrates and proteins found in SDA media.

Fungi Inoculation into Alternative Media Source

Inoculation of the fungus *Trichophyton rubrum* uses the single dot method. This method is done by inserting a needle nose in the middle of the surface so that the media SDA, green bean media varieties Vima 1 and local variations of the mass of 6 grams and 7 grams (Ahmad et al., 2019).

Statistical Analysis

The research Data obtained in the form of measuring the diameter of the growth of Trichophyton rubrum fungus for 14 days was then converted into a table. The Data was processed using the SPSS 25 for Windows statistics application program. The data obtained were a normality test using the Kolmogorov-Smirnov (K-S) test followed by homogeneity test using a homogeneity of Variances test. After that, parametric statistical tests were conducted using an independent t-test with a significance level of 5% (Novri et al., 2018).

RESULT

Based on Table 1 obtained data measuring the diameter of the growth of *Trichophyton rubrum* fungus with an average final diameter of growth in positive control media of 11.65 mm; green bean media varieties Vima 1 variation of 6 grams by 11 mm; green bean media varieties Vima 1 variation of 7 grams

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by 14 mm, green bean media local varieties variation of 6 grams by 7.5 mm; and green bean media local varieties variation of 7 grams by 11.5 mm.

Table 1. Average growth Diameter of the fungus *Trichophyton rubrum*

Media Replication	Colony Diameter (mm)				
	SDA	Vima 1		Local	
		6 g	7 g	6 g	7 g
I	12	10	13	7,5	12
II	11,5	11,5	15	6,5	12
III	12	11	13,25	7,5	11,5
IV	11	10,5	14,25	8,5	10
V	11,75	12	14,5	7,5	12
$\sum$	58,25	55	70	37,5	57,5
$\overline{X}$	11,65	11	14	7,5	11,5

The statistical analysis results using an independent t-test of the diameter of the growth of *Trichophyton rubrum* fungus in alternative media of green beans varieties Vima 1 and local variations in mass of 6 grams and 7 grams and positive control media SDA was described in table 2. The results result was Pvalue < α (0.05) on alternative media green bean varieties Vima 1 and local variations of the mass of 7 grams. It significantly influences the diameter of colony growth with positive control media SDA.

Table 2. Test results Independent T-test

Variable	P-*value*	Description
Media Vima 1 variation mass 6 g	0,143	There is no influence
Media Vima 1 variation mass 7 g	0,01	There is influence
Media Local variation mass 6 g	0,736	There is no influence
Media Local variation mass 7 g	0,00	There is influence

DISCUSSION

This study aims to determine the use of green beans as an alternative medium in the growth of trichophyron rubrum fungus, with varieties of green beans used are varieties of green beans Vima 1 and local. The study was compared the diameter of fungal growth colonies and the growth rate of *Trichophyton rubrum* fungi on SDA-positive control media and alternative media of Vima 1 and local mung bean varieties.

Based on Table 1 shows that the fungus *Trichophyton rubrum* can grow well on alternative media varieties of green beans Vima 1 mass variation of 7 grams and alternative media varieties of local green beans mass variation of 7 grams. In alternative media varieties of green beans Vima 1 and local, there is an increase in the diameter of the growth of fungal colonies Trichophyton rubrum along with an increase in the mass variation of green bean seed flour used as a base material in the manufacture of alternative media.

Trichophyton rubrum fungus can grow on each media on the fifth day, both on positive control media SDA and alternative green bean varieties Vima 1 and local. The content of nutrients contained in the

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alternative media of green beans is higher when compared with the content of nutrients contained in the media SDA. In 100 grams of green beans, local varieties contain carbohydrates 67.22 grams; protein 27.1 grams; fat 1.78 grams; fiber 8.88 grams; calcium 263.91 grams; vitamin C 11.83 grams; calories 345 kcal; and water 15.5 grams (Indonesia, 2013). While in 100 grams of green beans varieties, Vima 1 contains carbohydrates 67.62 grams of protein 28.02 grams and fat 0.40 grams (Balitkabi, 2016).

Each litre of SDA media is peptone 10.0 grams, dextrose 40.0 grams and agar 15.0 grams (Nuryati & Sujono, 2017). Fungi use dextrose as a source of carbon. At the same time, sugar and protein are used as a source of energy in the growth of fungs (Sophia & Yogica, 2021).

Trichophyton rubrum mushroom colonies grown on alternative media of green beans of Vima 1 and local varieties have a complete morphological structure of the fungus. The nutrients in green bean varieties Vima 1 and local were high enough, with carbohydrates and proteins in question. Carbohydrates have an essential function in the growth of fungi, and proteins function as structural molecules that are useful in forming cell layers, functional molecules, and enzymes and help the metabolic process (Wantini & Octavia, 2018). Mushrooms will use the carbohydrate content found in green beans in excreting enzymes α-amylase to convert amylum into glucose, whereas fungi will absorb glucose to assist in the growth process (Ahmad et al., 2019).

Table 2 shows the Independent T-test results obtained p-value significance value of $0.00 < \alpha$ (0.05) on alternative media green beans Vima 1 and local variations in the mass of 7 grams. These values indicate if there is a significant difference in the diameter of the growth of *Trichophyton rubrum* mushroom colonies on alternative media of green bean varieties Vima 1 and local variations in the mass of 7 grams with positive control media SDA.

Based on previous research on using green beans against the growth of the *Aspergillus flavus* fungi. Shows the diameter of *Aspergillus flavus* mushroom column that grows on mung bean media of 6.7 cm and mentions if the mung bean media can be used as an alternative media to replace SDA media (Nuryati & Sujono, 2017).

The growth of *Trichophyton rubrum* fungus on green bean media of Vima 1 variety with a mass variation of 7 grams has a final diameter of 14 mm colony. This is because the alternative media green beans varieties Vima 1 variation of 7 grams of mass has a high carbohydrate content, amounting to 4.73 grams. In addition, the growth of *Trichophyton rubrum* fungi can be influenced by other factors, including light intensity, temperature, pH, humidity, carbon, nitrogen, sulfur, phosphorus, and mineral (Yuniliani et al., 2018).

Subsequent studies are expected to conduct more research on the manufacture of alternative media using natural materials. So that natural products can be better utilized. The limitation of this study is the

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sampling of *Trichophyton rubrum* fungus that will be inoculated on growth media. It is feared that the small number of samples inoculated will affect the growth diameter of the fungal colony *Trichophyton rubrum*.

CONCLUSION

Based on the results of research on the use of Vima 1 and local green beans as an alternative media to SDA media in the growth of *Trichophyton rubrum* fungus showed that there was no significant difference in the growth of *Trichophyton rubrum* fungus on each media. The alternative media green beans Vima 1 and local mass variation of 6 grams and 7 grams can be used as an alternative media substitute for Natural Resources media. However, the same fungus's growth is not better or worse than the media SDA.

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