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Research Article

"Exploring Locally Sourced Cereal Grains As Alternative Media For Fungal Growth: A Comparative Study With Potato Dextrose Agar"

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ABSTRACT
This study explores the feasibility of utilizing locally available cereal grains as cost-effective alternatives to Potato Dextrose Agar (PDA) for fungal cultivation. Parboiled rice and wheat, rich in carbohydrates and essential nutrients, were used to develop Rice Dextrose Agar (RDA) and Wheat Dextrose Agar (WDA). The growth performance of *Aspergillus niger, Neurospora crassa*, and *Fusarium* sp. was evaluated by measuring mycelial growth diameter on RDA, WDA, and PDA (control). Cereal-based media were tested at reduced concentrations (2.9 g and 1.9 g per 100 mL), while PDA was consistently maintained at 3.9 g per 100 mL throughout the experiment as a control. *Aspergillus niger* exhibited optimal growth on WDA across all concentrations. *Neurospora crassa* rapidly colonized all media, covering the full petri dish within 24 hours. For *Fusarium* sp., PDA showed the highest growth, though RDA and WDA performed comparably with slight reductions in mycelial diameter. These findings suggest that cereal grains can serve as viable, affordable alternatives to PDA, providing a sustainable solution for microbiological research and large-scale fungal applications.

KEYWORDS: Cereal grains, Potato dextrose agar (PDA), Fungal growth, Mycelial growth diameter, Alternative culture media, Fungal culture media,

ABBERIVATIONS
PDA – Potato Dextrose Agar
RDA – Rice Dextrose Agar
WDA – Wheat Dextrose Agar
MGD – Mycelial Growth Diameter
SDA – Sabouraud Dextrose Agar

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INTRODUCTION

Microbiological research relies heavily on the ability to cultivate and maintain microorganisms in laboratory settings, which is made possible by providing suitable culture media that mimic favorable environmental conditions (MB352 General Microbiology Laboratory 2021 (LEE), 2021). The pioneering work of Louis Pasteur (Gerald L. Geison, 1955) and his colleagues in France, as well as Robert Koch and his team in Germany, enabled the in vitro cultivation of microorganisms in the late 19th century (Collins, 2004). Scientific discoveries have revealed that the cellular composition of microorganisms primarily comprises a few key elements including carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. Notably, each of these macro elements serves a distinct purpose in supporting the various biological processes that occur within microorganisms. Phosphorus is essential for the production of nucleic acids, and potassium is necessary for enzymatic activity and maintaining osmotic balance. These "macro elements" or "macronutrients" are essential for microorganism growth and development, and are required in significant amounts(Collins, 2004).

Throughout history, a complex and fascinating relationship has existed between food, microorganisms, and humans. Foods provide essential nutrients, but also create an ideal culture media for microorganism growth (Willey et al., 2008), with cereals being a prime example of this symbiotic relationship. Cereals rich in carbohydrates (50-80% of their weight) also contain small but significant amounts of proteins (5-6%) and lipids (1-10%), making them an attractive option for supporting microbial growth. Furthermore, whole grains are a valuable source of mineral salts (1.5-2.5%), including phosphorus, calcium, magnesium, potassium, iron, zinc, and copper, as well as various vitamins (Garutti et al., 2022), which are essential for microbial development. The unique combination of nutrients and moisture-holding capacity in cereal grains, particularly parboiled rice and wheat, makes them ideal culture media for microorganisms. Moreover, the widespread cultivation of these grains across the world ensures their availability and affordability, rendering them a cost-effective alternative to traditional culture media. This convergence of nutritional value, availability, and affordability makes cereal grains a promising substrate for microbial growth.In the captivating realm of microbiological research, the prohibitive cost and limited availability of traditional culture media have posed significant challenges(Adesemoye & Adedire, 2005). To address this, our study considered the potential of parboiled rice and wheat as cereal grain-based alternatives to the commonly used potato dextrose agar (PDA). By creating rice dextrose agar (RDA) and wheat dextrose agar (WDA), we aimed to assess their effectiveness in supporting fungal growth, emphasizing the cost-effectiveness and widespread availability of these grains.

MATERIAL AND METHODS

Sample collection

Parboiled rice and wheat samples were obtained from a government ration shop (also known as a fair price shop) in Chennai, Tamil Nadu, India. The samples were meticulously washed with distilled water, air-dried under shade, and then ground into a fine powder using a pulverizer.

Added nitrogen source

Ammonium sulfate was purchased from an online store. Glucose is often used as a carbon source, whereas ammonium salts or nitrates are used as inorganic nitrogen sources in growth media (MB352 General Microbiology Laboratory 2021 (LEE), 2021). The nitrogen source plays a crucial role in cell behavior and production performance; ammonium sulfate is a commonly used nitrogen source for microbial cultivation (Konzock et al., 2022).

Formulation of media

Under the media formulation process, different concentrations of Rice Dextrose Agar (RDA) and Wheat Dextrose Agar (WDA) were prepared, each containing cereal grain and ammonium sulfate in varying amounts, along with 2 g of agar-agar in 100 mL of distilled water. The media formulations were designed with three distinct concentration levels: 3.9 g, 2.9 g, and 1.9 g of total media components.

Table 1 presents the media formulation at a total concentration of 3.9 g per 100 mL, consisting of 3.47 g of cereal grain, 0.43 g of ammonium sulfate, and 2 g of agar-agar. **Table 2** outlines the formulation at a reduced concentration of 2.9 g, with 2.58 g of cereal grain and 0.32 g of ammonium sulfate. Similarly, **Table 3** details the lowest concentration (1.9 g), comprising 1.69 g of cereal grain and 0.21 g of ammonium sulfate per 100 mL."After preparing the media, the components were autoclaved to ensure sterility. Once sterilized, the media were poured into Petri dishes and allowed to solidify. After solidification, the details of the plates are marked.

Table 1: Formulation of media with a total 3.9 g concentration in 100 mL distilled water

Serial number	Name of the formulated media	Amount of cereal grain (g)	Amount of ammonium sulfate (g)	Amount of agar-agar (g)	Total volume of distilled water (mL)
1.	RDA	3.47	0.43	2	100
2.	WDA	3.47	0.43	2	100

Table 2: Formulation of media with a total 2.9 g concentration in 100 mL distilled water

Serial number	Name of the formulated media	Amount of cereal grain (g)	Amount of ammonium sulfate (g)	Amount of agar-agar (g)	Total volume of distilled water (mL)
1.	RDA	2.58	0.32	2	100
2.	WDA	2.58	0.32	2	100

Table 3: Formulation of media with a total of 1.9 g concentration in 100 mL distilled water

Serial number	Name of the formulated media	Amount of cereal grain (g)	Amount of ammonium sulfate (g)	Amount of agar-agar (g)	Total volume of distilled water (mL)
1.	RDA	1.69	0.21	2	100
2.	WDA	1.69	0.21	2	100

Preparation of control media and standardization (Potato Dextrose Agar)

To prepare the control medium, 3.9 g of Potato Dextrose Agar (PDA) was mixed with 100 mL of distilled water. The mixture was sterilized in an autoclave at 121°C for 20 minutes at 15 psi pressure. After sterilization, the media were poured into sterile petri dishes and allowed to cool and solidify.

"This concentration of PDA remained constant throughout the research, serving as a benchmark for comparison with the experimental media, Rice Dextrose Agar (RDA) and Wheat Dextrose Agar (WDA), which were tested at three distinct concentrations: 3.9 g, 2.9 g, and 1.9 g per 100 ml. The consistent use of PDA as a control established a stable reference point for evaluating fungal growth across the differentially formulated media at varying concentrations."

Comparison of physico-chemical properties of formulated media and control

Table 4: Physico-chemical properties of Potato Dextrose Agar (PDA), Rice Dextrose Agar (RDA), and Wheat Dextrose Agar (WDA)

Media	Colour	Consistency
PDA	Light yellow	Firm and gel-like
RDA	Milky white	Gelatinous
WDA	Brownish	Granular

Test organisms

The microorganisms used in this study were isolated from agricultural field soil samples. Soil samples (100 g) were collected from a depth of 1 m close to the plant roots and stored in a sterile zip-lock bag to maintain sample integrity. To obtain pure cultures, the soil sample was serially diluted and plated on Sabouraud Dextrose Agar (SDA), a selective medium for fungal growth. The isolated fungal species were identified as follows:

- *Aspergillus niger*
- *Neurospora crassa*
- *Fusarium* sp

Inoculation and incubation of culture media

To evaluate the efficacy of the formulated media compared to the control PDA media, samples were obtained from pure cultures of the isolates at the 96th hour of growth during the logarithmic phase, when cells exhibited consistent physiological characteristics. Using a cork borer, a small implant was carefully extracted from the pure culture plate and transferred into the formulated media RDA, WDA and control PDA media using sterile forceps. Before commencing the experimental work, the laminar flow was pre-sterilized using UV light, and all the equipment was thoroughly sanitized by dipping in 70% ethanol and flaming to minimize the risk of contamination. The culture plates were incubated at 32°C.

Evaluation of fungal growth on formulated media

Growth was monitored every 24 h by measuring the diameter of fungal colonies using a digital Vernier caliper. The development of fungal species was quantified by converting the average diameter into the area of a circle, providing a comprehensive assessment of the performance of the media. The mycelial growth diameter (MGD) was calculated as the average of two perpendicular diameters (D1 and D2) and expressed in millimeters (mm) (Werghemmi et al., 2022).

The MGD was calculated using the following formula:

MGD (mm) = (D1 + D2)/2

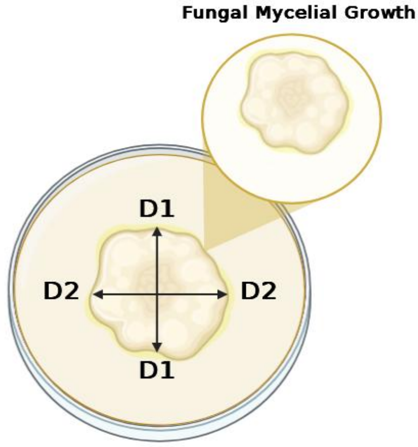

Fig 1. Mycelial growth diameter calculation

Chemical analysis of dehydrated media

The macronutrient content of the dehydrated media samples was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and a UV Spectrophotometer.

RESULTS

Comparison of physico-chemical properties of formulated media and control

The physico-chemical properties of the formulated media, Rice Dextrose Agar (RDA) and Wheat Dextrose Agar (WDA), were comprehensively evaluated in comparison to the standard control, Potato Dextrose Agar (PDA), In terms of color, PDA exhibited a light-yellow hue, while RDA appeared milky white and WDA had a brownish tint. PDA was consistently firm and gel-like, RDA displayed a gelatinous texture, and WDA was granular in nature.

Chemical analysis of dehydrated media

An analysis of the mineral content in Rice Dextrose Agar (RDA) and Wheat Dextrose Agar (WDA) was conducted, with the results presented in **Table 5**. The findings reveal that WDA exhibits higher levels of key minerals in comparison to RDA. In particular, the potassium concentration in WDA (2977 mg/kg) was found to be substantially greater than in RDA (1363.8 mg/kg). WDA also demonstrated elevated levels of magnesium and phosphorus, measuring 1066 mg/kg and 1136.05 mg/kg respectively, while RDA contained 324.5 mg/kg and 746.86 mg/kg of these minerals. Additionally, WDA showed slightly higher concentrations of calcium (334.7 mg/kg) and iron (40.9 mg/kg) compared to RDA (130.4 mg/kg and 35.2 mg/kg).

Table 5: Mineral composition of Rice Dextrose Agar (RDA) and Wheat Dextrose Agar (WDA) in mg/kg

Mineral	RDA	WDA	Unit
Potassium	1363.8	2977	mg/kg
Magnesium	324.5	1066	mg/kg
Iron	35.2	40.9	mg/kg
Calcium	130.4	334.7	mg/kg
Phosphorus	746.86	1136.05	mg/kg

Growth of the selected fungi on the formulated media and the control media Figures 2-4 are graphical representations of the data obtained from culturing three test fungi on the two formulated alternative culture media and PDA (mycelial growth diameter taken every 24 through 72 h).

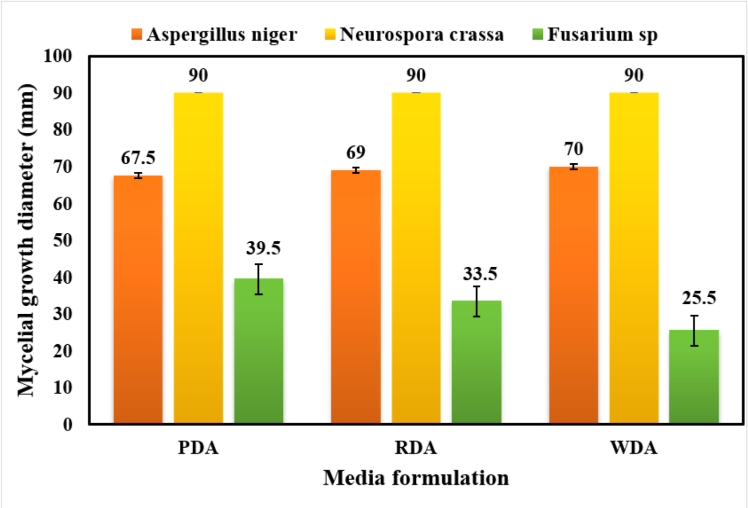

Fig 2. Mycelial growth on different formulated media at 3.9 g concentration

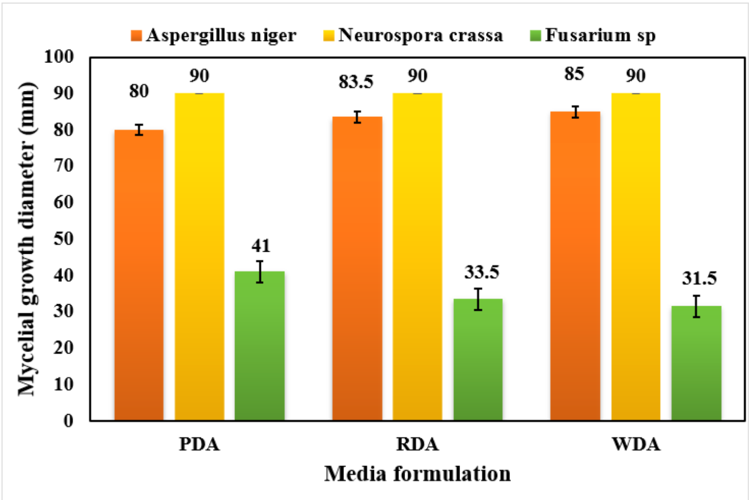

Fig 3. Mycelial growth on different formulated media at 2.9 g concentration

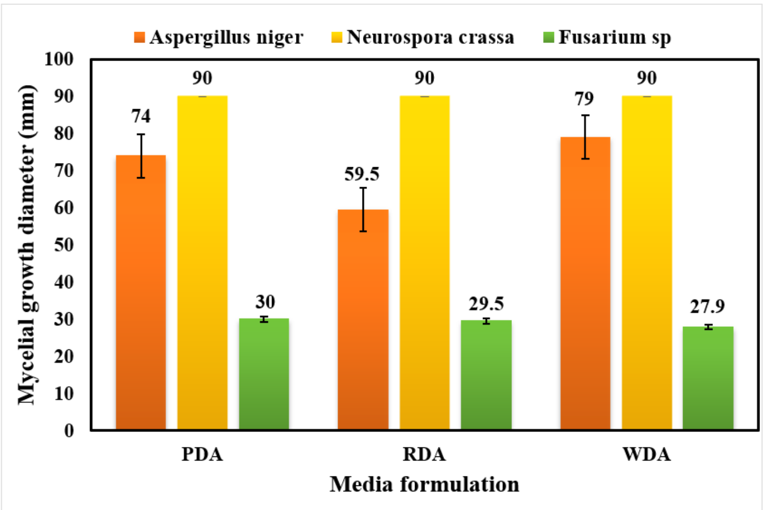

Fig 4. Mycelial growth on different formulated media at 1.9 g concentration

The formulated media at a 3.9 g concentration supported the growth of all test organisms (Figure 2). As depicted in Figure 2, *Aspergillus niger* displayed the most extensive mycelial growth on WDA (70 mm), RDA (69 mm), and PDA (67.5 mm) following closely behind, suggesting that WDA is particularly effective for this species. *Neurospora crassa* showed consistent growth across all media, reaching the full diameter of the petri plate (90 mm) without demonstrating a clear preference for any specific medium. In the case of *Fusarium* sp, PDA yielded the greatest growth (39.5 mm), whereas growth was relatively reduced on RDA (33.5 mm) and minimal on WDA (25.5 mm). These results underscore the varying media preferences among fungal species, with WDA proving the most beneficial for *Aspergillus niger*, PDA being ideal for *Fusarium* sp, and *Neurospora crassa* exhibiting uniform growth across all tested media.

The formulated media, at a concentration of 2.9 g, proved effective in supporting the growth of all test organisms, even with reduced nutrient content (figure 3). As illustrated in Figure 3, Among the tested fungi, *Aspergillus niger* displayed the most significant mycelial expansion on WDA (85 mm), with RDA (83.5 mm) and PDA (80 mm) following suit, suggesting that WDA is particularly beneficial for this species at the given concentration. *Neurospora crassa* demonstrated remarkable adaptability, achieving full petri plate coverage (90 mm) across all media types, indicating its versatility in various formulations. In the case of *Fusarium* sp, PDA yielded the greatest growth (41 mm), whereas RDA (33.5 mm) and WDA (31.5 mm) resulted in comparatively less expansion.

At the lowest tested concentration (1.9 g, the formulated media supported the growth of all test organisms (figure 4), albeit with some variation, as shown in Figure 4 *Aspergillus niger* exhibited the highest mycelial growth on WDA (79 mm), followed by PDA (74 mm) and RDA (59.5 mm), demonstrating WDA's efficacy even at reduced concentrations. *Neurospora crassa* maintained robust growth, covering the entire petri plate diameter (90 mm) across all media, showcasing its adaptability at lower nutrient availability. For *Fusarium* sp, PDA supported the maximum growth (30 mm), followed closely by RDA (29.5 mm) and WDA (27.9 mm), with relatively minor differences between the media. These observations further illustrate media-specific responses among the fungal species at lower nutrient levels, with WDA favouring *Aspergillus niger,* PDA being slightly more conducive to *Fusarium* sp, and *Neurospora crassa* thriving uniformly across all media.

Figures 5–7 illustrate the growth of three fungal species (*Aspergillus niger, Neurospora crassa, and Fusarium* sp) on Rice Dextrose Agar (RDA) and Wheat Dextrose Agar (WDA) at varying concentrations (1.9 g, 2.9 g, and 3.9 g).

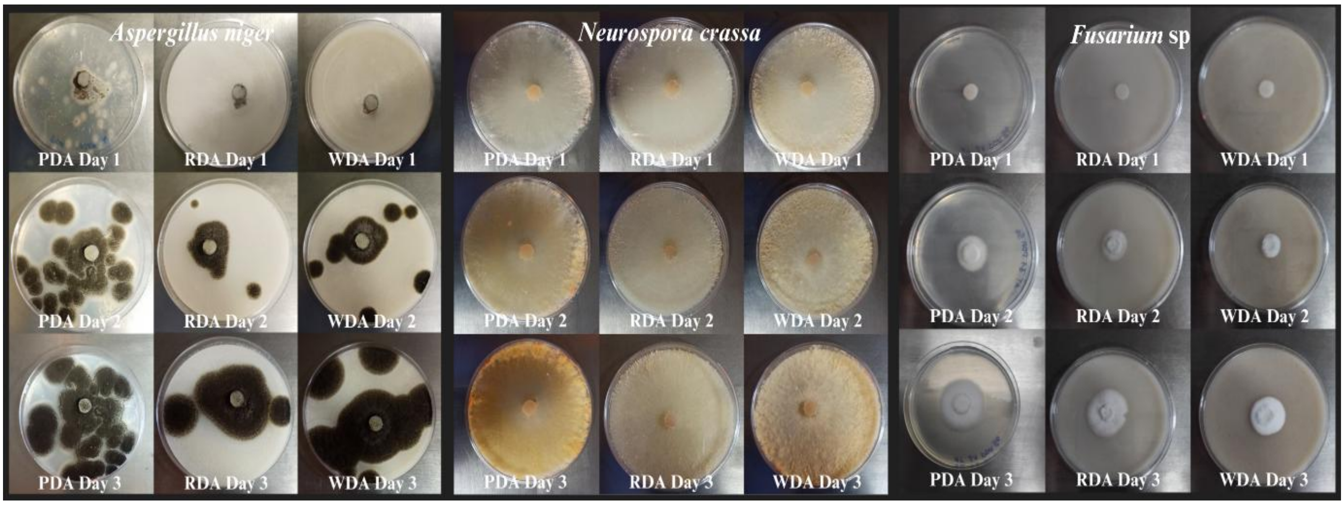

Fig 5. Mycelial growth of test fungi on formulated media and control at 3.9 g concentration

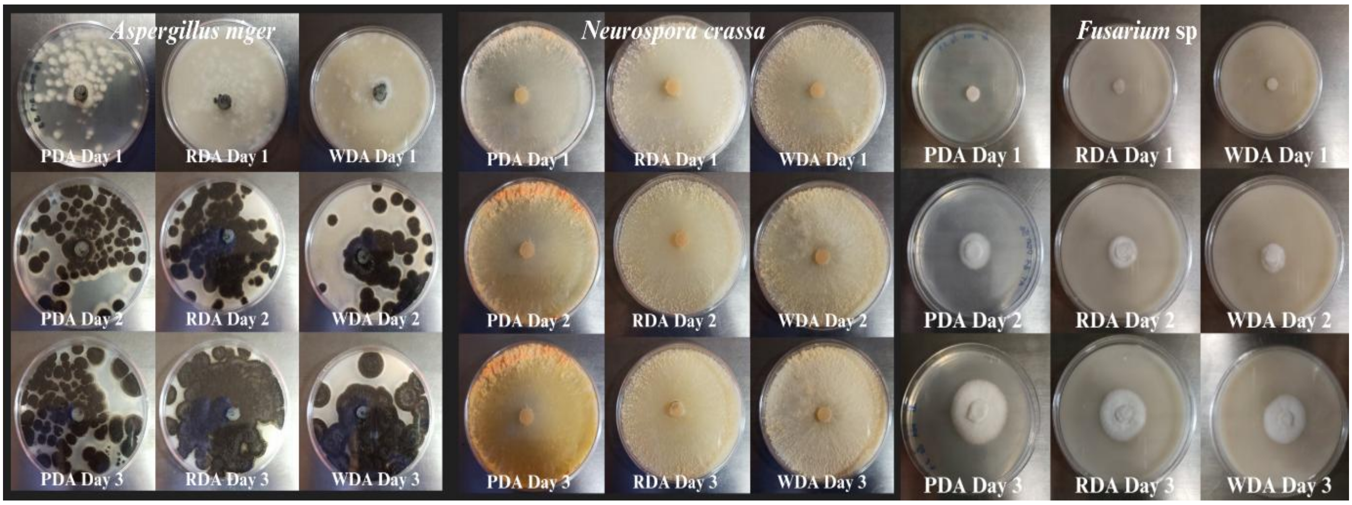

Fig 6. Mycelial growth of test fungi on formulated media and control at 2.9 g concentration

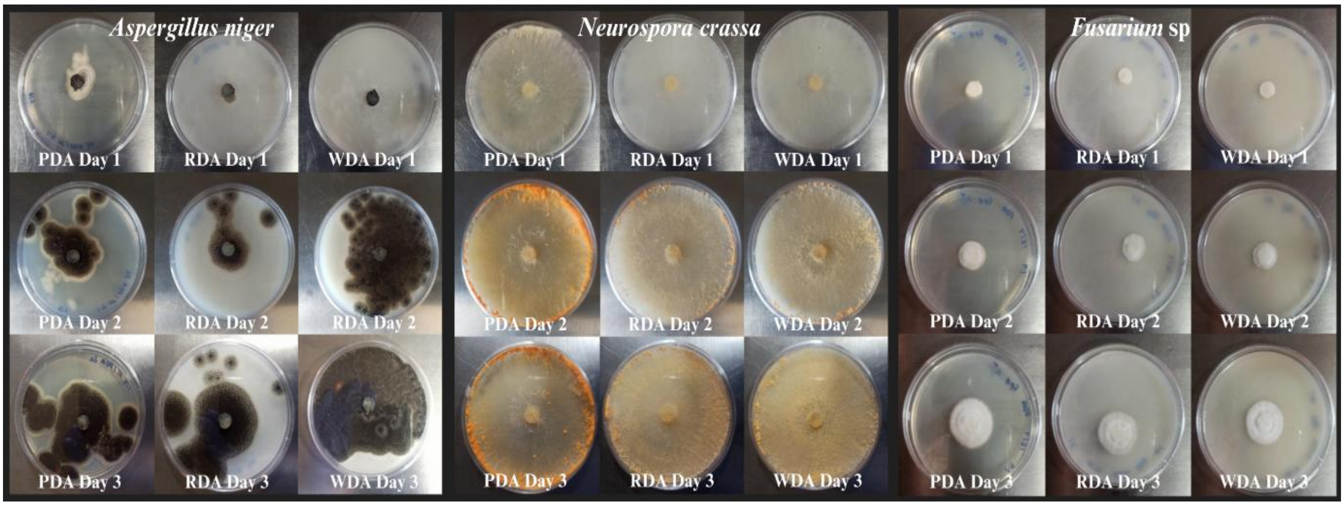

Fig 7. Mycelial growth of test fungi on formulated media and control at 1.9 g concentration

DISCUSSION

The findings of this study demonstrate the potential of Wheat Dextrose Agar (WDA) and Rice Dextrose Agar (RDA) as cost-effective and accessible alternatives to the conventional Potato Dextrose Agar (PDA) for fungal cultivation. The superior growth of *Aspergillus niger* on WDA, particularly at higher mineral concentrations (e.g., potassium, magnesium, and phosphorus), highlights the importance of nutrient-rich substrates in supporting robust fungal growth. This aligns with previous studies that emphasize the role of essential minerals in enhancing fungal metabolism and biomass production (Sośnicka et al., 2022; Lal et al., 2019; Liu et al., 2020). In contrast, *Fusarium sp.* exhibited a preference for PDA, suggesting that this species may require specific nutrients or a balanced composition that is uniquely provided by the standard medium. This finding underscores the species-specific nature of fungal growth requirements, as noted in earlier research on the variability in fungal responses to alternative media formulations (Agu, K.C. et al., 2023; Sośnicka et al., 2022; Monis et al., 2024). For instance, the use of alternative ingredients like beetroot extract and brewer's yeast tailings has shown promise but requires careful optimization to meet the nutritional needs of different fungal species (Shaban et al., 2024; Lal et al., 2019). The exceptional adaptability of *Neurospora crassa* across all media types and concentrations is particularly noteworthy. Its consistent growth, even at lower nutrient levels (1.9 g), suggests a high degree of metabolic flexibility, which may be attributed to its ability to utilize a wide range of substrates efficiently. This observation supports the idea that certain fungi can thrive on less refined or alternative media, reducing dependency on expensive traditional formulations. The use of parboiled rice and wheat from government ration shops as base materials for WDA and RDA not only ensures affordability but also addresses the challenge of scalability, a critical factor for large-scale applications. The granular and gelatinous texture of WDA and RDA, along with their reduced transparency, presents practical challenges for laboratory use. These issues could be mitigated through further optimization of the media's physical properties, such as adjusting the gelling agents or refining the preparation process (Sośnicka et al., 2022; Liu et al., 2020).

Despite these challenges, the higher mineral content and nutritional profile of WDA and RDA make them promising candidates for fungal cultivation. Future research should focus on refining the formulations to enhance their suitability for a broader range of fungal species, including those with more specific nutrient requirements. Additionally, combining RDA and WDA to create hybrid media could leverage the strengths of both substrates, potentially improving growth outcomes for species like *Fusarium sp* (Gamit et al., 2023; Shaban et al., 2024).Finally, the affordability and accessibility of these cereal-based media offer significant advantages for resource-limited settings, making them a sustainable alternative to conventional media. Expanding the application of WDA and RDA to bacterial studies could further validate their versatility and cost-effectiveness, paving the way for their widespread adoption in both research and industrial contexts.

CONCLUSION

This study demonstrates that Wheat Dextrose Agar (WDA) and Rice Dextrose Agar (RDA) are viable, cost-effective alternatives to Potato Dextrose Agar (PDA) for fungal cultivation. WDA, in particular, supported superior growth of *Aspergillus niger*, likely due to its higher mineral content, while *Neurospora crassa* exhibited remarkable adaptability across all media types and concentrations. Although *Fusarium sp.* showed a preference for PDA, both WDA and RDA provided comparable growth, highlighting their potential as substitutes for conventional media. The chemical analysis revealed that WDA and RDA are rich in essential minerals such as potassium, magnesium, and phosphorus, which are critical for fungal growth. This, combined with their affordability and accessibility, makes them highly suitable for large-scale applications. However, the granular and gelatinous texture of the formulated media, along with reduced transparency, presents practical challenges that warrant further optimization. Overall, the use of rice and wheat as base materials for fungal media offers a sustainable and economical solution for microbial research and industrial processes. Future studies should focus on refining the formulations to improve their physical properties and expand their applicability to a wider range of microorganisms. By addressing these challenges, WDA and RDA have the potential to revolutionize fungal cultivation, particularly in resource-limited settings, and contribute to the advancement of microbial biotechnology.

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Conflicts of interest
The authors declare that they have no conflict of interest. All authors have approved the manuscript for submission.

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