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Morphological and Molecular Characterization of the Fungi Isolated from Infected Wheat Plant

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ABSTRACT

Background: *Aspergillus aflatoxiformans, Fusarium humuli* and *Curvularia geniculata* are fungal pathogens of agricultural and clinical significance, associated with crop diseases, mycotoxin production and opportunistic human infections. Accurate identification is essential for understanding their taxonomy, pathogenic potential and ecological roles.

Methods: Infected wheat grains and leaves were collected from the Meerut region, India. Fungi were isolated using surface sterilization and cultured on potato dextrose agar. Morphological identification was performed via colony characterization and microscopic examination. Molecular identification was conducted through internal transcribed spacer region amplification, sequencing and BLAST analysis. Phylogenetic relationships and genetic diversity were assessed using Neighbor-Joining and Maximum Composite Likelihood methods.

Result: *Fusarium spp.* exhibited rapid growth with sickle-shaped macroconidia; *Aspergillus spp.* formed yellow-green, powdery colonies with globose conidia; *Curvularia spp.* produced dark brown, velvety colonies with geniculate conidia. Internal transcribed spacer sequencing yielded amplicons of 553 bp (*Fusarium humuli*), 588 bp (*Aspergillus aflatoxiformans*) and 618 bp (*Curvularia geniculata*), showing high homology to reference strains (98–100%). Phylogenetic analysis clustered isolates with their respective species, revealing clear genetic distinctiveness and potential regional adaptations. Integration of morphological and internal transcribed spacer-based molecular approaches effectively identified *Fusarium humuli*, *Aspergillus aflatoxiformans* and *Curvularia geniculata* highlighting their pathogenic potential and genetic distinctiveness. These findings underscore the importance of combined diagnostic methods for accurate fungal identification, which is crucial for crop protection, food safety and public health.

Key words: Fungi, ITS sequencing, Molecular identification, Phylogenetic analysis.

INTRODUCTION

Fungi are most important species which are significant in crops, food and health. Diverse and ubiquitous fungi can be found in agricultural fields as soil act as a reservoir for fungi, which can enter and colonize a variety of beneficial crops (Khuna *et al.*, 2022). *Fusarium* species have a broad geographical presence, colonizing soil, plants and the atmosphere. In several countries, the insect pest directly affects a large number of crops such as corn, sorghum, rice, wheat, papaya, grass, grapes, oranges and various ornamental vegetable crops (Sa *et al.*, 2025). Many different types of fungus in the genus *Fusarium* are phytopathogenic to a variety of plants in a range of environmental settings. Ear rot of maize, foot rot, *Fusarium* head blight (FHB, sometimes referred to as “scab” or ear blight) and seedling blight are among the diseases of small-grain cereals caused by *Fusarium* fungi. *Fusarium graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum* and *Microdochium nivale* are the most prevalent pathogens (Deng *et al.*, 2024). Other fungi such as *Fusarium humuli*, *Aspergillus aflatoxiformans* and *Curvularia geniculata* have also been reported as important plant pathogens capable of infecting cereals and other crops, thereby contributing to significant yield losses and contamination with harmful mycotoxins (Nkuwi *et al.*, 2023).

A saprotrophic fungus, *Aspergillus* reproduces through asexual mode and is widely known for the production of a large variety of aflatoxins (Zhang *et al.*, 2025) and also causing superficial infection. The toxin produced by fungal species pose significant health risks to human life and can harm plants during the growth season as well as after harvest. Due to the decrease in harvest yields and quality standards, this species causes enormous losses that may have an effect on the economy (Xue *et al.*, 2025). A number of studies have been done which focusses on the characterization of aflatoxigenic *Aspergillus* species, due to their high incidence and potential to produce aflatoxin. Some of them produce several mycotoxins, such as aflatoxins, 3 nitropropionic acid, tenuazonic acid and cyclopiazonic acid (Cho *et al.*, 2022; Frisvad *et al.*, 2019). The genus *Curvularia* consist of dematiaceous species of the class Dothideomycetes of Pleodporales fungi in different ecosystem where they occupy saprophytic, endophytic, phytopathogenic niches (Fukuto *et al.*, 2025). This genus contains species that induce *Curvularia* spot, crucial for the

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crops of corn, rice, oats, sorghum, wheat and barley (de Siqueira *et al.*, 2021; Tawfike, 2018). Fungal pathogens belonging to the genera *Fusarium*, *Aspergillus* and *Curvularia* are of significant agricultural and clinical importance due to their wide host ranges, pathogenic potential and toxin production (Antônio *et al.*, 2016; Tann and Soytong, 2017). Their identification and classification rely on both morphological and molecular markers, which provide robust tools for distinguishing closely related species, understanding their evolutionary relationships and also for developing effective disease management strategies and food safety interventions. As plant pathogens and mycotoxin producers, they represent significant threats to global agriculture, trade and human health, underscoring the need for integrative approaches combining classical mycology with molecular diagnostics (Rout *et al.*, 2025). Accurate identification and characterization are essential for understanding their biology and managing their impacts. This study aims to detail the morphological features and genetic profiles of isolated fungal pathogens to aid in accurate identification and understanding their ecological roles.

MATERIALS AND METHODS

These studies were conducted at the Department of Microbiology, IIMT University Ganga Nagar, Meerut. The sample was collected from the region around Meerut between April-August; therefore, most of the experiments were carried out between April- September 2024-2025. The Biokart Genomic Lab in Bengaluru, Karnataka, India, is where the molecular characterization of fungi was done.

Collection of the samples

Infected wheat grains and leaves showing typical symptoms of fungal infestation were collected during the cropping season, from cultivated fields of the Meerut region in Uttar Pradesh, India. The plant parts exhibiting visible signs of infection such as discoloration, necrotic lesions or shrivelling were selected for study. The samples were placed in sterile polythene bags to prevent secondary contamination and under ambient storage conditions (Verma *et al.*, 2017; Waqas *et al.*, 2023).

Isolation fungal pathogen

Surface debris was removed from infected plant parts using running tap water, followed by sterilization. Small segments of infected tissue were surface sterilized in 70% ethanol solution for 1-2 minutes, rinsed thoroughly in sterile distilled water (3-4 times) and then blotted dry using sterile filter paper. The sterilized fragments were transferred aseptically onto potato dextrose agar plates modified with streptomycin sulfate (50 mg/L) to suppress the growth of bacteria. The inoculated plates were incubated at 25±2°C for 5-7 days. Emerging fungal colonies were purified through repeated sub-culturing using the hyphal tip method until pure cultures were obtained (Khalaf *et al.*, 2024; Sharma *et al.*, 2024). The isolates were preserved at 4°C. To ensure culture viability, preserved isolates were sub-cultured at regular intervals of 30-45 days. Each isolate was labelled with a unique identification code and catalogued for further morphological and molecular studies.

Morphological Identification

Macroscopic features such as colony colour, texture and growth were observed directly through naked eyes. Microscopic examination involved staining with lactophenol cotton blue and observation under a light microscope to assess spore morphology, hyphal structure and conidiophore characteristics. The isolated fungal cultures were identified on the basis of their cultural and microscopic characteristics using standard taxonomic keys (de Siqueira *et al.*, 2021). To confirm the morphological features of the isolate, it was compared with the previous work done by other researchers (Fukuto *et al.*, 2025; Mehta *et al.*, 2022).

Molecular Identification

The isolated fungal pathogens were identified and later confirmed using molecular characterization of the internal transcribed spacer region of rDNA. The genomic DNA of pure cultures was extracted using the Xploregen Plant gDNA Extraction Kit. DNA quality was verified through spectrophotometric quantification and agarose gel electrophoresis (Kanipriya *et al.*, 2024; Khattak *et al.*, 2021).

Agarose gel electrophoresis

Genomic DNA quality and integrity were initially evaluated through agarose gel electrophoresis. Distinct genomic DNA bands were visualized against a 100 bp molecular weight ladder to confirm intactness and absence of degradation. The quantified DNA was subsequently used as a template for polymerase chain reaction amplification using the internal transcribed spacer primer pair, a universal marker commonly employed for fungal species identification. Polymerase chain reaction products obtained were again analyzed through gel electrophoresis, enabling visualization of discrete amplified bands corresponding to the expected internal transcribed spacer fragment sizes. Each gel image was documented for verification and quality assurance. Samples that exhibit clear, sharp amplification bands were further sequenced and analysed through Phylogenetic tree. Specifically, samples labelled ITLA-01, ITTF-02 and ITLA-03 all met the required amplification standards confirming their suitability for downstream sequencing or molecular analysis (Chinnasamy *et al.*, 2023).

Polymerase chain reaction amplification of the internal transcribed spacer region (ITS1-5.8s-ITS2) was performed using universal primers internal transcribed spacer1 (5′-TCCGTAGGTGAACCTGCGG-3′) and internal transcribed spacer4 (5′-TCCTCCGCTTATTGATATGC-3′). The polymerase chain reaction reaction mixture (50 µL) contained 1 µL of genomic DNA (≈170 ng), 2 µL of each primer (10 pM), 4 µL of dNTPs (2.5 mM each), 10 µL of 10× Taq buffer with MgCl, (3.2 mM), 1 µL of high-fidelity Taq DNA polymerase (3 U/µL) and nuclease-free water to volume. The cycle was started with an initial denaturation at 94°C for 3 minutes which was

followed by 30 cycles of denaturation at 94°C for 1 minute. After that annealing was done at 50°C for 1 minute and extension at 72°C for 2 minutes, with a final extension at 72°C for 7 minutes (Alam *et al.*, 2023; Hassan and Marzani, 2024).

Analysis of the genetic diversity

The amplified internal transcribed spacer fragments (~0.7 kb) were visualized on 1.5% agarose gels and subsequently purified for bidirectional sequencing on an ABI 3130xl Genetic Analyzer using the BigDye Terminator v3.1 cycle sequencing kit. The obtained sequences were assembled, edited and subjected to BLASTN searches against the NCBI GenBank database for species-level identification.

RESULTS AND DISCUSSION

Identification of the fungi

The cultural and morphological features of the fungal isolates were initially studied on potato dextrose agar medium and later under compound microscopy using lactophenol cotton blue. Based on standard descriptive keys, the isolates were identified as *Aspergillus spp., Fusarium spp.* and *Curvularia spp*.

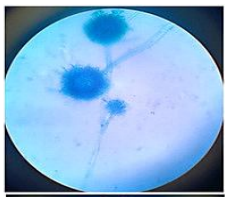
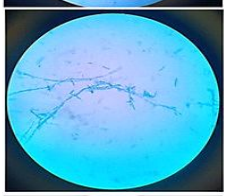
Morphological Identification

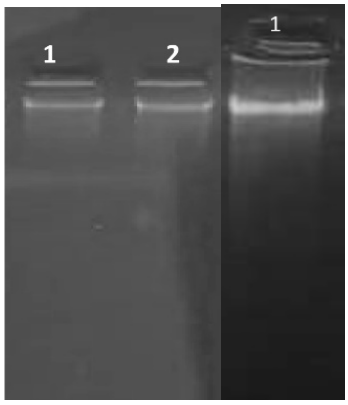
Isolates of *Aspergillus spp.* developed compact, granular to powdery colonies showing yellow-green pigmentation on potato dextrose agar. Under the microscope, unbranched conidiophores terminating in globose vesicles were observed, bearing biseriate phialides that produced rough-walled globose conidia in chains. These characteristics closely matched the descriptions of aflatoxin-producing *Aspergillus spp*. (Malik *et al.*, 2025; Zhang *et al.*, 2025). Colonies of *Fusarium spp.* exhibited rapid growth with abundant aerial mycelium, initially white in colour, which gradually turned pinkish to reddish-brown. Microscopic examination revealed sickle-shaped, multi-septate macroconidia and oval to elliptical microconidia borne on short monophialides, which are diagnostic of the genus *Fusarium* (Deng *et al.,* 2024; Nkuwi *et al.,* 2023). Colonies of *Curvularia spp.* grew moderately fast, appearing dark brown to black with a velvety texture. The conidia were curved, multi-septate and exhibited distinctly swollen central cells, imparting a geniculate appearance, which is considered a key feature of this species (de Siqueira *et al.*, 2021; Tann and Soytong, 2017). The morphological characteristics of all three isolates are shown in Table 1.

Agarose gel electrophoresis

The electrophoresis profiles demonstrated that all processed samples exhibited high-quality genomic DNA with no signs of fragmentation or smearing, indicating excellent sample integrity. The subsequent polymerase chain reaction amplification yielded distinct internal transcribed spacer amplicons, consistent with successful target region amplification. The appearance of strong, single bands in all tested lanes validated both primer specificity and reaction efficiency (Fig 1). The uniformity of results across all three samples (ITLA-01, ITTF-02 and ITLA-03) shows the reproducibility of the polymerase chain reaction process and confirms that the extracted DNA was free from inhibitory substances that could affect amplification. The QC status “PASSED” for each sample underscores the reliability of the DNA extraction and amplification (Fig 2). This successful amplification suggests that the samples are of adequate purity and concentration for subsequent molecular identification or sequencing-based phylogenetic analysis. The electrophoretic band intensities were observed and they aligned with expected internal transcribed spacer fragment

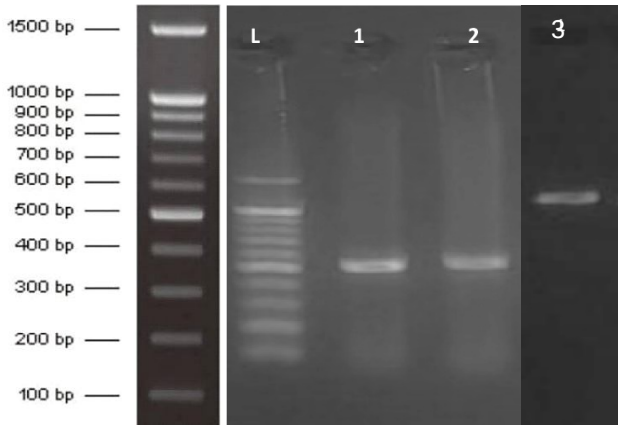
Table 1: Cultural and microscopic morphology of the isolated strains.

Strain no.	Name of microorganism	Cultural morphology	Microscopic morphology
ITLA-01	*Aspergillus aflatoxiformans*		
ITTF-02	*Fusarium humuli*		
ITLA-03	*Curvularia geniculata*		



Lanes 1, 2 and 3 showing intact high-molecular-weight bands with minimal smearing, indicating successful extraction.

Fig 1: Agarose gel electrophoresis result of genomic DNA extracted from the samples.



Lane L represents the 100 bp molecular weight DNA ladder; Lanes 1, 2 and 3 correspond to amplified internal transcribed spacer (ITS) regions of *fungi* isolates, showing clear DNA bands. The distinct amplification bands confirm the successful amplification and purity of the genomic DNA used for molecular characterization.

Fig 2: Agarose gel electrophoresis image of polymerase chain reaction-amplified products of fungal isolates.

sizes (~500-650 bp), further verifying target region specificity. Overall, the QC results affirm that the molecular preparation and polymerase chain reaction amplification methods employed were effective and yielded high-quality outputs, ensuring experimental reproducibility and reliability for downstream genetic characterization (Chaliha *et al.*, 2023).

rDNA analysis

To confirm the identity of the isolated fungus, rDNA analysis was performed which targeted the internal transcribed spacer region. Amplicons of 588 bp, 553 bp and 618 bp were successfully obtained for *Aspergillus, Fusarium* and *Curvularia,* respectively and the sequences were subsequently submitted to the NCBI GenBank database under accession numbers PV789437, PV789438 and PV789439. BLASTN analysis of the internal transcribed spacer sequences revealed high homology: 98.21% with *Fusarium humuli* CGMCC 3.19374, 100% with *Aspergillus aflatoxiformans* CBS 143679 and 98.68% with *Curvularia geniculata* isolate Cgen1, which led to the confirmation of the identity of the isolates (Table 2) (Sa *et al.*, 2025).

To infer the phylogenetic relationship of these isolates with related taxa, approximately optimal phylogenetic trees were constructed based on the internal transcribed spacer region. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method was employed to visualize clustering, while evolutionary distances were computed using the Neighbor-Joining method (NJM) and the Maximum Composite Likelihood method. Bootstrap analysis with 1,000 replicates provided strong statistical support for the clades. The isolates clustered closely with their respective reference strains of *Aspergillus aflatoxiformans, Fusarium humuli* and *Curvularia geniculata*, thereby confirming their molecular placement within these taxa.

Genetic diversity analysis

Genetic diversity among the isolates of *Aspergillus aflatoxiformans, Fusarium humuli* and *Curvularia geniculata* was assessed using internal transcribed spacer region-based molecular characterization. The internal transcribed spacer amplicons (588 bp for *Aspergillus aflatoxiformans*, 553 bp for *Fusarium humuli and* 618 bp for *Curvularia geniculata*) were sequenced and analyzed using BLASTN and phylogenetic methods. The UPGMA-generated dendrogram, supported by Neighbor-Joining (NJ) and Maximum Composite Likelihood (MCL) methods, revealed clear clustering of each species with their respective reference sequences from GenBank. *Aspergillus aflatoxiformans* aligned with CBS 143679, *Fusarium humuli* isolate grouped closely with *Fusarium humuli* CGMCC 3.19374, while *Curvularia geniculata* clustered with isolate Cgen1, confirming their taxonomic placement in Fig 3.

The clustering patterns indicated genetic distinctiveness among the isolates, consistent with internal transcribed spacer. Such intraspecific variability has been widely reported in fungal pathogens, where molecular markers such as internal transcribed spacer and ISSR reveal population-level diversity (Moussaid *et al.*, 2025; Vaghasiya and Parmar, 2023). Previous studies have emphasized combining morphological keys with molecular markers for precise fungal characterization (Alam *et al.*, 2023; Dettman *et al.*, 2023), a principle supported by the present results.

The findings demonstrated that *Aspergillus aflatoxiformans, Fusarium humuli* and *Curvularia geniculata* form distinct genetic groups compared with their closest relatives in GenBank, highlighting the regional diversity of these pathogens. Geographic origin appeared to influence genetic clustering, particularly for *Curvularia geniculata*, which displayed high similarity to Indian isolates but distinct separation from isolates reported elsewhere. This suggests

Table 2: Molecular identification of fungal isolates based on ITS region sequencing and BLAST analysis showing the matching organisms from the NCBI GenBank database, percentage similarity and assigned accession numbers.

Identification of fungi				
Code of sequence isolate	Matching organism in NCBI gene bank database with accession number	Identity of the sequenced *spp.*	Percentage similarity code	Accession number
ITLA-01	*Aspergillus aflatoxiformans NR_171606.1*	*Aspergillus aflatoxiformans*	99-100	PV789437
ITTF-02	*Fusarium humuli NR_164598.1*	*Fusarium humuli*	98-99	PV789438
ITLA-03	*Curvularia geniculate PP961894.1*	*Curvularia geniculate*	98-99	PV789439

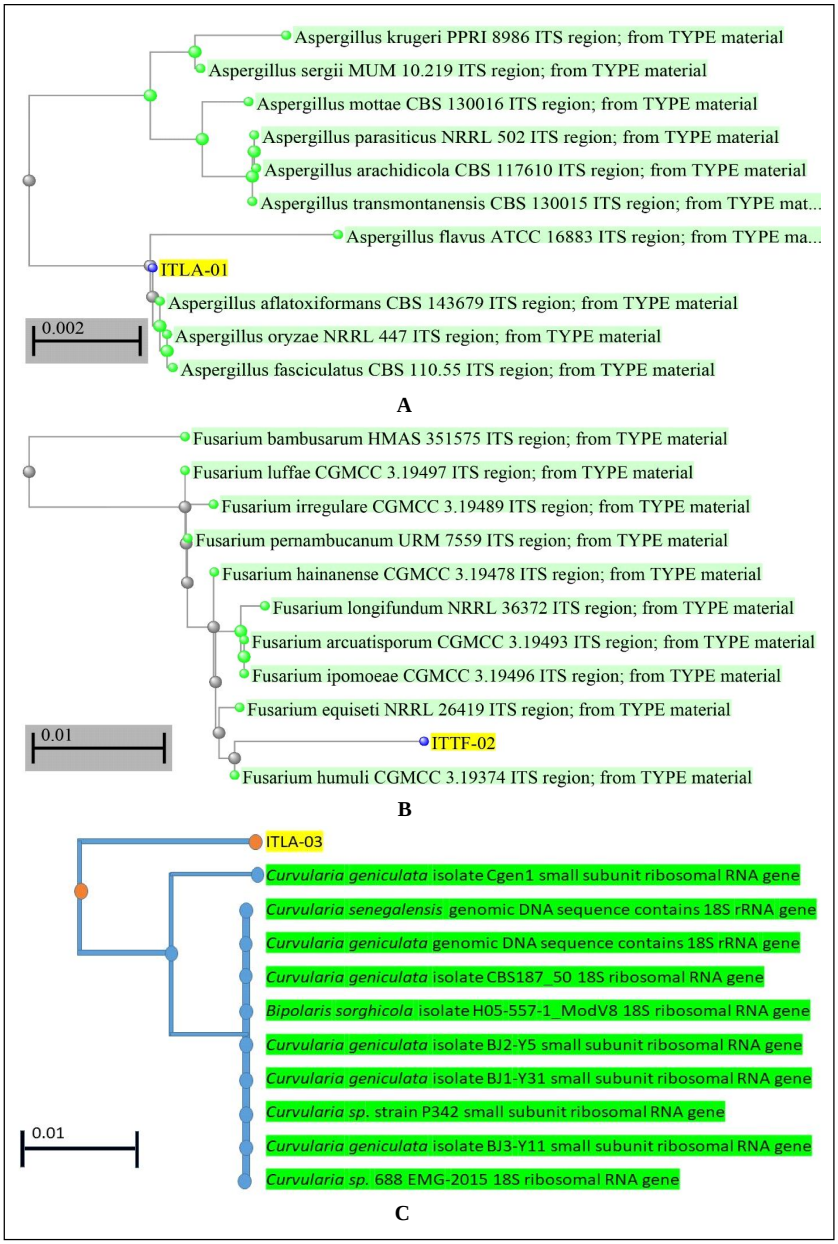


Fig 3: Phylogenetic tree based on ITS region sequences showing the relationship of the isolate, A. *Aspergillus aflatoxiformans*, B. *Fusarium humuli* and C. *Curvularia geniculate*.

possible local adaptations favouring pathogenicity on wheat and related crops (Wang *et al.*, 2019). These results are compared with earlier studies where internal transcribed spacer and 28S rDNA markers were applied to distinguish closely related fungal species (Beemrote *et al.*, 2024; Okioma *et al.*, 2023). Overall, the integration of morphological observations with internal transcribed spacer-based molecular characterization confirmed the genetic identity of *Aspergillus aflatoxiformans, Fusarium humuli* and *Curvularia geniculata*. Their distinctiveness supports their role as important pathogens of cereals and underscores their potential impact on crop health.

CONCLUSION

The present study successfully characterized *Aspergillus aflatoxiformans, Fusarium humuli* and *Curvularia geniculata* using both morphological and molecular approaches. Morphology, internal transcribed spacer such as colony growth, pigmentation and conidial structures provided initial identification, which was further confirmed by internal transcribed spacer region-based rDNA sequencing. BLAST analysis and phylogenetic tree construction placed the isolates within their respective clades, with strong sequence homology to reference strains. Genetic diversity analysis revealed that each isolate clustered distinctly with Internal transcribed spacer closest relatives, highlighting species-level resolution and indicating potential regional adaptations. These findings confirm the reliability of internal transcribed spacer-based molecular tools in complementing classical mycological methods for accurate fungal identification. Given the pathogenic potential of *Fusarium humuli* as a destructive cereal pathogen, *Aspergillus aflatoxiformans* as a potent aflatoxin producer and *Curvularia geniculata* as both a phytopathogen and opportunistic human pathogen, their correct identification holds significant implications for crop protection, food safety and public health. The integration of morphological and molecular diagnostics thus provide a robust framework for monitoring and managing these fungi in agricultural systems.

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Disclaimers

The statements, opinions and conclusions presented in this article are entirely those of the authors and do not necessarily represent the official views, policies, or positions of their affiliated institutions. The authors assume full responsibility for the accuracy, validity and integrity of the data and interpretations included. Although utmost care has been taken to ensure the reliability of the information, the authors disclaim any responsibility for errors, omissions, or any consequences arising from its use. Additionally, the authors declare that there are no financial, commercial or personal relationships that could influence the research outcomes reported in this manuscript.

Informed consent

No animal study.

Conflict of interest

The authors declare that there are no conflicts of interest related to the publication of this article. No external funding, financial support or sponsorship influenced the study design, data collection, data analysis, interpretation of results, decision to publish or preparation of the manuscript. The research was conducted solely with institutional support and reflects the independent work and judgment of the authors.

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