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Incidence, Symptomology, Morpho-molecular Characterization of *Agroathelia rolfsii* (*Sclerotium rolfsii* Sacc.): The Causal Agent of Basal Rot in Chabaud Carnation

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ABSTRACT

Background: Basal rot or sclerotium rot is one of the most serious and deadly disease in wide range of crops, including carnations. In carnations, it causes significant damage and economic loss.

Methods: The *Agroathelia rolfsii*, basionym: *Sclerotium rolfsii* Sacc., the causal agent of basal rot in Chabaud carnation was ioslated from diseased plant parts collected from the Horticultural Research Farm, Assam Agricultural University, Jorhat, in 2023-2024 consequently, the study on the disease incidence (%), symptoms and morphomolecular characterization of the pathogen was carried out.

Result: The incidence of basal rot was recorded the maximum in April (25.78%). The symptoms first appeared in the basal region, just above the ground, which gradually progressed upward over time. Colonizing white mycelia were conspicuous, producing white smooth fruiting bodies that turned brown, resembling mustard seed grains. The disease caused constriction at the infected point, resulting in decaying of the tissue and collapse of the plant eventually. The culture exhibited compact, whitish, cottony mycelia with sclerotia grown over time. Additionally, the DNA sequence of fungal isolate was submitted to NCBI, with an accession No. PP 203038. The fungal isolate had 92.99% similarity with *A. rolfsii* and a phylogenetic tree was constructed according to its nearest species with a bootstrap value.

Key words: *Dianthus caryophyllus*, Diseases in carnation, Fungus diseases, Mycelia, Phytopathogens, Sclerotia.

INTRODUCTION

Sclerotium rot or basal rot caused by *Agroathelia rolfsii* (basionym: *Sclerotium rolfsii* Sacc.) is a serious and destructive soil-borne phytopathogen (Kator *et al.,* 2015). It can cause diseases in a wide range of crops, at all stages, including pre-mergence, post-emergence and in adult plant (Gandhi *et al.,* 2017). It is one of the most destructive diseases in crops worldwide. It attacks different parts of hosts under favorable conditions and occurs in the tropics, subtropics and other warm temperate regions of the world. The sclerotia produced by the pathogen, which constitute the primary inoculum, can be easily spread to uninfected areas by different means. Globally, it is estimated that sclerotium rot can cause large losses of 10-20 million dollars and reduce crop yields by 1-60% in the field (Kator *et al.,* 2015) and yield loss increase with increase in disease severity (Tamang and Saha, 2023).

Sclerotium rot generally affects the basal region just above the soil surface. Infected plants exhibit white, cottony mycelial growth in the collar region, which leads to rotting of the stem at the soil level. Girdling starts in the collar region and progresses upward along with mycelia (Tabing *et al.,* 2018). Mycelial networks and white smooth sclerotia that turn brown later on both ground and infected plant parts are conspicuous (Sharma and Sharma, 2008 and Nakkeeran *et al.,* 2018). Eventually, infected plants become pale green and dry, culminating in the death of the stem and plants within 3-5 days (Tabing *et al.,* 2018). Basal rot is largely caused by *Sclerotium rolfsii*, however, Duarte *et al.* (2022) reported that *Fusarium verticillioides* also causes basal rot in carnation in Colombia, implying that it can also be caused by other fungal pathogens.

Carnations (*Dianthus caryophyllus* L.) one of the leading cut flowers in the global floriculture market are susceptible to various fungal diseases that greatly impact their growth and yield. Of numerous fungal diseases reported in carnations, basal rot caused by *A. rolfsii* (basidionym: *S. rolfsii*) is one of the major problems in its cultivation. It is considered one of the most prominent diseases of the carnations and main obstacle in its

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production. Nevertheless, the literature concerning basal rot in carnations from Northeast India (current study region) is still lacking. In fact, the crop, carnations and its cultivation itself is still new to these parts of India, which necessitates an in-depth study of the associated diseases for a better understanding and to prepare growers with better management strategies to protect crops from this deadly disease in the future. Thus, when basal rot was first detected in the experimental plot of Chabaud carnation at Horticultural Research Farm, Assam Agricultural University, Jorhat, Assam (India), during 2023-2024, further studies, incidence, symptoms, morphological and molecular characterization was felt necessary for systematic documentation. Hence, this paper presents details of the incidence of disease, symptoms of the disease and the morphomolecular characterization of the pathogen. To the best of our knowledge, considering the lack of studies on this particular crop in the region, this might be the first such report of Sclerotium basal rot in carnation (*Dianthus caryophyllus* L.) from Northeast India.

MATERIALS AND METHODS

Location of the study

The present study was carried out in the Department of Horticulture and Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, Assam (India) during 2023-2024. The field study was conducted at the Experimental Research Farm of AAU, located at 26°43′40.57″N latitude and 94°12′08.09″E longitude and at 113 MSL, in the Upper Brahmaputra Valley Agroclimatic Zone. The experimental site is characterized by a subtropical climate with humid summers and comparatively dry and cool air during winter and receives uneven rainfall throughout the year, with annual rainfall ranging between 1,875 mm and 2,146 mm. The laboratory study, isolation, culture, microscopic examination of pathogen and pathogenicity test was carried in the Department of Plant Pathology, AAU.

Incidence (%) of *Agroathelia rolfsii*

A month old Chabaud carnation seedlings raised from seeds were transplanted in an experimental plot during the first week of December 2023 and it started bearing flower buds from end of February 2024 and attained full bloom stage in March 2024. In between, incidence of basal rot was noticed from full bud formation stage. Subsequently, the diseased plants infected with *Agroathelia rolfsii* were inspected and examined during the field trial of Chabaud carnation. A total of 525 plants were randomly selected and considered as sample size to study the incidence (%) of the disease during the experimentation. Regular inspection was conducted from January 2024 onward at intervals of 15 days. The infected plants were counted and tagged. The incidence (%) was calculated *via* the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{No. of plants examined}} \times 100$$

Symptoms of basal rot

The symptoms of basal rot in carnation were recorded by closely monitoring and observing each and every plant infected with *A. rolfsii* from the beginning of the symptoms to the death of the plant. The infected plant parts, mycelia, sclerotia and nature of dissemination of the inoculum were observed and recorded. The symptoms were then compared with those reported in the literature on sclerotium rot in carnations and other crops.

Isolation and characterization of pathogens

The diseased plant samples were collected from the experimental plots at the Experimental Research Farm, Department of Horticulture, Assam Agricultural University, Jorhat. The diseased samples were examined for the presence of sclerotia. The pathogens were subsequently isolated *via* the methods described by Damm *et al.* (2012) and Weir *et al.* (2012).

For culture, a portion of tissue from diseased plants was excised with a sterilized blade and then sterilized by dipping it in 1% NaOCl solution for 30 seconds. The plant tissue was rinsed at least three times with sterilized distilled water to remove traces of NaOCl. Excess water was removed and the sample was dried by soaking with sterilized blotting paper. The sterilized cut pieces of tissue were then aseptically transferred to potato dextrose agar (PDA) in Petri dishes (20 ml/Petri dish) and incubated for 3 days at 28±1°C for mycelia formation.

Morphocultural identification

The cultural characteristics, such as the type of margin, texture and colony, of the pathogens were studied on the basis of their growth pattern in PDA media. A slide culture method was used for microscopic examination, in which a 2 cm^2 pathogen block from an isolated plate was inoculated on a glass slide *via* a sterile needle in a moistened petri dish. A sterilized cover slip was placed over the block and incubated for 3 days at 25°C (Rosana *et al.,* 2014). The Coslab View (COSHDUSB 5000) was used for microscopic examination of the pathogen.

Molecular identification

DNA extraction

Seven-day-old fungal cultures were used for genomic DNA extraction by modifying the protocol described by Gul *et al.* (2017). Mycelia were scraped from the plate surface and ground with 200 mg of sterilized quartz sand and 600 µL of 2X CTAB extraction buffer (2% w/v CTAB, 100 mm Tris HCl, 1.4 m NaCl, 20 mm EDTA, pH 8) in a 1.5-mL Eppendorf tube. This was followed by incubation for 60 minutes at 60°C in a water bath with occasional swirling. The samples were subsequently macerated and the resulting homogenized fungal tissues were centrifuged for 10 minutes at 13,000 rpm. The supernatant was collected and transferred to a fresh microcentrifuge tube. RNase A (10 mg/ml) was added to the supernatant and the mixture was incubated at 37°C for 15 minutes. An equal

concentration of phenol:chloroform: isoamyl alcohol at a 25:24:1 ratio was subsequently added and the mixture was mixed properly and centrifuged at 13,000 rpm for 10 minutes. This process was repeated to remove protein and cell debris. The aqueous layer obtained was carefully transferred to a fresh microcentrifuge tube and an equal volume of ethanol (100%) was added. The mixture was allowed to precipitate for at least 3 minutes at -20°C and then centrifuged at 12,000 rpm for 10 minutes to allow the DNA to settle in pellet form. The DNA pellets were then collected, washed with ethanol (70%) and centrifuged for 5 minutes at 12,000 rpm. The DNA was then air-dried for several minutes and dissolved in 1 × TE buffer for further use. Afterwards, the quality and quantity of the DNA pellets were determined *via* 0.8% agarose gel electrophoresis and absorbance at 260 nm, respectively, with a Thermo Scientific NanoDrop 1000 spectrophotometer.

PCR and sequencing

The ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′ -TCCTCCGCTTATTGATATATGC-3′) universal primers were used for PCR amplification of fungal genomic DNA as templates. DNA extraction and PCR amplification were performed following the methods described by Mills *et al.* (1992). The obtained DNA sequences were subjected to analysis *via* the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and similar sequences were obtained and compared with our sequence *via* nucleotide BLAST (basic local alignment search tool); accordingly, a phylogenetic tree was constructed to examine their relationships. The phylogenetic tree was constructed on the basis of the relationships of the 18S rDNA gene sequences and the neighbor joining method with 1000 bootstrap replications.

Pathogenicity test

The pathogenicity test of fungal isolate was performed by following the methods described by Tabing *et al.* (2018) and Mondal *et al.* (2022). A sterilized soil (150 g) was used as the media and was placed in a plastic pot 10 cm in diameter. Healthy and disease-free young seedlings of Chabaud carnation were planted. Some mycelia and sclerotia were inoculated in the soil after light irrigation to provide ambient moisture conditions (room temperature) for fungal growth and one control was maintained without inoculation. Both inoculated and noninoculated pots were replicated five times. The potted plants were kept under controlled conditions at 28±2°C with a 14 h photoperiod and 80-85% RH as described by You *et al.* (2024).

RESULTS AND DISCUSSION

Incidence of *A. rolfsii*

It can be seen from the Table 1 that until January, the plants were free from *A. rolfsii* infection. In the second half of February, when the plants reached the full bud stage, basal rot started to appear, with a small percentage (9.67%) of the plants becoming infected. By the time the plant reached the paintbrush stage (harvesting stage), it became widespread (March) and at the full bloom stage (April), it took aggressive turns and became problematic (25.78%). This increase was aggravated by the downpour of the winter monsoon, which facilitated the transfer of the inoculum to uninfected areas. Notably, by the end of the cropping season, nearly half of the crops were infected with *A. rolfsii.* Similar results of incidence of *S. rolfsii* were reported by Babu and Deepika (2022) in groundnuts (5.39-23.56%) from Andhra Pradesh, whereas Ambika *et al.* (2023) reported a significantly greater incidence (18.89-36.28%) in groundnuts from the Karnataka region.

Symptoms of basal rot caused by *A. rolfsii* in carnation

The first symptoms appeared on the collar region of the stem, just above the ground. The pathogen produced white mycelia and smooth fruiting bodies arised from hyphae that turn brown and resemble the grains of mustard seeds. Later, at the base of the stem, infected parts appear brown and constriction occurs. As a result, the collar region of the plant softens and decays and the entire plant withers and collapses.

Furthermore, the adjoining point of the branches at aerial parts (infected) breaks easily due to severe colonization by the pathogen. In addition, hyphae expanding their network in soil as well as sclerotia can be observed with the naked eye (Fig 1). Disease can spread quickly to uninfected areas, as its sclerotia and mycelia are easily transferred. Similar symptoms of diseases caused by *A. rolfsii* were recently reported by Sikder *et al.* (2024) in sunflower in Bangladesh and Huang *et al.* (2024) in cowpea in China.

Morphocultural characteristics of *Agroathelia rolfsii*

The morphology of the pathogen isolate was examined in PDA culture media (Fig 2). The culture of *A. rolfsii* showed profuse mycelium growth within a day and sclerotia formed within a week of inoculation. Similar results of fast growing nature of mycelia and sclerotia development has been reported by (Srividya *et al.,* 2022). The isolate had cottony whitish and compact mycelia. Initially, sclerotia appear whitish in color and turn into dark brown, mustard, seed-like grains at later stages. The morpho-cultural observations resemble those of Huang *et al.* (2024) and You *et al.* (2024), who reported similar characteristics.

Table 1: Incidence (%) of *A. rolfsii* during the study period.

Period (2024)	Incidence of *A. rolfsii* (%)
1-15th Jan	0.00
16-31st Jan	0.00
1-15th Feb	9.67
16th Feb- 3rd Mar	14.33
4th Mar-19th Mar	18.01
20th Mar-5th Apr	21.47
16th Apr-21st Apr	25.78

The growth rate of mycelia in culture was approximately 25-28 mm/day, which coincides with those of Paparu *et al.* (2020) and You *et al.* (2024), who reported values of 25.2±0.67 and 26.86±0.06 mm/day, respectively. On the other hand, Huang *et al.* (2024) reported comparatively different and slower growth rates of 12.00 to 21.30 mm/day. The microscope view of the isolate is shown in Fig 3.

Molecular identification of *Agroathelia rolfsii*

The fungal isolate was subjected to BLAST from NCBI GenBank and was found to have 92.99% similarity with *Agroathelia rolfsii* (*Agroathelia rolfsii* isolate LTR1, *Agroathelia rolfsii* isolate SrKK17_1090, *Agroathelia rolfsii* strain CSAEGro-CaDIA and *Agroathelia rolfsii* isolate GAHaSr-2) and 93.01% similarity with *Athelia rolfsii* isolate SS-16, a synonym for *Sclerotium rolfsii*. The identification of the pathogen as *A. rolfsii* was confirmed at the Indian Type Culture Collection (ITCC), Department of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi. A phylogenetic tree of the same type was subsequently constructed with bootstrap values, revealing its closeness with the other species of *Agroathelia* (Fig 4). In addition, the DNA sequence of *A. rolfsii* was subsequently submitted to the NCBI Gene Bank and accession no. PP 203038 was obtained (https://www.ncbi.nlm.nih.gov/nuccore/PP203038.1).

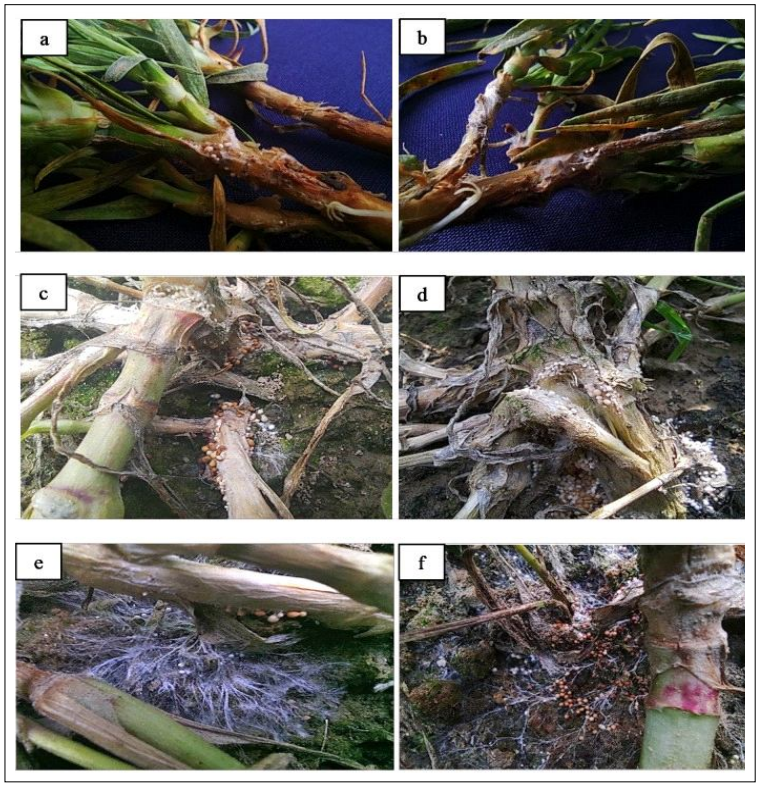
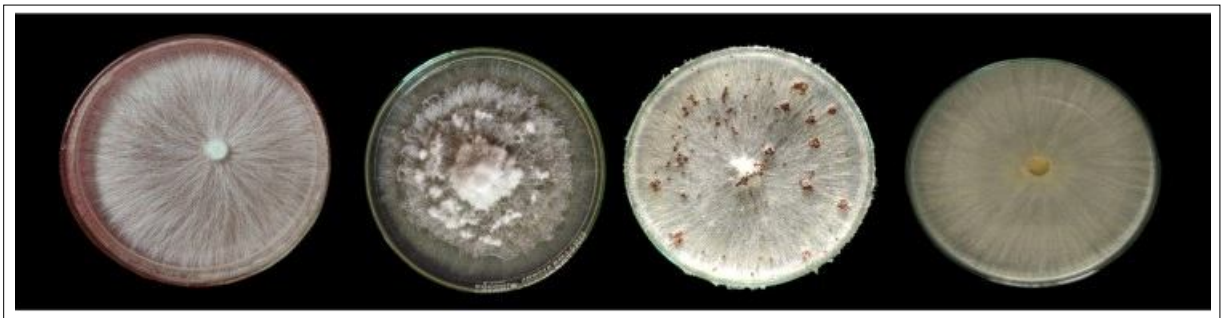


Fig 1: Symptoms of basal rot (*A. rolfsii*) disease in carnation: a and b) Constriction and decaying of the collar region; c and d) split branches and stems exposing sclerotia; e and f) mycelial network on the ground.



a) initial stage, b) cottony mycelium formation, c) culture with sclerotia and d) backside of the culture.

Fig 2: Culture of *Agroathelia rolfsii,* the causal organism of collar rot.

Pathogenicity assay of *A. rolfsii*

The first symptoms of basal rot of Chabaud carnation in the pathogenicity test appeared within one week, which was much earlier than those reported by Tabing *et al.* (2018), who reported 15 days in brinjal. This disparity might be due to the different crops used for the study and the virulency of the pathogen toward the host or the susceptibility of the crop to the pathogen. The noninoculated plants remained disease free and exhibited no signs of any symptoms of basal rot. Thus, the pathogen was reisolated, confirming Koch's postulates. Microscopic examination confirmed the presence of *A. rolfsii*, confirming that it was the causal agent of basal rot in carnation (Fig 5).

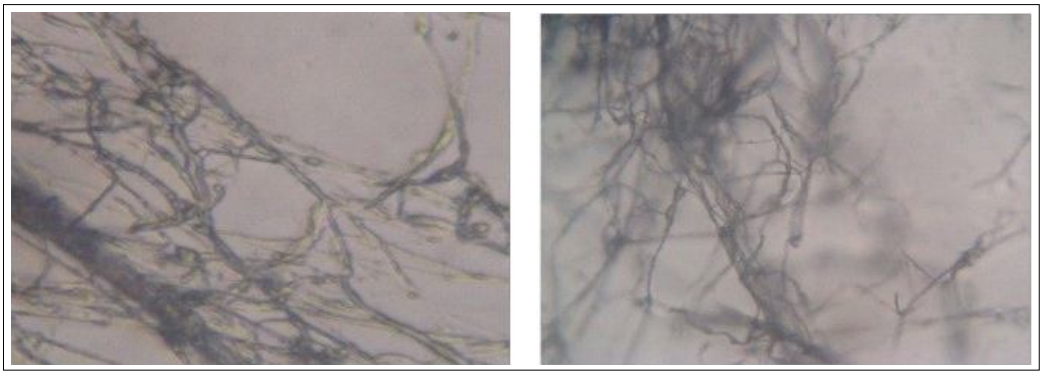


Fig 3: Microscopic view of mycelia of *A. rolfsii.*

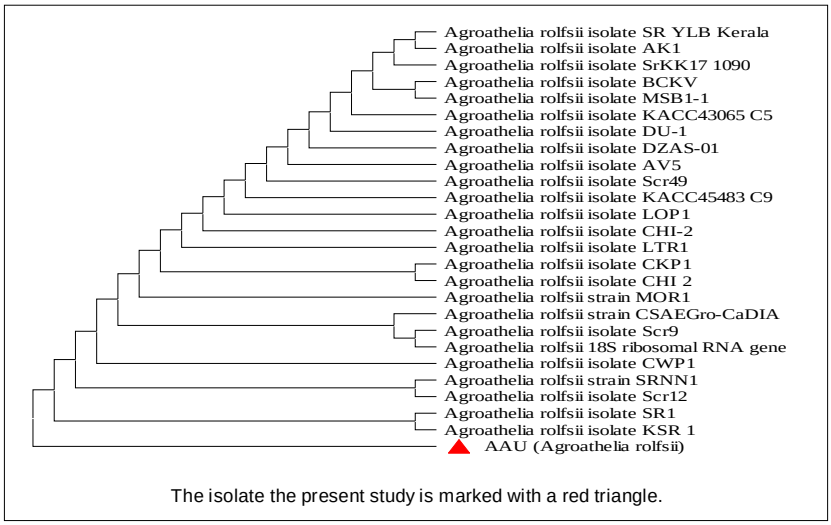


Fig 4: Phylogenetic tree based on the sequences of the ITS region from the *Agroathelia rolfsii* isolate.

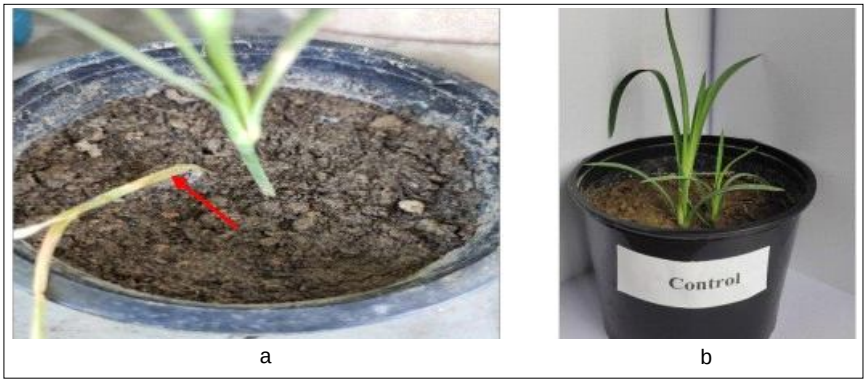


Fig 5: Pathogenicity assay of *A. rolfsii.* a) Close view of inoculated plants with decaying collar region and mycelia, indicated with red arrow. b) Control after 1 week.

CONCLUSION

The present study provides details of the characterization of *A. rolfsii*, a causal agent of basal rot, its incidence, symptoms and morphomolecular identification. Basal rot is destructive and can cause significant economic losses. The incidence of disease started in February and reached the maximum incidence percentage in April (full bloom stage). Pathogens infected plants just above the soil surface, causing constriction that led to decaying of the collar region. Later, it progressed to the aerial parts as well, infecting leaves and branches. Mycelial networks and sclerotia, sizes of mustard grains, were found to be conspicuous in infected plant parts as well as in their surroundings, as they spread quickly and easily and were accentuated and facilitated by the winter monsoon downpour. The culture of the isolate was compact and whitish and produced sclerotia and the mycelia had a fast growth rate. The pathogen was identified molecularly as *Agroathelia rolfsii,* with 92.99% similarity with other *A. rolfsii* isolates. The fungal isolate was submitted to the NCBI GenBank with accession No. PP 203038. Considering the lack of systematic studies of carnation in the northeastern Indian region, we believe that this is probably the first report of basal rot in carnation from the region.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

N/A.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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