PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 29 (3) 841-847 Mar. 2025

Isolation of Native Fungi from Sawdust Collected at a Sawmill from Adeleke Area, Ogbomosho, Oyo State, Nigeria

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ABSTRACT: Sawdust is an agricultural waste and could be utilized in several productive and beneficial entities. Therefore, the objective of this paper is to assess the Isolation of Native Fungi from Sawdust collected at sawmill from Adeleke area, Ogbomosho, Oyo state, Nigeria using appropriate standard procedures. Results were obtained at 25°C and 35°C in this study. At 35°C, the result reveals that *Aspergillus carbonarius* cultured with sawdust had the highest enzymatic activity of 3.65±1.414 than with CMC where it had an enzymatic activity of 2.9±0.070. *Rhizopus nigrican* had an enzymatic activity of 3.5±1.414 with sawdust than with CMC where it had an enzymatic activity of 2.7±1.414. *Aspergillus flavus* had an enzymatic activity of 2.45±0.707 with sawdust than with CMC where it had an enzymatic activity of 1.95±0.707. *Aspergillus Niger* had an enzymatic activity of 2.4±1.414 with sawdust than with CMC where it had an enzymatic activity of 1.6±1.414. *Trichoderma harzianum* had an enzymatic activity of 2±1.414 with sawdust than with CMC where it had an enzymatic activity of 1.3±1.414. In this study, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus carbonarius*, *Rhizopus nigrican*, and *Trichoderma harzianum* showed significant cellulolytic ability to degrade sawdust. *Rhizopus nigrican* showed the highest fermentation ability and producing clear zones of 40mm (25°C) and 34mm (35°C) **(**$p<0.001$**).** Hence, the use of *Rhizopus nigrican* in the production of cellulase using sawdust as an agro waste is possible because of its high cellulolytic ability which was observed at both temperatures.

DOI: https://dx.doi.org/10.4314/jasem.v29i3.20

Cite this Article as: AKOMOLAFE, A. P; ODELADE, K. A; SULAIMON, S. O. (2025) Isolation of Native Fungi from Sawdust Collected at a Sawmill from Adeleke Area, Ogbomosho, Oyo State, Nigeria. *J. Appl. Sci. Environ. Manage.* 29 (3) 841-847

Dates: Received: 20 February 2025; Revised: 13 March 2025; Accepted: 18 March 2025; Published: 31 March 2025

Keywords: Sawdust; cellulose; fermentation; biodegradation; cellulolytic ability

Agricultural wastes are the byproducts from growing crops and/or the initial processing of unprocessed agricultural goods, including fruits, vegetables, dairy, meat, poultry, and other products (El-Ramady *et al*., 2022a). There are three categories of agro-waste: liquid, solid, and slurry (El-Ramady *et al*., 2022b). Agro-wastes make up close to 30% of all agricultural products generated worldwide. Several studies have quantified agricultural waste production and environmental effects, such as the global nutritional and environmental losses caused by food waste (Chen *et al*., 2020). As a more economical and environmentally friendly alternative to treatment, sustainable management with an emphasis on reducing food waste is recommended (Ogunmoroti *et al*., 2022). Wood dust is also the byproduct of a few animals, birds, and insects that live in wood, such as the carpenter ants and woodpeckers. The production

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of briquettes and sawdust bioremediation reduces air pollution while yielding a useful and valued end product. Sawdust becomes biofertilizers by composting, which binds nitrogen to the soil for 180 days (Asadu *et al*., 2019). Because of its high lignin and cellulose concentration and low nitrogen level, it is an organic waste that does not degrade in particular, certain microbes. Lignin and cellulose must be completely hydrolyzed by ligninase and cellulase for sawdust to decompose. Both cellulose and lignin are more complex polysaccharides with a high molecular weight; cellulose has a molecular weight of 2 x105 to 2.4 x 106 mole and approximately 2 x103 to 104 glucose monomers (Rusdianto *et al*., 2021). Numerous Streptomyces species can flourish on potato dextrose agar and Sabouraud agar with different sawdust concentrations, *including S. purpureus, S. albidoflavus, S. antibioticus, S. albus,* and *S. cyaneus* (Ejaz *et al*., 2021). Sawdust biodegradation requires the release of several hydrolyzing enzymes, such as cellulase and ligninase, which hydrolyze cellulose and lignin, respectively (Singhania *et al*., 2021). Sawdust-decomposing microorganisms thrive in polysaccharide-rich environments like soil, where efficient hydrolyzing enzymes are continuously generated (Sharma *et al*., 2019). Hence, the objective of this paper is to assess the Isolation of Native Fungi from sawdust collected at sawmill from Adeleke area, Ogbomosho, Oyo state, Nigeria.

MATERIALS AND METHODS

Sample collection and preparation: Sawdust was aseptically collected from a sawmill, Adeleke area, Ogbomosho, Oyo state, ($8^{o}7^{l}29^{"}$ N $4^{o}13^{'}32^{"}$E) Nigeria. The sawdust was pretreated by weighing 100g, sun drying, and oven drying at 80°C for 1h and then cooling. The pretreated sawdust was blended with a blender. The blended sample was filtered to small particles sizes with 0.1mm mesh sieve. The filtered sample was then stored in an airtight polythene bag at room temperature until required.

Isolation and Characterization of Fungi: Five grams (5g) of soil sample was collected aseptically into sample bottles from the Precious Cornerstone University disposal site. The soil sample collected was serially diluted and the dilution factor of 10^{-3} and 10^{-5} was used. For five days, the inoculum was incubated at 28±2°C on Potato Dextrose Agar (PDA) supplemented with 0.001mg/l of chloramphenicol. A pure culture was obtained by subculturing the colonies from the culture. Stock cultures were kept on kept on PDA slants and stored at 28°C. The isolates were identified and characterized by phenotypic characteristics which included colony colour observation and growth pattern studies, as well as microscopic characteristics (Gurung *et al*., 2020).

Screening for Cellulase Producing Fungi: Spot inoculations of pure cultures' spore suspension were made on plates using a minimal salt medium that contained 1g of carboxymethyl cellulose (CMC) agar, 0.25g of $NaNO_3$, 0.1g of KH_2PO_4, 0.05g of $MgSO_4$, 0.06g of $CaCl_2$, 1.5g of agar agar powder, and 0.001g chloramphenicol in 100ml of H_2O. The plates were then incubated for five days at 28±2°C. Spot inoculations of pure cultures' spore suspension were made on other plates using a minimal salt medium that contained 1g of sawdust, 0.25g of $NaNO_3$, 0.1g of KH_2PO_4, 0.05g of $MgSO_4$, 0.06 of $CaCl_2$, 1.5g of agar agar powder, and 0.001g chloramphenicol in 100ml of H_2O to induce cellulase secretion. The plates were then incubated for five days at a temperature of 28±2 °C. Following incubation, the plates were stained for 15 minutes with 1% Congo red solution and then 10 minutes later with 1M NaCl solution. Each colony's zone of clearance was measured in diameter. To produce cellulase, fungal isolates with the highest zone of clearance were chosen. The fungi isolated are *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus carbonarius*, *Rhizopus nigrican and Trichoderma harzianum.*

Fermentation Process for Cellulase Activity of the fungal isolates: The fermentation process was done by comparing the organisms incubated at different temperatures. To determine which temperature had the highest zone of clearance and to assess the fungal isolates' optimal growth, the isolates were incubated at 25°C and 35°C.

Screening of cellulolytic enzyme activity: Microorganisms that produced cellulases were screened on agar plates that were enriched with only CMC as a carbon source. This plate was used as a control. The microorganisms were then screened on another agar plate that included sawdust as the sole carbon source. The plates were kept at 28± 2 °C for five days of incubation. The basis for this qualitative examination is the way congo red interacts with cellulose and its broken-down components, allowing the dye to be retained in the integral biopolymer whereas areas containing cellulose that has been hydrolyzed by enzymes produce clear zones. The enzymatic activity (index) was subsequently calculated by measuring the clear zones using the formula described by Rosa *et al*. (2020).

$$EA = \frac{Diameter\ of\ clear\ zone}{Diameter\ of\ inoculum\ (colony)} \quad (1)$$

Statistical Analysis: Graphpad Prism 5 was used for the analysis of the fungal isolates on sawdust and C.M.C at 25^{o}C and 35^{o}C. Two-way ANOVA test was applied to set the degree of significance of the fermentation activity. Also, comparison of the enzyme activity of different isolates was done statistically. In this study, P<0.05; P<0.01 and P<0.001 were used to disapprove the null hypothesis (Oladeji *et al*., 2020).

RESULTS AND DISCUSSION

The fungi isolated are *Aspergillus niger, Aspergillus flavus, Aspergillus carbonarius, Rhizopus nigrican and Trichoderma harzianum*

Characterization of the fungi isolates:

Aspergillus niger
Colony diameter: 4.5-6.5 cm.
Obverse: Blackish-brown often with yellow mycelium.
Reverse: Creamish-yellow to yellow or orange.
Head: Globose, splitting with age.
Stipe: long, smooth, hyaline to brownish.
Vesicle: Globose, thick-walled, brownish, and fertile on entire surface.
Metulae: present, long, closely packed, brownish.
Phialides: shirt, thick, ampulliform, brownish.
Conidia: Globose to elliptical, rough, dark brown to black.
Mycotoxins: Malformin C, naphthoquinones, nigragillin.
Aspergillus flavus
Colony diameter: 4.0-4.5.
Obverse: Yellowish-green becoming green with age.
Reverse: Creamish-yellow
Head: Radiating, becoming loosely columnar with age.
Stipe: long, hyaline.
Vesicle: Dome-shaped, fertile on the entire surface.
Metulae: present, small.
Phialides: small, ampulliform.
Conidia: Globose to subglobose, usually yellow-green.
Mycotoxins: Aflatoxins B1 and B2, paspalini.
Aspergillus carbonarius
Morphology: Hyphae that form mycelium
Colony: Dark black
Shape: Filamentous with hyphae
Conidiophores: unbranched and may have vesicles at their tips from 3-5 micrometers.
Rhizopus nigrican
Colour**:** Black or dark gray.
Morphology: Mold-like appearance with a mycelium that branches out and spreads.
Reproduction: sexual or asexual.
Trichoderma harzianum
Colour: Greenish hue.
Conidiophores: Elongated and none branching with structures that emerge from the mycelium
Conidia: Oval or ellipsoidal
Size: 3-5 micrometer
Colony Texture: Colonies often exhibit a fluffy or cotton-like texture.

Comparative analysis of the fermentative activity of the fungal isolates at 25^{o}C and 35^{o}C: During the fermentation of sawdust at 25^{o}C and 35^{o}C, the cellulolytic organisms isolated showed significant fermentative ability. The fungal isolates grown at 25oC with sawdust were compared statistically with the fungal isolates grown with CMC at 25^{o}C and the fungal isolates grown with sawdust at 35^{o}C were compared with the isolates grown with CMC at 35^{o}C. The graph (Figure 1-2) below shows the significance of each fungal isolate when compared with CMC. Values with (*, **, and ***) are significantly higher than the control at P < 0.05; P < 0.01, and P < 0.001. The statistical analysis of the fermentation activities on sawdust and carboxymethyl cellulose for the cellulolytic activity of the organisms is shown in Figures 1 and 2. In the fermentation process, it was observed that all the fungal isolates showed a significant ability to biodegrade sawdust at P<0.05, P<0.01, P<0.001, although at different rates. Figure 1 at 25^{o}C shows that all the fungal isolates grown with sawdust had the highest fermentation activities than fungal isolates grown with CMC. The fungal isolates showed clear zones ranging from 27mm-40mm. *Rhizopus nigrican* showed the highest fermentation activity with a clear zone of 40mm at P<0.001 with sawdust than with CMC where it had a clear zone of 32mm. *Aspergillus niger* had a clear zone of 27mm at P<0.05 with sawdust than with CMC where it had a clear zone of 23mm. *Aspergillus flavus* had a clear zone of 30mm at P<0.001 with sawdust than with CMC where it had a clear zone of 17mm. *Aspergillus carbonarius* had a clear zone of 37mm at P<0.01 with sawdust than with CMC where it had a clear zone of 30mm. *Trichoderma harzianum* had a clear zone of 30mm at P<0.001 on sawdust than with CMC where it had a clear zone of 22mm.

Figure 2 at 35^{o}C shows that fungal isolates grown with sawdust had the highest fermentation activity than the fungal isolate grown with CMC. The fungal isolates showed clear zone ranging from 19mm-34mm. *Rhizopus nigrican* showed the highest fermentation activity with a clear zone of 34mm at P<0.001 than with CMC where it had a clear zone of 28mm. *Aspergillus niger* had a clear zone of 23mm at P<0.001 than with CMC where it had a clear zone of 17mm.

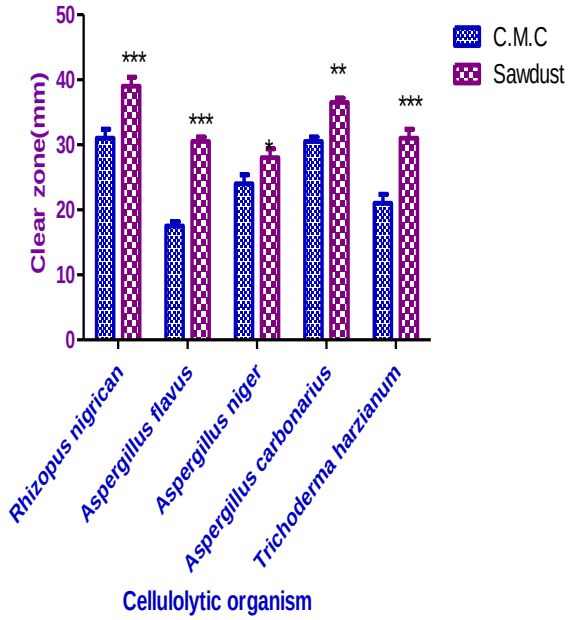

Fig 1: Fermentation activity of cellulolytic organism on sawdust against C.M.C at 25°c

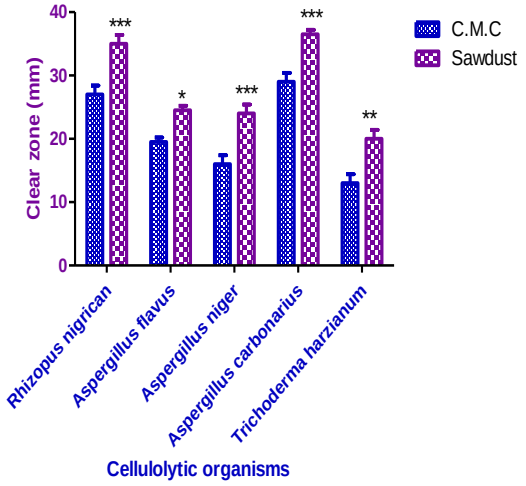

Fig. 2: fermentation activity of cellulolytic organism on sawdust against C.M.C at 35°c

Aspergillus flavus had a clear zone of 25mm at P<0.05 with sawdust than with CMC where it had a clear zone of 20mm. *Aspergillus carbonarius* had a clear zone of 37mm at P<0.001 with sawdust than with CMC where it had a clear zone of 30mm. *Trichoderma harzianum* had a clear zone of 19mm at P<0.01 with sawdust than with CMC where it had a clear zone of 12mm.

In Table 1, values of the enzymatic activities of the cellulolytic organisms at 25°C and 35°C are expressed as mean ± SD for n=2 for each concentration. Values with (*, **, ***) are significantly higher than the standard at P<0.05, P<0.01, P<0.001 respectively.

Comparative analysis on the enzymatic activity of the fungal isolates on sawdust and CMC at 25°C and 35°C: The enzymatic activities of the fungal isolates grown with sawdust at 25°C were compared statistically with the fungal isolates grown with CMC at 25°C. Also, the fungal isolates grown with sawdust at 35°C were compared with the isolates grown with CMC at 35°C (Table 1). This analysis was statistically analyzed at a significance level of P < 0.05, P < 0.01 and P < 0.001. To minimize errors, the mean ±SD for each concentration was determined. At 25°C the enzymatic activity of *Rhizopus nigrican* cultured with sawdust was statistically higher having an enzymatic activity of 3.9±0.414 at P < 0.001 than with CMC where it had an enzymatic activity of 3.1±0.141. *Aspergillus flavus* had an enzymatic activity of 3.05±0.707 with sawdust than with CMC where it had an enzymatic activity of 1.75±0.070. *Aspergillus niger* had an enzymatic activity of 2.8 ±0.141 with sawdust than with CMC where it had an enzymatic activity of 2.4 ±0.141. *Aspergillus carbonarius* had an enzymatic activity of 3.65±0.070 with sawdust than with CMC where it had an enzymatic activity of 3.05±0.070. *Trichoderma harzianum* had an enzymatic activity of 3.1±1.414 with sawdust than with CMC where it had an enzymatic activity of 2.1±0.141.

Table 1: comparative analysis of the enzymatic activity of the fungal isolates at 25°c and 35°c

Fungal Isolates	CMC at 25°C	Sawdust at 25°C	CMC at 35°C	Sawdust at 35°C
Rhizopus nigrican	3.1±0.141	3.9±0.414***	2.7±1.414	3.5±1.414***
Aspergillus flavus	1.75±0.070	3.05±0.707***	1.95±0.707	2.45±0.707*
Aspergillus niger	2.4±0.141	2.8±0.141*	1.6±1.414	2.4±1.414***
Aspergillus carbonarius	3.05±0.070	3.65±0.070**	2.9±0.707	3.65±1.414***
Trichoderma harzianum	2.1±0.141	3.1±1.414***	1.3±1.414	2±1.414**

At 35°C *Aspergillus carbonarius* cultured with sawdust had the highest enzymatic activity of 3.65±1.414 than with CMC where it had an enzymatic activity of 2.9±0.070. *Rhizopus nigrican* had an enzymatic activity of 3.5±1.414 with sawdust than with CMC where it had an enzymatic activity of 2.7±1.414. *Aspergillus flavus* had an enzymatic activity of 2.45±0.707 with sawdust than with CMC

where it had an enzymatic activity of 1.95±0.707. *Aspergillus niger* had an enzymatic activity of 2.4±1.414 with sawdust than with CMC where it had an enzymatic activity of 1.6±1.414. *Trichoderma harzianum* had an enzymatic activity of 2±1.414 with sawdust than with CMC where it had an enzymatic activity of 1.3±1.414.

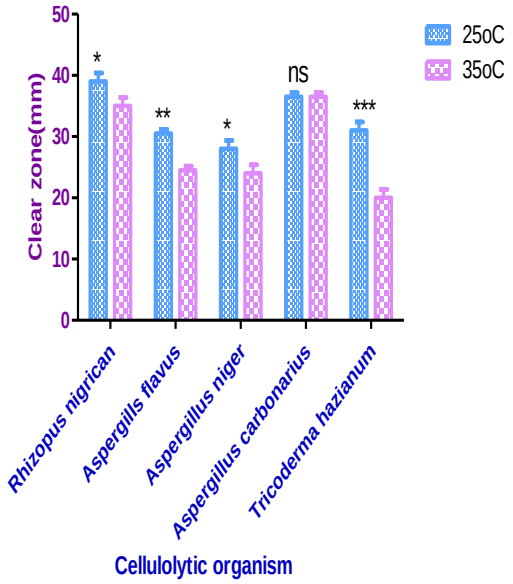

Fig. 3: comparison of the cellulolytic activity of the fungal isolates at 25°c and 35°c on sawdust.

The statistical analysis of the cellulolytic activity of the fungal isolates on sawdust was done by comparing their cellulolytic activity at 25°C and 35°C. Figure 3 shows that *Trichoderma hazianum* was statistically higher at 25°C at p<0.001 than at 35°C. While *Aspergillus carbonarius* had no significant value at p>0.05. *Aspergillus flavus* had a significant value of p<0.001 at 25°C than at 35°C *Aspergillus niger* had a significant value of p<0.05 at 25°C than at 35° and *Rhizopus nigrican* had a significant value of p<0.05 at 25°C than at 35°C.

In this study, different fungal isolates were used in the biodegradation of sawdust to produce cellulase because of its high cellulolytic compound in conjunction with (Christopher *et al*., 2023). The fungal isolates identified and characterized from the sawdust decomposing soil were *Aspergillus niger, Aspergillus flavus, Rhizopus nigrican, Trichoderma harzianum, Aspergillus carbonarius* in contrast with Oshoma *et al*., (2020) that used fungal isolates from contaminated soils. As adaptable cellulase producers in the bioprocessing business, these fungi have been reported by several authors (Tomico-Cuenca *et al*., 2021). In contrast to (Fierro *et al*., 2022) findings, which suggested that *P. chrysogenum* is a suitable choice for cellulase high productivity yield among the Penicillium species, this study found that Rhizopus nigrican is a good microorganism to produce cellulase. From the screened fungal isolates result all isolates had varied zones of clearance for the cellulase production profile (Carbonero-Pacheco *et al*., 2023). According to the results of the screening for cellulolytic activity, Rhizopus nigrican had the highest zone of clearance (40 mm) and is considered to be a highly productive and efficient species of cellulolytic fungus. The enzymatic activity of the fungal isolate was done following (Coronado-Ruiz *et al*., 2018)

The statistical analysis of the fermentation activities on sawdust and carboxymethyl cellulose to the cellulolytic activity was carried out between the organisms. In the fermentation process, it was observed that all the fungal isolates showed a significant ability to biodegrade sawdust at P<0.05, P<0.01, and P<0.001 although at different rates (Oshoma *et al*., 2020).

According to this study, the temperature at 25°C shows that all the fungal isolates grown with sawdust had the highest fermentation activities than fungal isolates grown with CMC. The fungal isolates showed clear zone ranging from 27mm-40mm. *Rhizopus nigrican* showed the highest fermentation activity with a clear zone of 40mm at P<0.001. *Aspergillus niger* had a clear zone of 27mm at P<0.05, *Aspergillus flavus* had a clear zone of 30mm at P<0.001 *Aspergillus carbonarius* had a clear zone of 37mm at P<0.01 *and Trichoderma harzianum* had a clear zone of 30mm at P<0.001. At 25°C fungal isolates grown with CMC showed low fermentation activity compared to those grown with sawdust (Jumare *et al*., 2022). The fungal isolates showed clear zones ranging from 18 mm to 32 mm at P<0.05, P<0.01, P<0.001. *Rhizopus nigrican* showed the highest fermentation activity with a clear zone of 32mm. *Aspergillus niger* had a clear zone of 23mm, *Aspergillus flavus* had a clear zone of 18mm, *Aspergillus carbonarius* had a clear zone of 31mm *and Trichoderma harzianum* had a clear zone of 22mm.

At the temperature 35°C it also shows that fungal isolates grown with sawdust had the highest fermentation activity than the plates containing fungal isolates grown with CMC. The fungal isolates showed clear zone ranging from 19mm-34mm. *Rhizopus nigrican* showed the highest fermentation activity with a clear zone of 34mm at P<0.001. *Aspergillus niger* had a clear zone of 23mm at

P<0.001, *Aspergillus flavus* had a clear zone of 25mm at P<0.05 *Aspergillus carbonarius* had a clear zone of 37mm at P<0.001 *and Trichoderma harzianum* had a clear zone of 19mm at P<0.01. At 35^{o}C fungal isolates grown with CMC showed low fermentation activity compared to those grown with sawdust 2025/3/13. The fungal isolates showed clear zones ranging from 12mm-30mm at P<0.05, P<0.01, P<0.001. *Rhizopus nigrican* showed the highest fermentation activity with a clear zone of 28mm *Aspergillus niger* had a clear zone of 17mm, *Aspergillus flavus* had a clear zone of 20mm, *Aspergillus carbonarius* had a clear zone of 30mm *and Trichoderma harzianum* had a clear zone of 12mm.

*Conclusion***:** This study demonstrated that sawdust, which is an agricultural waste, can promote the growth of fungal isolates by facilitating the breakdown of cellulose, the main component required for cellulase production, under various temperature conditions. This finding suggests that utilizing sawdust for cellulase production is a promising approach that can effectively address environmental pollution caused by improper waste disposal.

Acknowledgments: The authors extend their appreciation to the Tertiary Education Trust Fund (TETFUND) for funding this research through a grant awarded under the Institution-Based Research (IBR) intervention, 2023, to the Federal Polytechnic Ayede, Oyo State, Nigeria.

Declaration of Conflict of Interest: The authors declare no conflicts of interest.

*Data Availability Statement***:** Data are available upon request from the second author.

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