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Effect of Nutritional and Environmental Conditions on Growth of *Colletotrichum gloeosporioides* Causing Anthracnose of Anthurium

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Isolates of *Collectotrichum gloeosporioides* causing anthracnose disease of anthurium were collected from five different locations of anthurium growing areas of Tamil Nadu and Kerala and their pathogenicity was established. Effect of different pH level, light intensity, media and carbon

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and nitrogen sources were tested against the growth of *C. gloeosporioides* under *in vitro* conditions. The results of experiment indicated that the growth of *C. gloeosporioides* was maximum in pH range of 6.0-6.5. The exposure of the fungus to alternate cycles of 12 hour light and 12 hour darkness resulted in the maximum mycelial growth of *C. gloeosporioides* compared to continuous light and darkness. Among the different media tested, potato dextrose agar medium increased the growth of mycelium followed by oatmeal agar. The maximum growth of *C. gloeosporioides* was observed in mannitol followed by starch and maltose as carbon source. Among the nitrogen sources, potassium nitrate was found by more mycelial growth of *C. gloeosporioides.*

Keywords: Colletotrichum gloeosporioides; mycelial growth; media.

1. INTRODUCTION

"Anthurium is one of the most valued tropical ornamental plants which has a great export potential as a cut flowers. Anthurium belongs to the genus Anthurium is a native of Central and South America which has over 700 species. Anthurium production has seen a significant increase in recent years, and it is now an important export-oriented crop. However, successful production requires managing various pests and diseases, including bacterial blight, anthracnose, bacterial wilt, root rot, and black nose" [1]. "Among the diseases, anthracnose or spadix rot caused by *Colletotrichum gloeosporioides* (Penz). Penz. & Sacc. in Penz., (Teleomorph *Glomerella cingulata* (Stonem) Spauld and Schreule) is the most important destructive pathogen that causes severe damage, inflicting severe qualitative and quantitative losses" [2,3,4]. "Anthracnose caused severe rotting symptom in anthurium resulting in 100 per cent death of plants" [5]. "Severity of anthracnose of anthurium ranged from 21.67 to 54.89 per cent in Tamil Nadu" [6]. "An array of opportunistic pathogens is debilitating the crop. Fungi are not exempt from it. Fungi usually obtain their nourishment from the substrate, on which they grow and multiply. To culture the fungi artificially in the laboratory, it is essential to provide all the basic nutritional elements easily accessible in the medium. All the media are not equally good for all the fungi nor is there any universal artificial media on which all the fungi can grow" [7]. Understanding the role of environmental conditions and their effect on infection and survival of the pathogen is necessary to develop cultural disease management practices.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Survey was conducted during 2010-2011 at five locations in Tamil Nadu and two places at Kerala to assess the intensity of anthracnose in anthurium. Disease severity was recorded in 0-9 scale [8] and a percent disease index (PDI) was calculated. Infected samples of leaves were collected from these areas.

2.2 Isolation and Identification of Pathogens

"The pathogens causing anthracnose in anthurium were isolated from the collected samples by tissue segment method and they were purified by single spore isolation and maintained on potato dextrose agar (PDA). The causal organisms were identified based on spore morphology" [9].

2.3 Pathogenicity Test

2.3.1 Preparation of spore suspension

The pathogenicity of purified cultures of *C. gloeosporioides* was confirmed by Koch's Postulates. Sporulating cultures of *C. gloeosporioides* were inoculated on PDA and incubated at room temperature (28 ± 2°C) to attain full growth. After incubation, the dishes were flooded with 10 ml of distilled water and the spores were collected using a small brush. The spore suspension was filtered through a six-layer-sterile cheese cloth to remove mycelial debris. Using a haemocytometer spore concentration of *C. gloeosporioides* was adjusted to 5×10^5 spores ml^{-1} respectively with distilled water.

2.3.2 Inoculation

Single leaf inoculation technique was followed and the spore suspension was sprayed using a syringe until run-off on to the leaves of anthurium raised in glasshouse. Such single leaves were covered with polythene bags and symptom expression was observed regularly. Proper controls were also maintained.

2.4 Effect of pH on the Growth of *C. gloeosporioides*

Sterilized PDA medium was distributed in 250 ml Erlenmeyer flasks @100 ml per flask and the pH of the medium was adjusted to p^H levels *viz*., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 with 0.1N HCl or 0.1N NaOH and autoclaved at 1.4 kg cm^{-2} for 20 min. Fifteen ml of the medium from each p^H level was poured onto sterilized Petri dishes and allowed to solidify. The pathogen was inoculated and the mycelial growth was measured after 9 days of inoculation of *C. gloeosporioides*.

2.5 Effect of Light Intensity on the Growth of *C. gloeosporioides*

The effect of light on the growth of the pathogens was studied by exposing them on PDA to alternate cycles of 12 h light, 12 h dark, continuous light and continuous darkness in an environment chamber maintained at 30°C. A mycelial disc of 9 mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in the environment chamber and light intensity was adjusted to the required level. The mycelial growth was recorded 10 days after inoculation.

2.6 Standardization of Culture Media for the Rapid Growth of *C. gloeosporioides*

Cultures of *C. gloeosporioides* were grown on PDA, oatmeal agar, carrot dextrose agar, beetroot dextrose agar, host leaf extract agar, Czapek's Dox agar, Richard's agar, starch agar and Martin's rose bengal agar to standardize the one which supported rapid growth of the pathogen. Sterilized warm medium was poured @15 ml in sterile Petri dishes and the medium was allowed to solidify. The pathogen was inoculated at the centre of the plate by placing a 9-day-old (*C. gloeosporioides*), 9-mm culture disc of the fungus. The plates were incubated at room temperature (28±2ºC) and three replications were maintained for each treatment. The radial growth was measured after 9 days of inoculation of *C. gloeosporioides* [10].

2.7 Growth of *C. gloeosporioides* on Different Liquid Media

Liquid broths *viz.,* potato dextrose, oatmeal, carrot dextrose, beetroot dextrose, host leaf extract, Czapek's Dox, Richard's, starch and Martin's rose bengal were prepared. From the prepared medium 100 ml was distributed in 250 ml Erlenmeyer flasks and autoclaved at 1.4 kg cm^{-2} for 20 min and cooled. The flasks were separately inoculated with 9-day-old (*C. gloeosporioides*), 9-mm culture disc of the pathogen. After the incubation period, the mycelial mat was filtered through pre-weighed Whatman No.1 filter paper, and dried in a hot air oven at 100ºC until constant weight was obtained.

2.8 Effect of Carbon, and Nitrogen Sources on the Growth of *C. gloeosporioides*

Richard's agar medium as well as broth was substituted with different carbon sources *viz.*, starch, mannitol, fructose, glucose, carboxy methyl cellulose (CMC), sucrose, maltose, and nitrogen sources such as ammonium nitrate, ammonium molybdate, ammonium oxalate, peptone, potassium nitrate, sodium nitrate, urea and sterilized. The medium without carbon and nitrogen sources served as control. The colony diameter was measured after 9 days of inoculation of *C. gloeosporioides*.

3. RESULTS AND DISCUSSION

3.1 Occurrence of Anthracnose

A survey was conducted during 2010-2011 to assess the severity of anthracnose in different anthurium growing areas of Tamil Nadu and Kerala. The severity of anthracnose as per cent disease index (PDI) ranged from 12.69 to 44.44 (Table 1). The causal organism isolated was identified as *Colletotrichum gloeosporioides* (Penz) Sacc. based on colony characters and spore morphology.

3.2 Pathogenicity Test

The pathogenicity of *C. gloeosporioides* was confirmed by artificial inoculation. The results indicated that the isolated pathogens reproduced the typical anthracnose symptoms in anthurium. The pathogens were reisolated from infected tissues and Koch's postulates were fulfilled.

3.3 Hydrogen Ion Concentration and the Growth of *C. gloeosporioides*

In our investigation, the ideal pH observed for culturing *C. gloeosporioides* was 7.0 with a mean mycelial growth of 8.90 cm followed by pH 6.5 (8.60 cm) and 6.0 (8.45 cm). The growth was

very slow at pH 4.0 with a mean colony diameter of 3.90 cm (Fig. 1). The observation is in agreement with the results of [6,11] who also had observed the maximum growth of the fungus at pH 7.0 followed by pH 6.0 which was preferred for sporulation. Gina [12] identified pH 6.0 as optimum for the growth of *C. gloeosporioides*.

3.4 Light Intensity and the Growth of *C. gloeosporioides*

Diurnal light when compared to continuous light and darkness was conducive for the growth of *C. gloeosporioides*. Exposure of the *C. gloeosporioides* to alternate cycles of 12 h light and 12 h darkness for 10 days yielded maximum mycelial growth and dry weight of the pathogen (8.80 cm and 448 mg). The mycelial growth and dry weight of the fungus when exposed to continuous light was 7.45 cm and 410 mg respectively. Continuous darkness recorded 6.50 cm growth and 338 mg dry mycelial weight respectively (Fig. 2). Similarly [13,14,15,16] observed that exposure of *C. gloeosporioides* to alternate cycles of 12 h light and 12 h darkness yielded maximum biomass and spores. Ravi Malipatil et al. [7] studied the mycelial growth of fungus each at five different range of temperatures (15, 20, 25, 30 and 35°C). Significantly, maximum mycelial growth of 88.63 mm and excellent sporulation was observed at 25°C followed by on par growth (86.65 mm) at 30°C and significantly least mycelial growth of 21.74 mm was recorded at 15°C. Excellent sporulation was observed at 25 and 20°C and moderate sporulation was noticed at 15, 30 and 35°C.

Table 1. Occurrence of anthracnose/spadix rot in anthurium growing areas of Tamil Nadu and Kerala

Sl. No.	Isolate code	Location	Variety	Disease severity (PDI)	Symptoms
1	CI_1	Shade net house, TNAU	Lady jane	44.44	Dark brown margin with grey brown centre on leaves
2	CI_2	Wyanad	Temptation	25.39	Spadix rot
3	CI_3	Munnar	Sweet orange	15.87	Small to irregular brown spots on leaves
4	CI_4	Yercaud	Temptation	20.63	Spadix rot
5	CI_5	Thadiyankudisai	Lady jane	12.69	Dark brown margin with grey brown centre on leaves

PDI: Percent disease index

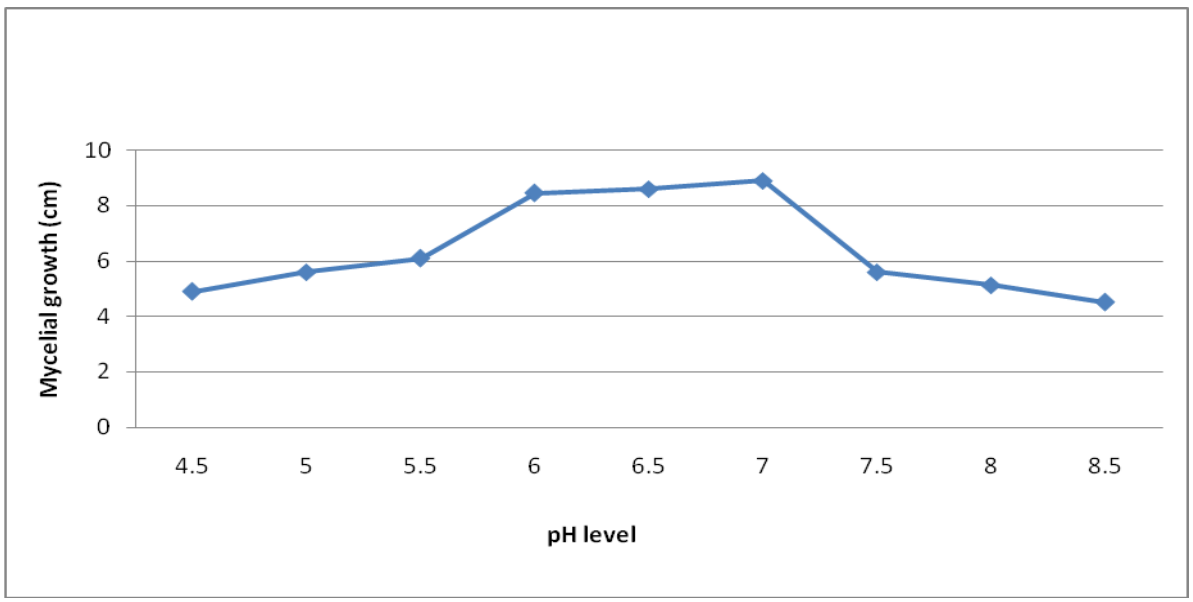

Fig. 1. Effect of Hydrogen ion concentration on the growth of *C. gloeosporioides*

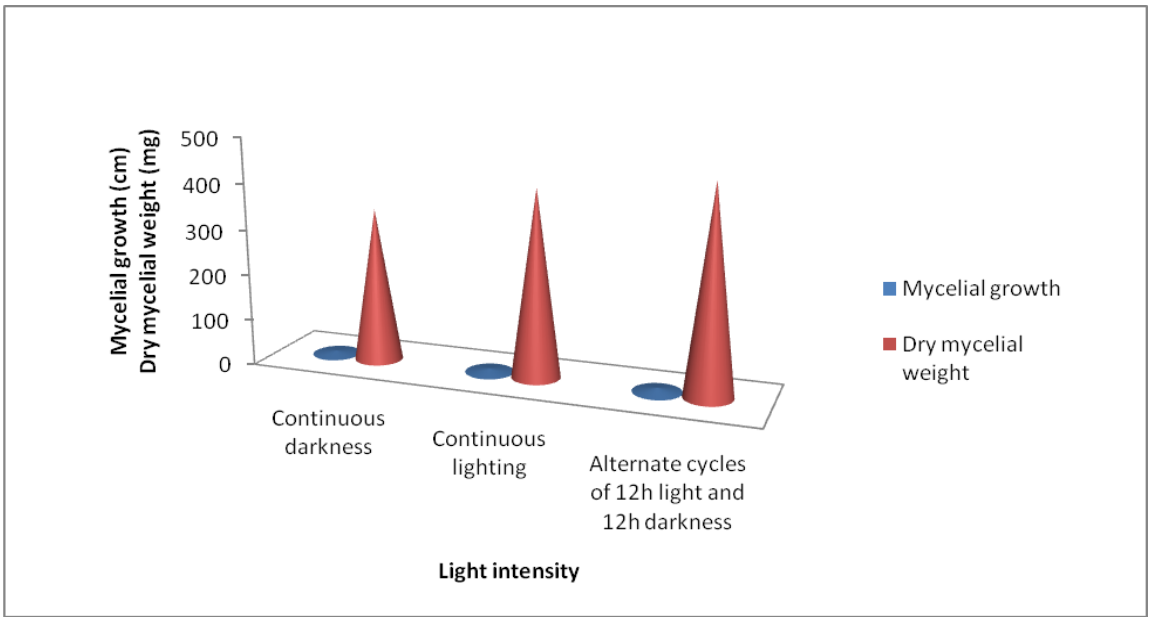

Fig. 2. Effect of light intensity on the growth and dry mycelial weight of of *C. gloeosporioides*

3.5 Culture Media for the Growth of *C. gloeosporioides*

"Every living being requires food for its growth and reproduction and fungi are not an exception to it. Fungi secure food and energy from the substrate upon which they live in nature. To culture fungus in the laboratory, it is necessary to furnish essential elements and compounds in the medium for their growth and other life processes. All media are not equally good for all fungi, nor there can a universal substrates or artificial media upon which all fungi grow well. So, different media including both synthetic and non-synthetic media were tried for *C. gloeosporioides* in the present investigation" [17].

Among the nine different media tested, PDA significantly supported the maximum growth of *C. gloeosporioides* with a mean mycelial growth of 9.00 cm which was on par with oatmeal agar wherein the fungal growth was 8.90 cm (Table 2). Lowest mycelial growth was recorded in Martin's rose Bengal agar (4.60 cm). Nine different liquid media were used for culturing *C. gloeosporioides* (Fig. 3). Among them oatmeal broth significantly was the best by recording maximum dry mycelial weight of the fungus (394 mg). These results conform with the findings of [18] who recorded the maximum growth of *C. gloeosporioides* of anthurium on PDA. Similarly, [19] also observed that the growth of *C. gloeosporioides* from chillies was more on PDA. Tasiwal and Benagi [16] recorded the maximum mycelial weight of the fungus in Richard's broth. Among the seven media tested for the growth of *C. gloeosporioides*, host leaf extract recorded the maximum mean colony diameter followed

Table 2. Growth of *C. gloeosporioides* on different solid media

Sl. No.	Media	Mycelial growth (cm)
		***C. gloeosporioides* (9 DAI)**
1	Potato dextrose agar	9.00
2	Oatmeal agar	8.90
3	Carrot dextrose agar	5.20
4	Beetroot dextrose agar	5.30
5	Host leaf extract agar	7.20
6	Czapek's Dox agar	6.10
7	Richard's agar	7.80
8	Starch agar	6.80
9	Martins rose Bengal agar	4.60
CD (0.05)		1.05

DAI - days after incubation

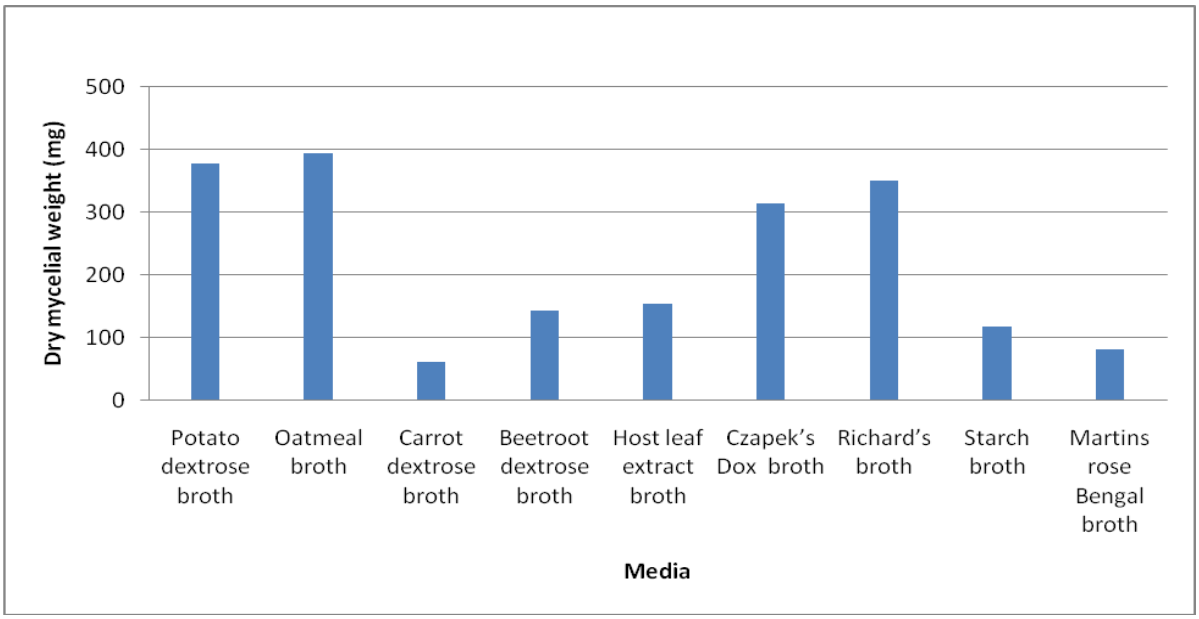

Fig. 3. Growth of of *C. gloeosporioides* on different liquid media

by PDA and corn meal agar [6]. Manjunath et al. [20] observed that host leaf extract agar followed by PDA supported the maximum growth of *C. gloeosporioides* that causes anthracnose of noni.

3.6 Carbon Sources and the Growth of *C. gloeosporioides*

"Carbon occupies a unique position among the essential elements required by the living organisms. Carbon utilization is speculated to be dependent upon enzyme system. The utilization of various carbon compounds may depend either on the activity of the fungus to utilize certain simple forms or on its power to convert the complex carbon compounds into simple forms which may be utilized. As a component of both structural and functional constituents carbon comprises about 50% of the total dry mycelia weight in fungi". Bilgrami and Verma [21] Monosaccharides are the better sources for the growth and sporulation of the pathogen. The fungus *C. gloeosporioides* varied in its ability to utilize different carbon sources. *C. gloeosporioides* grew well in all the carbon sources tested (Table 3). Mannitol ranked first in promoting the mycelial growth of the pathogen with a mean growth of 8.90 cm. It was followed by starch (8.80 cm), maltose (8.70 cm) and fructose (8.50 cm) that were at par with mannitol. Our results are in agreement with the observations by [22-24]. Chaturvedi [25] also noticed that starch and fructose induced more growth and sporulation in *C. gloeosporioides* than other carbon sources. Sucrose was found to be a better carbon source followed by glucose and dextrose for the growth of betel vine anthracnose pathogen C. *gloeosporioides* [26]. Hegde et al. [27] observed dextrose and sucrose as good carbon sources for *C. gloeosporioides* isolated from arecanut. Enhanced mycelial growth and sporulation of bean anthracnose pathogen *C. gloeosporioides* was recorded in starch added medium while poor sporulation was recorded in galactose and lactose [28].

3.7 Nitrogen sources and the Growth of *C. gloeosporioides*

"The fungi for their structural and functional processes use nitrogen, like carbon. Nitrogen is very important element in the protein synthesis. But all the sources of nitrogen are not equally good for the growth of all fungi" [29]. Purkayastha and Sengupta [30] "found that peptone, casamino acid and potassium nitrate were favourable for both mycelial growth and sporulation of *C. gloeosporioides* the incitant of jute anthracnose. *C. gloeosporioides* of mango utilized potassium nitrate more efficiently while ammonium nitrate was used less effectively for its growth and sporulation" [31]. In the present investigation, Potassium nitrate was found to promote more mycelial growth in *C. gloeosporioides* with a mean colony diameter of 8.70 cm and least growth was observed in urea (4.20 cm) (Table 4). Saxena [32] "reported potassium nitrate as the best source for growth

Table 3. Effect of carbon sources on the growth of *C. gloeosporioides*

Sl. No.	Carbon source	Mycelial growth (cm)
		***C. gloeosporioides* (9 DAI)**
1	Starch	8.80
2	Mannitol	8.90
3	Fructose	8.50
4	Glucose	8.30
5	Carboxy methyl cellulose	8.10
6	Sucrose	8.00
7	Maltose	8.70
8	Control	1.20
CD (0.05)		0.59

DAI - days after incubation

Table 4. Effect of nitrogen sources on the growth of *C. gloeosporioides*

Sl. No.	Nitrogen source	Mycelial growth (cm)
		***C. gloeosporioides* (9 DAI)**
1	Ammonium nitrate	6.60
2	Ammonium sulphate	8.10
3	Ammonium oxalate	4.50
4	Peptone	6.40
5	Potassium nitrate	8.70
6	Sodium nitrate	8.20
7	Urea	4.20
8	Control	1.40
CD (0.05)		0.49

DAI - days after incubation

and sporulation of *C. gloeosporioides* isolated from betelvine and pomegranate". Deshmukh et al. [28] observed that maximum growth and sporulation of *C. gloeosporioides* causing anthracnose of bean was recorded in potassium nitrate.

4. CONCLUSION

The results of present study are concluded that the *Colletotrichum gloeosporioides* (Penz) Sacc. was confirmed as the causal agents of anthracnose of anthurium based on colony characters, spore morphology and pathogenicity. *C. gloeosporioides* were found to grow well in pH ranging from 6.0 to 7.0. Exposure of *C. gloeosporioides* to alternate cycles of 12 h light and 12 h darkness yielded maximum mycelial growth and mycelial dry weight of the pathogens. Among the culture media tested, Potato dextrose agar and oat meal broth was found to promote maximum growth and dry weight of *C. gloeosporioides.* mannitol as carbon sources and potassium nitrate as sources of nitrogen were found to be equally effective for the growth of *C. gloeosporioides*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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