Trop J Nat Prod Res, December 2025; 9(12): 6209 - 6213
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Rice Bran Dextrose Agar as a Cost-Effective Alternative to Standard Fungal Culture Media

Rafika Rafika[1]*, Rahman Rahman[1], Ridho Pratama[1], Artati Artati[1], Mursalim Mursalim[1], Muhammad Nasir[1], Widarti Widarti[1], Nuradi Nuradi[1], Nurdin Nurdin[1], Asyhari Asyikin[2], Nurisyah Nurisyah[2], Tajuddin Abdullah[2], Asmawati Asmawati[2]

[1]*Department of Medical Laboratory Technology, Poltekkes Kemenkes Makassar, Jl. Wijaya Kusuma, Banta-Bantaeng, Makassar City, South Sulawesi, Indonesia*
[2]*Department of Pharmacy, Poltekkes Kemenkes Makassar, Jl. Baji Gau Mamajang, Makassar City, South Sulawesi, Indonesia*

ARTICLE INFO

Article history:
Received 01 October 2025
Revised 25 November 2025
Accepted 27 November 2025
Published online 01 January 2026

ABSTRACT

Microorganisms require carbohydrate-rich media for growth. Rice bran, a carbohydrate-dense by-product, offers a cost-effective alternative to synthetic media like Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA), making it a promising ingredient for mushroom cultivation in laboratories. This research evaluated the performance of rice bran dextrose agar (RBDA) as a culture medium compared to standard SDA and PDA media for fungal growth. Rice bran dextrose agar was formulated (A1, A2, A3, B1, B2, B3, C2, and C3) and optimized by varying dextrose and agar concentrations to support fungal growth. *Aspergillus niger, Candida albicans*, and *Aspergillus fumigatus* were tested, and media performance was assessed by measuring colony diameters on day five. A statistical analysis was conducted to compare the fungal growth on RBDA with that on standard media, SDA, and PDA. On day five, the *Candida albicans* colony on RBDA (C1) showed a significantly larger diameter (16.46 mm, $p < 0.05$) compared to other RBDA formulations and SDA. For *Aspergillus niger*, no significant difference was observed ($p > 0.05$), but RBDA C1 (75.1 mm) showed better growth than the control (SDA), although lower than A2 (76.5 mm) and C3 (79.9 mm). *Aspergillus fumigatus* grown on RBDA (pH 5.91) showed significantly better growth (28.55 mm, $p < 0.05$) than SDA and PDA. The study' s findings indicated that RBDA medium demonstrated effective performance in supporting the growth of *Candida albicans*, *Aspergillus niger*, and *Aspergillus fumigatus*, suggesting that rice bran is a viable natural alternative to synthetic mushroom culture media.

Keywords: *Aspergillus niger, Aspergillus fumigatus, Candida albicans,* Culture Media, Rice Bran Dextrose Agar.

Introduction

Fungi are eukaryotic microorganisms consisting of mould and yeast.[1] They generally reproduce asexually and sexually.[2] Fungi are classified as chemoheterotrophic microorganisms that require organic compounds as a source of energy, carbon, and electrons for fungal growth. The nutrients needed are in the form of macronutrients and micronutrients for the continued growth and development of fungi.[3] In carrying out metabolism, fungi use more carbohydrates but also require other organic compounds, such as lignocellulose, protein, and lipids.[4,5] Apart from fulfilling nutritional requirements, appropriate pH, incubation period, temperature, and sterile media conditions must also be considered.[6] A medium is a nutrient material for the growth of microorganisms in the laboratory. The growth of microorganisms in artificial media is influenced by several physical and chemical factors. Nutrient materials prepared for the growth of microorganisms in the laboratory are called culture media, and the nutrient composition of the culture media plays a major role in microbial growth.[7]

*Corresponding author. Email: rafikauddinramli02@gmail.com
Tel: +62 823-4555-3522

Citation: Rafika R, Rahman R, Pratama R, Artati A, Mursalim M, Nasir M, Widarti W, Nuradi N, Nurdin N, Asyikin A, Nurisyah N, Abdullah T, Asmawati A. Rice Bran Dextrose Agar as a Cost-Effective Alternative to Standard Fungal Culture Media. Trop J Nat Prod Res. 2025; 9(12): 6209 – 6213 https://doi.org/10.26538/tjnpr/v9i12.39

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Mushroom culture requires a medium containing high carbohydrate nutrients, nitrogen for fungal growth in the pH range of 5 to 6, and a temperature range from 15 to 37°C.[8,9] Sabouraud dextrose agar (SDA) is a synthetic medium composed primarily of mycological peptone and dextrose.[10,11] Potato dextrose agar (PDA) is the main medium for the isolation and cultivation of fungi under laboratory conditions,[12– 14] and has a well-defined composition. It contains potato extract, glucose (dextrose), and agar (Oxoid, UK).[12] The use of synthetic media is an obstacle in school and university laboratories because of the high cost of purchasing such media.[15] Consequently, researchers have developed mushroom culture media using natural, readily available, and cost-effective agricultural products as alternatives for mushroom cultivation. Some examples of such products include flour,[16.] sago, Palmyrah tuber flour, sweet potato, cassava, breadfruit, potato, beetroot (*Beta vulgaris*), carrot (*Daucus carota*), sweet potato (*Colocasia esculenta*), and sweet potato (*Ipomoea batatas*).[17] Rice bran is a fine-textured by-product of rice processing. It is widely used in many communities, particularly as animal feed and in the pharmaceutical industry. The nutritional content of complete rice bran includes carbohydrates, protein, water, fat, vitamins, minerals, and fiber. In previous research,[18– 20] proximate analyses of rice bran powder showed that it contains 33.72% carbohydrates, 11.17% protein, 14.26% fat, 6.38% crude fiber, 9.5% moisture, and 9.2% ash. The nutritional composition of rice bran is more complex, as it also contains B vitamins that serve as essential growth factors for fungi.[18– 20].

Several investigations have employed rice bran as a medium for fungal growth. For example, rice bran combined with SDA supported the growth of *Aspergillus* sp.[21] Additionally, comparisons of *Aspergillus niger* and *Candida albicans* grown on bran dextrose agar (BDA) and PDA showed no significant differences in growth ($p < 0.05$).[22]. Another study tested variations in the concentration of BDA media on the growth

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

of *Candida albicans*, resulting in concentrations of 10% and 15% of rice bran producing larger colonies.[23] The present study formulated a ready-to-use laboratory medium from rice bran, providing a cost-effective alternative to expensive commercial synthetic media. However, previous studies generally used freshly prepared rice bran media rather than a laboratory-ready formulation. The present study addresses this gap by developing a ready-to-use rice bran dextrose agar (RBDA) formulated through multistep processing and freeze-drying. This approach is expected to provide a practical and cost-effective alternative to expensive commercial synthetic media while maintaining adequate fungal growth performance. The current research aimed to assess the effectiveness of the RBDA formulation as a fungal culture medium by comparing its performance with standard synthetic media (SDA and PDA) in supporting the growth of *Candida albicans*, *Aspergillus niger*, and *Aspergillus fumigatus*.

Materials and Methods

Sample source and preparation

The sample, in the form of rice bran powder, was obtained as the final milling by-product from a rice milling factory in Gowa Regency, Indonesia (https://maps.app.goo.gl/nuAg7GtEhgo5BqQV7). The rice bran was dried for about one week in an open room. After the sample was dried, it was sieved to ensure uniform particle size and remove impurities.

Source of test fungi

The test fungi used were *Aspergillus niger*, *Aspergillus fumigatus*, and *Candida albicans*, which were obtained from the culture collection of the Microbiology Laboratory, Poltekkes Kemenkes Makassar, Indonesia.

Preparation of rice bran filtrate extract

A 20% rice bran was prepared with 1 mL of pretreatment enzyme. The filtrate was prepared using a stratified pretreatment method starting with heating, followed by enzymatic treatment. In the first step, 200 g of rice bran were weighed into a beaker, 1 L of distilled water was added, and the mixture was homogenized with a stirring rod until a liquid bran slurry was formed. The rice bran solution was placed into a 500 mL beaker and covered with aluminum foil, then heated for 60 minutes. Then, the heated rice bran solution was supplemented with 5 mL of pretreatment enzymes. The chemically pretreated bran was then filtered to remove the residual bran solids. The clear bran filtrate was supplemented with a solidifying agent, Bacto agar, at 20 g/L and heated until completely dissolved.[24] Then the RBDA medium was cooled at room temperature to solidify. The RBDA medium was freeze-dried for 3-4 weeks and then ground into a powder.[25]

Fungal growth assessment on the freeze-dried medium

The freeze-dried RBDA medium was prepared for testing fungal growth using the culture method. The RBDA medium was supplemented with varying concentrations of dextrose to determine the optimal composition for fungal growth. The tested RBDA formulations were as follows: A series (rice bran 4 g + dextrose 0.5 g [A1], 1 g [A2], 1.5 g [A3]), B series (rice bran 4.5 g + dextrose 0.5 g [B1], 1 g [B2], 1.5 g [B3], and C series (rice bran 5 g + dextrose 0.5 g [C1], 1 g [C2], 1.5 g [C3]). Sabouraud dextrose agar was used as the control medium. Two fungal isolates, *Candida albicans* and *Aspergillus niger*, were used to evaluate growth across all treatments. The test was conducted over 5 days, with colony diameters measured at 24-hour intervals. Further testing was performed on *Aspergillus fumigatus* using the RBDA formulation that showed optimal fungal growth, with SDA and PDA serving as comparison media. All the experiments were replicated three times. Chemical analysis was performed using a proximate test based on the Luff-Schoorl titrimetric method to determine the levels of glucose, fat, protein, ash, crude fiber, moisture, and pH in RBDA, SDA, and PDA media, with SDA and PDA serving as comparison media.[26]

Statistical analysis

The data were analyzed descriptively and subjected to normality and analysis of variance (ANOVA) tests, with significance set at $p \leq 0.05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS; version 26, IBM Corp., 2019).[27]

Results and Discussion

The results (Figures 1 and 2) of testing the RBDA media treatment formulas (A1, A2, A3, B1, B2, B3, C1, C2, C3) and SDA (control) for *Candida albicans*, repeated in three replicates, showed that fungal colonies on all RBDA media were larger from day one to day five. On the fifth day, colony diameter measurements revealed a significant difference ($p < 0.05$; $p = 0.000$) among treatments. Most treatments (A1, A2, A3, B1, B2, B3, C2, and C3) had significantly different colony diameters compared to the control, with C1 showing the greatest difference. Nevertheless, the average colony diameter on RBDA C1 remained higher than on the control (Figure 1). These results indicated that RBDA consistently promotes superior fungal proliferation relative to SDA, confirming the enhanced nutritional suitability of rice-bran-based formulations. The growth of *Candida albicans* on the RBDA media formulation C1 was optimal. In contrast, a previous study,[23] using only rice bran agar, reported slightly smaller average colony diameters than the control medium (SDA). However, when biomass was measured using liquid rice bran media (without agar), the study found a significantly higher biomass of *Candida albicans* compared to SDA ($p < 0.05$). The RBDA formulation C1 includes added dextrose as an energy source. Borah *et al.* (2020),[17] reported that fungal growth media containing high carbohydrate and nitrogen sources support enhanced fungal growth. Therefore, the combination of rice bran nutrients and dextrose supplementation likely acts synergistically to enhance colony expansion, which explains the significantly higher growth observed in this study.

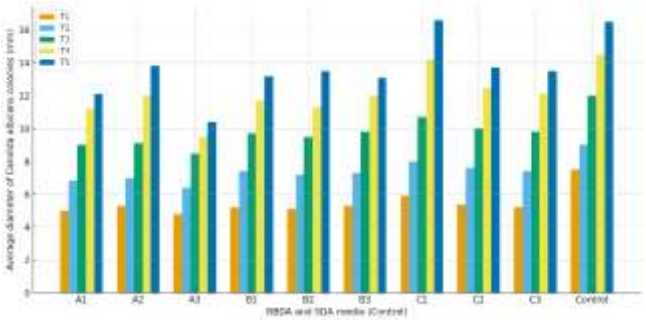

Figure 1: Growth performance of *Candida albicans* on RBDA compared with SDA (control) over a 5-day incubation period.
RBDA: Rice bran dextrose agar; SDA: Sabouraud dextrose agar; A series (rice bran 4 g + dextrose 0.5 g [A1], 1 g [A2], 1.5 g [A3]); B series (rice bran 4.5 g + dextrose 0.5 g [B1], 1 g [B2], 1.5 g [B3]; C series (rice bran 5 g + dextrose 0.5 g [C1], 1 g [C2], 1.5 g [C3])T1 – T5: Days 1-5.

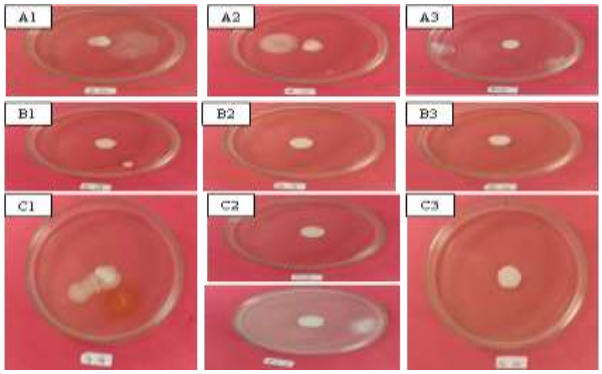

Figure 2: *Candida albicans* colonies on RBDA and SDA media.
RBDA: Rice bran dextrose agar; SDA: Sabouraud dextrose agar; A series (rice bran 4 g + dextrose 0.5 g [A1], 1 g [A2], 1.5 g [A3]); B series (rice bran 4.5 g + dextrose 0.5 g [B1], 1 g [B2], 1.5 g [B3]; C series (rice bran 5 g + dextrose 0.5 g [C1], 1 g [C2], 1.5 g [C3])T1 – T5: Days 1-5.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

On the fifth day of observation, the growth of *Aspergillus niger* showed no significant difference in average colony diameter across all media treatments ($p > 0.05$; $p = 0.570$), as shown in Figures 3 and 4. The treatments A2, C1, and C3 exhibited slightly larger colony diameters compared to other RBDA formulations and the control. Nevertheless, RBDA media demonstrated good overall performance comparable to the control. Moreover, the RBDA C1 media formulation showed higher colonies than the control. These results indicated that multicellular fungi, such as moulds, can effectively grow on RBDA media.

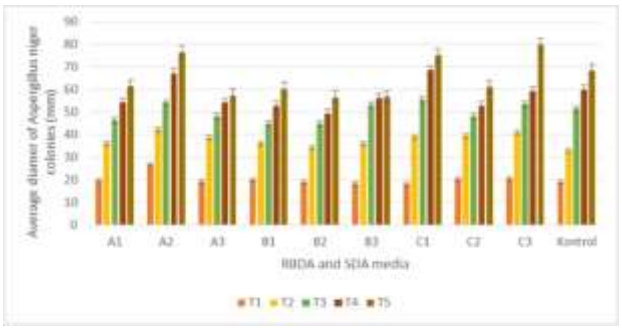

Figure 3: Growth performance of *Aspergillus fumigatus* on RBDA compared with SDA (control) over a 5-day incubation period.
RBDA: Rice bran dextrose agar; SDA: Sabouraud dextrose agar; A series (rice bran 4 g + dextrose 0.5 g [A1], 1 g [A2], 1.5 g [A3]); B series (rice bran 4.5 g + dextrose 0.5 g [B1], 1 g [B2], 1.5 g [B3]; C series (rice bran 5 g + dextrose 0.5 g [C1], 1 g [C2], 1.5 g [C3])T1 – T5: Days 1-5.

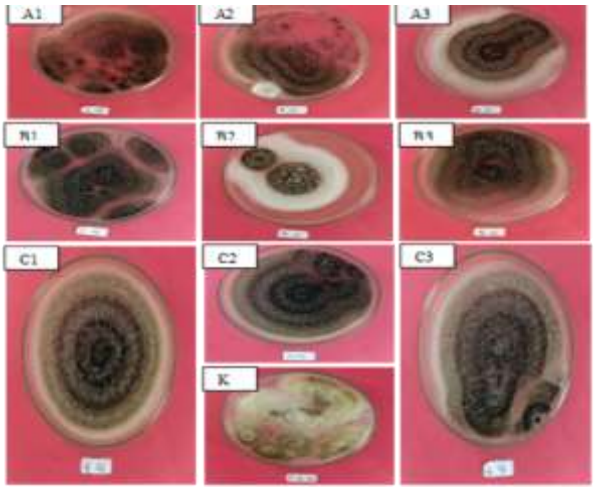

Figure 4. *Aspergillus niger* colonies on RBDA and SDA media.
RBDA: Rice bran dextrose agar; SDA: Sabouraud dextrose agar; A series (rice bran 4 g + dextrose 0.5 g [A1], 1 g [A2], 1.5 g [A3]); B series (rice bran 4.5 g + dextrose 0.5 g [B1], 1 g [B2], 1.5 g [B3]; C series (rice bran 5 g + dextrose 0.5 g [C1], 1 g [C2], 1.5 g [C3])T1 – T5: Days 1-5.

In a previous study,[23] *Aspergillus niger* showed significantly larger colony diameters on RBA media ($p < 0.05$). Similarly, biomass measurements in liquid rice bran revealed higher *Aspergillus niger* growth compared to SDA media. Although the ANOVA results for this study were not significant for *Aspergillus niger*; the consistent trend of higher colony diameter on RBDA suggests nutritional competitiveness comparable to commercial media. This trend aligns with previous findings showing rice-bran– based media support robust mould biomass production. Several studies have developed alternative media for mushroom cultivation, as supported by the findings of this study.[8] The colony diameters of *Aspergillus flavus* on rice bran media prepared from the Situ Bagendit variety were 79.24 mm, 81.47 mm, and 81.68 mm for 5%, 10%, and 15% concentrations, respectively, compared to an average of 75.03 mm on PDA. This indicates that rice bran media was more effective for fungal growth than PDA. Another study investigated the optimal extraction process from Hommali brown rice flour (HMBRF) to evaluate its suitability as a culture medium for *Aspergillus niger*.[22] These comparisons reinforce that rice-derived substrates can serve as reliable and economical alternatives to conventional synthetic media. Proximate chemical analysis was conducted to determine the nutritional composition and pH of RBDA media. The parameters tested included carbohydrates, moisture, ash, fat, protein, fiber, and pH. Analyses were performed on both solid (hydrated) and powdered forms of RBDA, SDA, and PDA media. The results (Table 1) showed that RBDA, in both forms, had higher carbohydrate content than SDA and PDA. Additionally, RBDA exhibited relatively high fat content, while its protein content was lower, reflecting the protein composition of pure rice bran. In contrast, the protein in SDA and PDA media is derived from peptone, a component of synthetic media formulations. The three components of macronutrients in RBDA trigger fungal growth and development. According to a study,[28] rice bran is generally cream or light brown in colour. It has a highly nutritious chemical composition, containing 12-17% protein, 13-23% fat, 34-54% carbohydrates, 6-14% fiber, and 8-18% ash. There are also vitamins, minerals, essential unsaturated fatty acids, and phenolics.[20] The pH of the solid RBDA media (5.91) and powdered form (5.89) was comparable to that of SDA and PDA media, falling within the optimal range for fungal growth (pH 5–6). Fungi generally grow well at this pH and at temperatures between 15 and 37 °C.[15] The high carbohydrate content and balanced pH of RBDA make it a nutritionally favourable medium, which likely accounts for its superior growth performance across multiple fungal species.

This study also evaluated the growth of the mould *Aspergillus fumigatus* on RBDA formulation C1, with SDA and PDA used for comparison. The growth results are shown in Figure 5, which illustrates colony development over 5 days (5 × 24 hours). Observations were made at 24-hour intervals to measure colony diameter across three replicates on RBDA, SDA, and PDA media. The results on the first day showed that the growth of fungal colonies on SDA was greater than that of RBDA and SDA. From the second to the fifth day, the growth of *Aspergillus niger* on RBDA media (28.55 mm on the fifth day) was faster, and the colonies were larger than those on SDA and PDA media (22.13 mm and 19.34 mm, respectively).

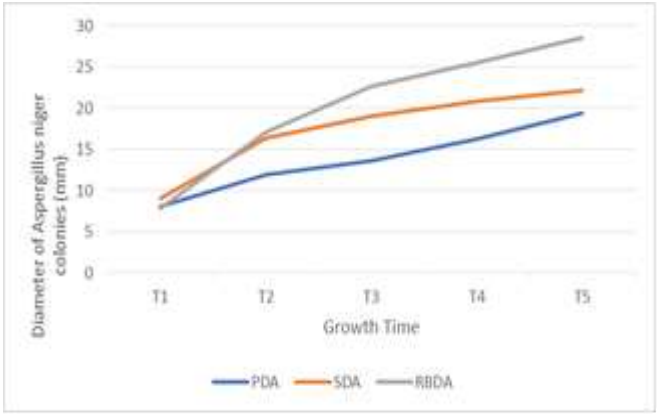

Figure 5: Growth performance of *Aspergillus fumigatus* on RBDA compared with SDA and PDA over five days of incubation.
RBDA: Rice bran dextrose agar; SDA: Sabouraud dextrose agar; PDA: Potato dextrose agar; A series (rice bran 4 g + dextrose 0.5 g [A1], 1 g [A2], 1.5 g [A3]); B series (rice bran 4.5 g + dextrose 0.5 g [B1], 1 g [B2], 1.5 g [B3]; C series (rice bran 5 g + dextrose 0.5 g [C1], 1 g [C2], 1.5 g [C3])T1 – T5: Days 1-5.

Table 1: Proximate analysis of RBDA medium (C1), SDA, and PDA media in both agar and powder forms.

Parameter	RBDA Media		SDA Media		PDA Media	
	congested	powder	congested	powder	Congested	powder
Carbohydrate	4.74	54.93	3.53	53.04	4.2	53.7
Water	94.35	6.02	95.14	3.52	96.14	2.39
Ash	0.33	6.69	0.09	2.82	0.08	2.4
Lipid	0.008	0.11	0.05	0.01	0.04	0.2
Protein	0.09	3.86	0.61	9.46	0.24	5.79
Fiber	0.51	0.61	0.52	0.92	0.61	0.9
pH	5.91	5.83	6.41	6.41	6.3	5.85

RBDA: Rice bran dextrose agar; C1: rice bran (5 g) + dextrose (0.5 g); SDA: Sabouraud dextrose agar; PDA: Potato dextrose agar.

The statistical analysis (ANOVA) results showed T1: $p > 0.05$ (0.87), T2: $p < 0.05$ (0.04), T3: $p < 0.05$ (0.002), T4: $p < 0.05$ (0.006), and T5: $p < 0.05$ (0.028), indicating that colony diameters increased from the first to the fifth day and that the average colony diameter differed significantly between RBDA, SDA, and PDA media. The post hoc Tukey test on the fifth day (T5) showed that in subset 1, the growth diameter of *Aspergillus fumigatus* on PDA and SDA media was similar, while in subset 2, the diameters on SDA and RBDA were comparable. Despite this, RBDA formulation C1 still supported faster growth of *Aspergillus fumigatus* compared to the other media. The significant differences observed on days 2– 5 further confirm the enhanced growth-supporting capacity of RBDA, demonstrating its potential for replacing more expensive synthetic media in fungal propagation workflows.

This research is supported by several studies exploring alternative media for fungal growth beyond synthetic SDA and PDA. One study reported that using legume seeds as a nutritional source for mushroom cultivation resulted in significantly higher growth of *Fusarium* sp. ($p < 0.05$) on black gram, whereas *Aspergillus* sp. showed significantly lower growth on PDA. Among all tested fungi, only *Trichoderma* sp. did not exhibit higher growth on PDA.[29] Research showed that *Sclerotium* sp. exhibited significantly higher growth on rice ($p < 0.05$), while *Penicillium* sp. grew significantly on both rice and maize ($p < 0.05$).[29] Sago and Palmyrah tuber media supported the growth of all tested fungi, with *Penicillium* sp. showing significantly greater growth on Palmyrah tuber media compared to PDA and sago media. Growth of *Trichoderma* sp. on sago media and *Mucor* sp. on sago and Palmyrah tuber media was relatively better than on PDA after 24 hours of incubation. However, over prolonged incubation, the growth rate on PDA increased more rapidly than on the tested media.[7] Sweet potato dextrose agar showed much higher mycelium growth than commercial PDA, while taro dextrose agar showed similar positive results, except for *F. semitectum* DOAC 1986. This study indicated that sweet potato and taro have strong potential as alternative nutritional sources for the production of fungal media supporting yeast and mould growth.[30,31] Overall, these comparisons demonstrate a growing trend toward low-cost, agriculture-based culture media that match or exceed the performance of commercial media, placing RBDA within this emerging category of efficient, accessible, and nutritionally rich fungal growth substrates.

Conclusion

This study demonstrates that rice bran dextrose agar (RBDA) is a viable and efficient alternative to conventional fungal media. RBDA, especially formulation C1, supported equal or superior growth of *Candida albicans*, *Aspergillus niger*, and *Aspergillus fumigatus* compared to SDA and PDA, confirming the strong nutrient potential of rice bran. The freeze-dried RBDA produced a stable, ready-to-use medium with practical storage benefits. Overall, RBDA is cost-effective, sustainable, and suitable for routine laboratory use, particularly in resource-limited settings. Further research should expand its application to broader fungal species, clinical, and industrial settings while assessing long-term stability.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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