


# Tryptophol Coating Reduces Catheter-Related Cerebral and Pulmonary Infections by *Scedosporium apiospermum*

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**Introduction:** Central venous catheter (CVC) is a medical device that is used to administer medication for a long duration. Colonization by an emerging opportunistic pathogen *Scedosporium apiospermum* in the CVC lumen is frequently reported to cause severe complications in patients. Here, we describe the effect of fungal quorum-sensing molecule (QSM) known as tryptophol (TOH) to control *S. apiospermum* colonization in catheter tube lumens in both in vitro and in vivo models.

**Methods:** Antifungal susceptibility of TOH against *S. apiospermum* was compared with voriconazole, and the colony diameter was determined on days 2, 4, and 6. Experimental catheterization rat model was conducted with pre-coating of TOH and voriconazole or an uncoated control and an infection with *S. apiospermum*. Biofilm formation on the catheter luminal surface was assessed using the scanning electron microscopy, crystal violet, and 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-(phenylamino)-carbonyl-2H-tetra-zolium hydroxide (XTT) reduction assays. Brain and lung samples of catheterized rats were histopathologically assessed. Serum samples from catheterized rats were injected into *Galleria mellonella* larvae. Survival of catheterized rats and *G. mellonella* was determined.

**Results:** TOH impeded the growth of *S. apiospermum* by reducing the colony diameter in a dose-dependent manner. TOH coating remarkably lessened *S. apiospermum* biofilm formation and fungal cell viability on the catheter luminal surface. Additionally, TOH coating lessens cerebral edema that is associated with abscess and invasive pulmonary damages due to *S. apiospermum* catheter-related infection. Furthermore, TOH coating also lessened the virulence of *S. apiospermum* in sera of experimental catheterized rats and extended the survival rate of larvae *Galleria mellonella* infection model.

**Conclusion:** An alternative modification of catheter by coating with TOH is effective in preventing *S. apiospermum* colonization in vivo. Our study gives a new strategy to control catheter contamination and prevents nosocomial diseases due to *S. apiospermum* infection.

**Keywords:** *Scedosporium apiospermum*, central venous catheter, quorum-sensing molecules, tryptophol, fungal biofilms, antifungal susceptibility, scedosporiosis, *Galleria mellonella*

## Introduction

Fungal infections in indwelling catheters, especially in long-term central venous catheters (CVC), are believed to be a chief cause of mortality among hospitalized patients.<sup>1</sup> Several studies showed various images of microorganisms growing in the contaminated site as a biofilm environment. It is interesting to note that results from electron microscopy have proposed the correlation between biofilm-related

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microorganisms on the surface of contaminated catheters and soft-tissue injuries.<sup>2</sup> Therefore, biofilm contamination on the luminal surface of the catheter can cause a high risk on human health for those who depend on the use of intravascular and other medical devices.

*Scedosporium apiospermum* was firstly isolated in Sardinia as the etiological cause of white mycetoma.<sup>3,4</sup> *Scedosporium apiospermum* was once considered to be the anamorph of *Scedosporium boydii* (formerly known as *Pseudallescheria boydii*). Until 2005, these two species were proven to be distinctly different species based on molecular, pathological, and biochemical data.<sup>5</sup> There is evidence that *S. apiospermum* is involved in opportunistic infections. In immunosuppressed patients, *S. apiospermum* can be the causative agent of invasive infections, which extremely spread to cause disseminated diseases.<sup>6</sup> In Thailand, *S. apiospermum* has been reported in brain abscesses of near-drowning and renal transplant patients.<sup>7,8</sup> Our previous study showed that *S. apiospermum* species complex can be found in soils across Bangkok and other major provinces of Thailand, with the predominance detection of *S. apiospermum sensu stricto*.<sup>9–11</sup> Furthermore, the increased incidence of *S. apiospermum* infections was reported to be because of the extensive use of corticosteroids, immunosuppressants, antineoplastics, broad-spectrum antibiotics, and indwelling catheters including long-term CVC.<sup>1,12–16</sup> In some cases, *S. apiospermum* infections following medical intervention were isolated from the insertion site of a catheter, indicating a significant evidence of nosocomial diseases.<sup>13</sup>

The recent study has exhibited that *S. apiospermum* can form biofilms on the surface of both polystyrene and polyurethane, a material of CVC.<sup>13,17</sup> *Scedosporium* spp. are demonstrated to be fundamentally resistant to antifungal amphotericin B and frequently respond more effectively to voriconazole.<sup>18</sup> On the contrary, biofilms formed by *Scedosporium* spp. are highly resistant to the azole class of antifungal drugs, such as caspofungin, fluconazole, itraconazole, and voriconazole,<sup>16,18</sup> although voriconazole, which has been used as the first-line antifungal drug, complications including severe phototoxicity, visual impairment, and elevated liver enzymes have been reported.<sup>19</sup> Because of antifungal resistance and complications, novel antifungal agents for prevention and/or control of *Scedosporium* spp. colonization in catheter tube lumens are poorly studied.

Fungal quorum-sensing molecules (QSMs) are signaling molecules that are used to communicate and/or control

behavior among fungi, bacteria, and other microorganisms.<sup>20</sup> Tryptophol (C<sub>10</sub>H<sub>11</sub>NO, TOH; molecular weight, 161.20) is a QSM that can be isolated from *Candida albicans* as well as *Saccharomyces cerevisiae*.<sup>20</sup> In *S. cerevisiae*, TOH is a synthesized alcohol that is derived from aromatic amino acid tryptophan through the Ehrlich pathway under a low-nitrogen condition.<sup>21</sup> In *C. albicans*, TOH was found to be an auto-antibiotic by inhibiting filamentation.<sup>22</sup> Our present study showed that TOH suppressed *C. albicans* biofilm formation by inducing a programmed cell death.<sup>23</sup> We also found that pretreatment of *C. albicans* with TOH remarkably lessened the pathogenicity and virulence of *C. albicans* infection in larvae of *Galleria mellonella*. Even though TOH holds a promising antifungal property, little is known about whether TOH can be utilized as an antifungal agent against *Scedosporium* spp. Furthermore, the effect of TOH for controlling *S. apiospermum* colonization in catheter tube lumens has not been explained. Thus, the current study's objective is to determine the antifungal effect of TOH against *S. apiospermum* infection. In addition, we explored the use of TOH as a coating agent for controlling catheter contamination and preventing nosocomial diseases due to *S. apiospermum* infection.

## Materials and Methods

### Ethical Statement

All animal experiments in this study were carried out in accordance with the Animals for Scientific Purposes Act, B.E. 2558 (A.D. 2015), in Thailand. For rat experiments, all animal experiments were performed with assistance from Dr. Nichapa Sansurin at Northeast Laboratory Animal Center (NELAC), Khon Kaen University, Thailand and were approved by the Institutional Animal Care and Use Committee (IACUC) of Khon Kaen University, Thailand (approval number: 0514.1.75/34). For *Galleria mellonella* experiments, all relevant international, national, and institutional guidelines for the care and use of *Galleria mellonella* were followed, and the study was approved by the research ethics committee of Mahidol University, Thailand (approval certificate number: MU-IACUC 2018/015).

### Fungal Strains and Growth Conditions

*Scedosporium apiospermum* CBS 117410 utilized in this study was kindly provided by Dr. Ana Alastruey-Izquierdo (Servicio de Micología, Instituto de Salud Carlos III,

Madrid, Spain).<sup>10</sup> *Scedosporium apiospermum* CBS 117410 was grown on Sabouraud dextrose agar (SDA) (Oxoid, Hampshire, UK) slant for 5 days at 37°C. Conidia were collected by washing with sterile phosphate-buffered saline (PBS, pH 7.2) and adjusted to a concentration of 10<sup>5</sup> conidia/mL.

## Analysis of Tryptophol (TOH) on *S. apiospermum* Growth

A stock solution of 1 M tryptophol (TOH) was prepared by dissolving TOH in ethanol. The stock solution was then diluted to the desired concentration with the use of SDA. Twenty microliters of *S. apiospermum* CBS 117410 conidia suspension were placed in each well of a six-well plate containing serial dilutions (1, 10, 100, and 1000 µM) of TOH (Sigma-Aldrich, St Louis, MO) and serial dilutions (1, 10, 100, and 1000 µM) of voriconazole (Sigma-Aldrich, St Louis, MO) in SDA. The plate was incubated at 25°C, and the colony morphology was observed, and the colony diameter was measured on days 2, 4, and 6. The plates were monitored for 10 days. Wells that contain 1% (v/v) ethanol (TOH diluent) and TOH-free SDA were also included as controls for TOH experiments. In the case of voriconazole, groups that were prepared with voriconazole-free SDA and 1% (v/v) RPMI (voriconazole diluent) were used as controls.

## In vivo Experimental Catheterization and Treatments

A sterilized polyethylene tube (1–1.5 mm in diameter) was utilized as a catheter in this study. The catheters were pre-coated with either 10 and 100 µM of tryptophol (TOH) or voriconazole overnight. Experimental catheterization rat model was conducted in female Mlac/Wistar rats (2-week-old, 350g of body weight, and 10 rats per group). Following the quarantine period, rats were anesthetized using an intraperitoneal injection (IP) (1 mg/kg) of a mixture of ketamine HCl 500 mg/10 mL at a concentration of 80 mg/kg (Bedford Laboratories, Bedford, OH) and xylazine at concentration of 8 mg/kg (Sigma-Aldrich, St Louis, MO), with a ratio of 1:2 (vol/vol). Next, the surgical incision was performed at the anterior neck just right to the midline, and a pre-coated catheter was inserted into the jugular vein.<sup>24,25</sup> The pre-coated catheters were placed 24 h prior to infection, allowing a conditioning period for deposition of host protein on the catheter surface. The inoculum of *S. apiospermum*

CBS 117410 at a concentration of 10<sup>8</sup> conidia/mL was instilled in the pre-coated catheter in a volume not greater than 700 µL (the entire catheter volume). Several inoculum conditions were instilled as follows: Group 1, pre-coated with TOH and inoculated with *S. apiospermum* CBS 117410; Group 2, pre-coated with voriconazole and inoculated with *S. apiospermum* CBS 117410; Group 3, uncoated catheter with inoculation of *S. apiospermum* CBS 117410; and Group 4, uncoated catheter with no *S. apiospermum* CBS 117410 inoculation. The inoculum was permitted to stabilize in the catheter lumen for 6 h, after which the catheter volume was withdrawn and the catheter was flushed and locked with sterile heparinized 0.85% NaCl. This infection was observed within 12 and 48 h. At the end point, the animals were sacrificed by CO<sub>2</sub> asphyxiation. After sacrificing, the catheter samples were aseptically collected. Blood samples were then collected into Eppendorf tubes and permitted to clot for 30 min, followed by centrifugation at 3000 g for 10 mins at 4°C to collect the serum fraction. Catheterized rat sera and their control sera were utilized for additional treatments with *Galleria mellonella*.

To determine the presence of *S. apiospermum* CBS 117410 in TOH-coated catheter samples, the fungal elements were carried out following treatment with 15% potassium hydroxide (KOH). After 48 h post-inoculation of *S. apiospermum* in catheterization rats, the catheter samples were inoculated on SDA (Oxoid, Hampshire, UK) and incubated at 25°C with daily observation for fungal colonies. After 30 days, the tubes with no visible fungal colonies were discarded as negative. The identification of genera and species of the fungal colonies was carried out with the use of lactophenol cotton blue staining.

## Analysis of *S. apiospermum* Biofilm Development by H-Score

To validate the presence of *S. apiospermum* germination inside the catheter lumen, a fine morphological study was conducted as formerly described.<sup>26</sup> The catheter was cut perpendicular to the catheter length (as doughnut segments). The catheter segments were primary fixed in 2.5% glutaraldehyde for 1 h at room temperature and then washed with 0.1 M sucrose phosphate buffer (SPB) thrice. The catheter samples were secondary fixed in 1% osmium tetroxide in SPB and then washed again. The tissues were dehydrated in a series of ethanol solutions

and air-dried overnight. The catheter segments were mounted on an aluminum stub and coated with a gold film (20 nm-thickness) using a sputter coater (Emitech K550, Ashford, UK). The luminal surface of catheter samples was imaged using a scanning electron microscope (JEOL JSM-6610LV, Japan) with 15 kV acceleration voltages.

Semi-quantitative examination of *S. apiospermum* CBS 117410 biofilm formation in the luminal surface of experimented catheters was measured using the H-score assay. The H-score was acquired from the biofilm coverage area (in percentage) and then multiplied by the score of biofilm thickness (1 = one layer, 2 = two layers, and 3 = three or more layers of biofilm thickness) using the ImageJ program (National Institutes of Health, Bethesda, MD, USA).

### Analysis of *S. apiospermum* Biofilm Development by Crystal Violet (CV) Staining

*Scedosporium apiospermum* CBS 117410 biofilm formation in the luminal surface of experimented catheters was assessed further with the use of CV staining as formerly described with some alterations.<sup>26</sup> The TOH-coated catheters and their controls were washed 2 times with PBS, air-dried, and then stained with 110  $\mu$ L of 0.4% aqueous CV solution for 45 min. The catheter samples were washed 5 times with 350  $\mu$ L sterile distilled water and destained with 200  $\mu$ L of 95% ethanol for 45 min. The destaining solution in each sample (100  $\mu$ L) was collected and then transferred into a new 96-well flat-bottom microtiter plate. The absorbance values were measured at 595 nm using a microtiter plate reader (Tecan, Sunrise, Austria). The absorbance values of treated groups were subtracted from those of the control groups to eradicate the background interference.

### Analysis of *S. apiospermum* Biofilm Development by XTT Reduction Assay

*Scedosporium apiospermum* CBS 117410 biofilm formation in the luminal surface of experimented catheters was examined using tetrazolium salt 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-(phenylamino)-carbonyl-2H-tetrazolium hydroxide or XTT (Sigma-Aldrich, St Louis, MO) reduction assay.<sup>27</sup> The XTT-menadione solution was freshly prepared by mixing the XTT solution (1 mg/mL in PBS) with the menadione solution (0.4 mM in acetone) at a 5:1 (v/v) ratio.<sup>28</sup> 12  $\mu$ L of the XTT-menadione

solution and 200  $\mu$ L of PBS were added to the 96-well microtiter plate containing catheter samples and incubated in the dark for 2 h at 37°C. Next, 100  $\mu$ L of the supernatant solution was transferred to a new microtiter plate, and absorbance values were measured at 490 nm. An arithmetic mean of the absorbance values was calculated. Any absorbance background was eliminated by subtracting the absorbance values of the control groups and the experimental groups.

### Histopathology Analysis

Histopathology was used to determine the severity of brain and lung impairments after *S. apiospermum* infection. Dissected brains and lungs from catheterized animals were collected and fixed in 10% neutral buffer formalin for 48 h. All tissue samples were dehydrated and embedded in paraffin. All the brain and lung sections (5  $\mu$ m) were stained with hematoxylin and eosin or Gomori methenamine silver (GMS) and examined under a light microscope. In the brain sections, the pathological changes were chiefly focused on microabscesses, perivascular cuffing with lymphocytes, perivascular edema, meningitis, and hemorrhage. In the lung sections, the pathological changes were chiefly focused on hemothorax, inflammatory zones, fibrosis, and granules. The amount of change in each histologic disease activity previously mentioned was scored on a four-tier system as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. Overall, the histopathologic score was calculated by the combination of each histological change.<sup>29</sup>

### *Galleria mellonella* and Maintenance

*Galleria mellonella* larvae were provided from Jerry<sup>®</sup> Wax Worms (Thailand). Larvae were grown on a bedding medium that consisted of bran, all-purpose flour, dried bakery yeast, liquid honey, and glycerol at room temperature in plastic boxes with grids prior to experiments. During the last-stage larvae, the worms that stopped feeding and started to produce a thin silk body cover were chosen, which were monitored approximately 2 months following the hatching from their eggs. At this stage, *G. mellonella* larvae with 200–400 mg body weight and lightly colored cuticles with no gray areas were chosen and transferred to sterile Petri dishes for 24 h with no food. Furthermore, their nascent protective cocoons were removed.<sup>23</sup>



**Table 1** Colony Diameters of Tryptophol (TOH)-Treated *Scedosporium apiospermum* CBS 117410 Following Incubation at 25°C

| Fungal Strain/Culture Condition       | Colony Diameter in mm (Mean ± SD) |                          |                          |
|---------------------------------------|-----------------------------------|--------------------------|--------------------------|
|                                       | Day 2                             | Day 4                    | Day 6                    |
| SDA                                   | 9.1 ± 0.71                        | 15.5 ± 0.83              | 21.0 ± 0.64              |
| SDA + RPMI                            | 9.9 ± 1.03                        | 17.7 ± 0.49 <sup>a</sup> | 21.0 ± 1.10              |
| SDA + 1 μM voriconazole               | 5.2 ± 0.98 <sup>a</sup>           | 13.6 ± 0.29 <sup>b</sup> | 15.0 ± 0.33 <sup>a</sup> |
| SDA + 10 μM voriconazole              | No growth <sup>b</sup>            | 5.4 ± 0.86 <sup>c</sup>  | 10.1 ± 0.74 <sup>b</sup> |
| SDA + 100 μM voriconazole             | No growth <sup>b</sup>            | No growth <sup>d</sup>   | 8.0 ± 0.55 <sup>c</sup>  |
| SDA + 1000 μM voriconazole            | No growth <sup>b</sup>            | No growth <sup>d</sup>   | 1.9 ± 0.74 <sup>d</sup>  |
| SDA                                   | 8.5 ± 0.95                        | 16.5 ± 0.47              | 21.0 ± 0.64              |
| SDA + ethanol                         | 8.0 ± 0.88                        | 14.7 ± 0.13 <sup>a</sup> | 21.0 ± 0.13              |
| SDA + 1 μM TOH                        | 7.6 ± 1.04 <sup>a</sup>           | 12.9 ± 0.09 <sup>b</sup> | 21.0 ± 0.09              |
| SDA + 10 μM TOH                       | 3.1 ± 0.37 <sup>b</sup>           | 10.0 ± 0.23 <sup>c</sup> | 15.5 ± 0.35 <sup>a</sup> |
| SDA + 100 μM TOH                      | No growth <sup>c</sup>            | 3.9 ± 0.91 <sup>d</sup>  | 13.1 ± 0.06 <sup>b</sup> |
| SDA + 1000 μM TOH                     | No growth <sup>c</sup>            | No growth <sup>e</sup>   | 9.8 ± 0.34 <sup>c</sup>  |
| Colony grown from catheter samples    |                                   |                          |                          |
| Inoculum of <i>S. apiospermum</i>     | 7.8 ± 0.19                        | 15.7 ± 0.34              | 20.9 ± 0.16              |
| Non-coated catheter                   | 6.2 ± 1.11                        | 11.8 ± 1.47 <sup>a</sup> | 19.1 ± 0.81              |
| Voriconazole-coated catheter (100 μM) | 2.3 ± 1.06 <sup>a</sup>           | 9.3 ± 0.79 <sup>b</sup>  | 13.6 ± 0.44 <sup>a</sup> |
| TOH-coated catheter (100 μM)          | 1.9 ± 0.15 <sup>b</sup>           | 4.2 ± 0.81 <sup>c</sup>  | 11.9 ± 1.17 <sup>a</sup> |
| Colony grown from sera samples        |                                   |                          |                          |
| Non-coated catheter                   | 4.5 ± 0.94                        | 10.9 ± 2.30              | 17.9 ± 1.46              |
| Voriconazole-coated catheter (100 μM) | 2.7 ± 0.31 <sup>a</sup>           | 7.0 ± 0.67 <sup>a</sup>  | 12.7 ± 1.14 <sup>a</sup> |
| TOH-coated catheter (100 μM)          | 2.1 ± 0.35 <sup>a</sup>           | 6.3 ± 0.55 <sup>a</sup>  | 11.1 ± 1.61 <sup>a</sup> |

Note: <sup>a-e</sup>Indicates that  $p < 0.05$  is statistically significant in each experimental condition by two-way ANOVA.

## Survival Analysis of *S. apiospermum*-Infected Rat Serum on *G. mellonella*

Moth larvae were split into three treatment groups, each with nine larvae. Larvae were anesthetized by placing at 5–8°C in Petri dishes for 1 day and then injected with sera acquired from *S. apiospermum*-infected, catheterized rats in several types of coated catheters such as TOH, voriconazole, and uncoated controls. In brief, moth larvae were held carefully between the thumb, index finger, and middle finger, and the injection area was cleaned with the use of 70% ethanol. Sera from catheterized rats that were collected at 12 and 48 h following catheterization were injected into the larvae immediately. Sera were injected into the larval hemocoel through the last proleg with the use of a 30G single-use sterile syringe. The larvae were further incubated in Petri dishes with no food at 37°C in the dark for survival analysis.

The survival measurements of treated larvae were monitored every day following post-injection until all worms died. Larvae were counted as dead when there

was no response to gentle stimulation with forceps and the cuticle color became totally black.

## Statistical Analysis

Each experiment was carried out in triplicate. The survivals of *G. mellonella* in all treatments were assessed using GraphPad Prism. Kaplan–Meier lifespan analysis was carried out, and  $p$ -values  $< 0.05$  were significantly different with the use of the Log-rank test. Data were presented as the mean ± standard deviation (SD). In other assays, significant differences at the  $p$ -values  $< 0.05$  were calculated by two-way ANOVA or independent Student's  $t$ -tests.

## Results

### Effect of Tryptophol (TOH) on *S. apiospermum* Growth

Colonies of *S. apiospermum* CBS 117410 were observed following 2 days of incubation. SDA media containing either 1% RPMI (v/v) or 1% ethanol (v/v) were utilized as controls for voriconazole or tryptophol (TOH), respectively. For voriconazole treatment, the colony diameter increased

from day 2 to day 4 in the same manner in 1% RPMI-treated group as in the colonies grown on SDA alone (Table 1). Treatment of voriconazole at the concentration ranging from 1 to 10  $\mu\text{M}$  remarkably lessened the growth rate of *S. apiospermum* CBS 117410. Nonetheless, the colony diameter remarkably lessened following treatment with moderate-to-high concentrations of voriconazole (100–1000  $\mu\text{M}$ ,  $p < 0.05$ ). Therefore, voriconazole at 100  $\mu\text{M}$  was chosen for further subsequent experiments. For TOH treatment, the colony diameter increased from day 2 to day 4 in the same manner with the 1% ethanol-treated group as in the colonies grown on solely SDA (Table 1). Treatment of TOH at the concentration ranging from 1 to 10  $\mu\text{M}$  remarkably lessened the growth rate of *S. apiospermum* CBS 117410. Additionally, the colony diameter was remarkably lessened following treatment with 100–1000  $\mu\text{M}$  of TOH ( $p < 0.05$ ). Therefore, TOH at 100  $\mu\text{M}$  was chosen for further subsequent experiments.

## General Animal Well-Being and Catheter Site Observations

Following catheterization, rats appeared well in the 96 h period of study (24 h pre-inoculation and up to 72 h post-inoculation). The surgical site and catheter exit site in all studied animals still had no signs of inflammation or purulence.

## Catheter Biofilm Imaging

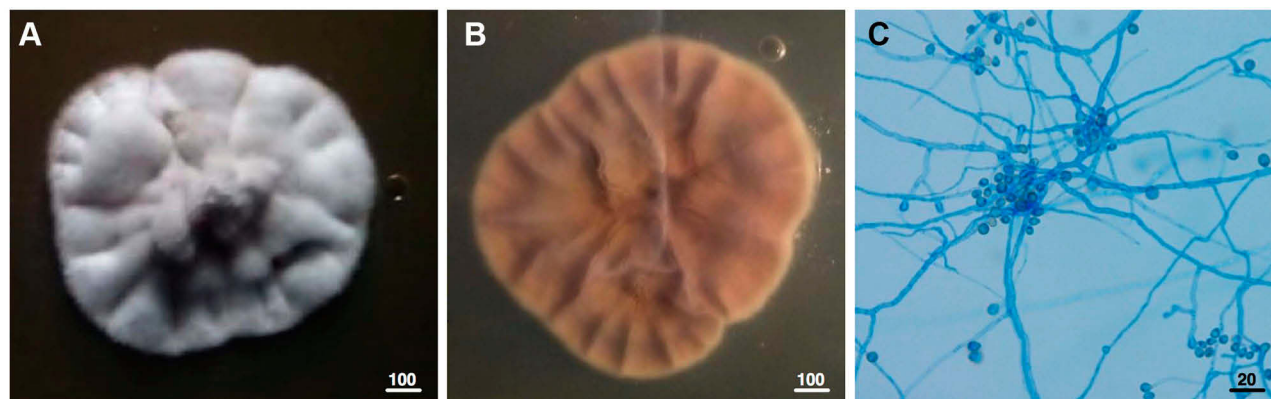
Catheter samples following 48 h post-injection with *S. apiospermum* CBS 117410 had a fungal growth on SDA. In an uncoated catheter, the colony diameter of *S. apiospermum* increased from day 2 to day 6 similar to the *S. apiospermum* inoculum alone (Table 1). Pre-coated catheter with voriconazole (100  $\mu\text{M}$ ) remarkably lessened the

*S. apiospermum* colony diameter from day 2 to day 6 in comparison to the uncoated control catheter ( $p < 0.05$ ). Nonetheless, pre-coated catheter with TOH (100  $\mu\text{M}$ ) remarkably lessened the *S. apiospermum* colony diameter from day 2 to day 4 in comparison with voriconazole-coated catheter (Table 1,  $p < 0.05$ ).

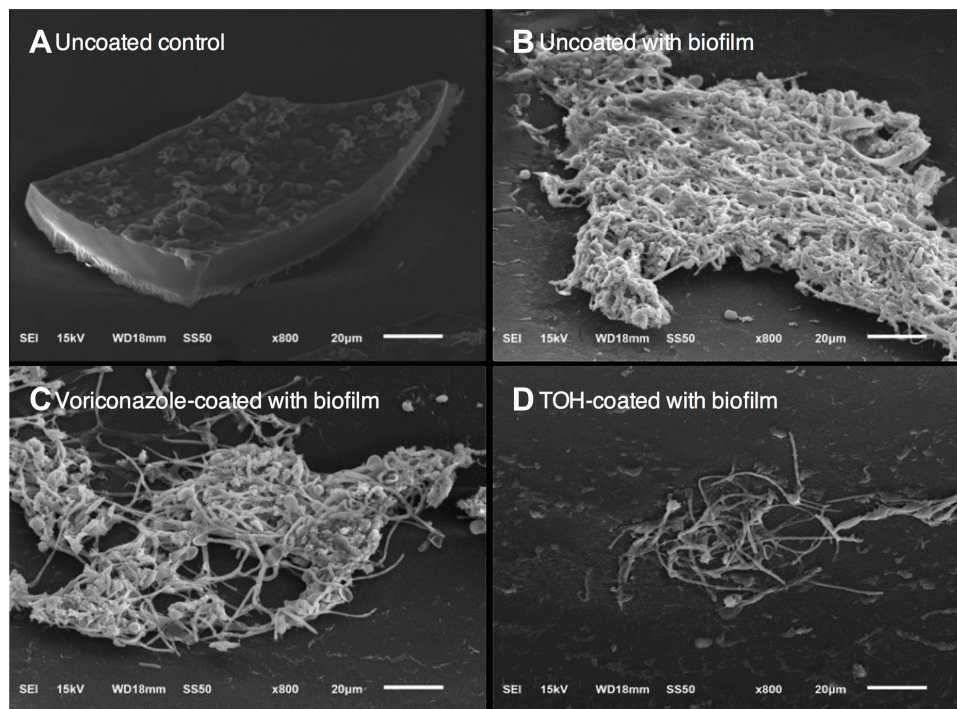
Colony and microscopic pictures of the *S. apiospermum* from TOH-coated catheter samples are shown in Figure 1A and B, and C using lactophenol cotton blue staining. Fungal biofilm on the luminal surface of the catheter segments was clearly visualized using a scanning electron microscopy (SEM). Uninoculated catheter with no infection was utilized as the control (Figure 2A). *Scedosporium apiospermum* biofilm was observed following 48 h post-injection from the top of the intraluminal biofilm surface (Figure 2B–D). Mature biofilm was characterized by both yeast and hyphal cell forms on the extracellular matrix networks. Reduction in *S. apiospermum* biofilm was observed in catheter lumen following treatments with 100  $\mu\text{M}$  of voriconazole or TOH. Nevertheless, it was clear that biofilm was remarkably lessened in the catheter coating with TOH in comparison with voriconazole (Figure 2C and D).

## Effect of TOH on *S. apiospermum* Biofilm Development

The fungal biofilm on the luminal surface of the catheter segments was further evaluated with SEM observations using the H-score determination. H-Scores were calculated using the percent coverage area of biofilm multiplied with the layer score of the *S. apiospermum* CBS 117410 biofilm. In the current study, the means of H-scores in uncoated catheter, voriconazole-coated-catheter (100  $\mu\text{M}$ ), and TOH-coated catheter (100  $\mu\text{M}$ ) were slowly decreased in a time-dependent



**Figure 1** Culture of *S. apiospermum* CBS 117410 on Sabouraud Dextrose Agar (SDA), (A) front view, (B) reverse view, and (C) macroconidia of *S. apiospermum* stained by lactophenol cotton blue. Scale bar of A, B = 100  $\mu\text{m}$  and C = 20  $\mu\text{m}$ .



**Figure 2** Coating catheter with Tryptophol (TOH) significantly reduced the intraluminal biofilm formation of *Scedosporium apiospermum* CBS 11741 ( $10^8$  conidia/mL) after 48 h post-inoculation as observed under a scanning electron microscope (SEM). Scanning electron micrograph in the luminal surface of (A) uncoated catheter, (B) uncoated catheter with *S. apiospermum*, (C) voriconazole-coated-catheter (100  $\mu$ M) with *S. apiospermum*, and (D) TOH-coated-catheter (100  $\mu$ M) with *S. apiospermum*. Scale bar (A–D) = 20  $\mu$ m.

manner from 12 h to 48 h post-inoculation. At 12 h post-inoculation, the results showed that the mean H-score of biofilm development in TOH-coated-catheter ( $88.66 \pm 18.09$ ) remarkably lessened in comparison with that in the uncoated catheter ( $316.39 \pm 42.63$ ,  $p < 0.05$ ) but not with voriconazole-coated catheter (Figure 3A,  $158.07 \pm 29.58$ ,  $p > 0.05$ ). Furthermore, the mean H-score of biofilm development in TOH-coated-catheter ( $137.29 \pm 16.74$ ) remarkably lessened in comparison with that in the uncoated catheter ( $296.64 \pm 35.41$ ) and voriconazole-coated catheter ( $234.11 \pm 55.03$ ) following 48 h post-inoculation (Figure 3A,  $p < 0.05$ ). The results proposed that TOH can be utilized as a coating agent to lessen the biofilm formation of *S. apiospermum* CBS 117410.

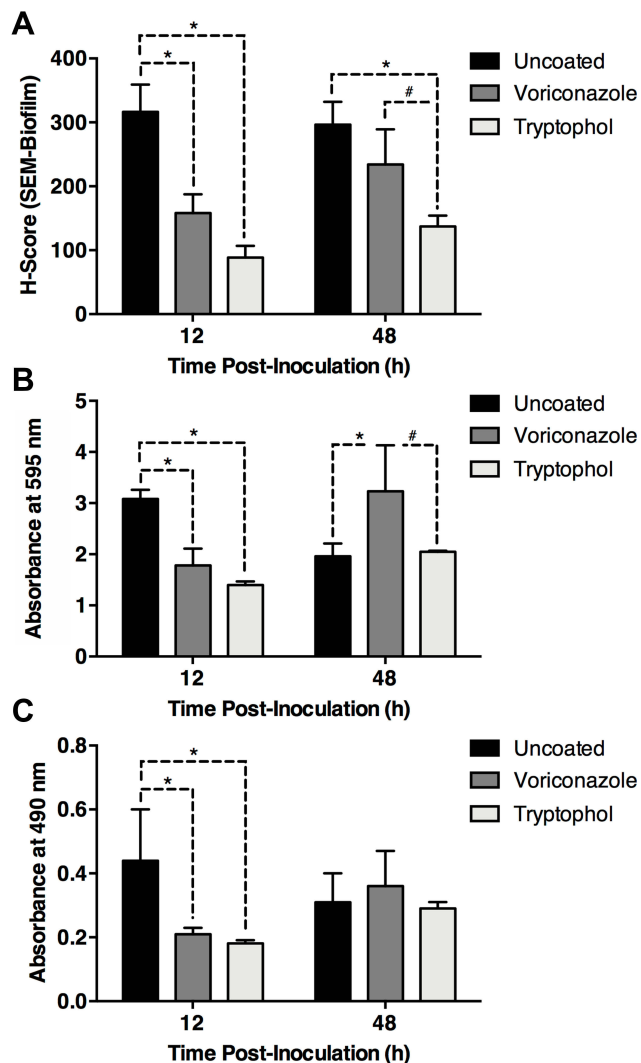
Fungal biofilm on the luminal surface of the catheter segments was further examined with the use of CV staining. At 12 h post-inoculation, the mean of A595 in TOH-coated catheter (100  $\mu$ M) ( $1.40 \pm 0.07$ ) was remarkably lessened in comparison with that in the uncoated catheter ( $3.08 \pm 0.18$ ,  $p < 0.05$ ) but not voriconazole-coated catheter (100  $\mu$ M) (Figure 3B,  $1.78 \pm 0.33$ ,  $p > 0.05$ ). At 48 h post-inoculation, the mean of A595 in TOH-coated catheter ( $2.05 \pm 0.07$ ) was remarkably lessened in comparison with that in the uncoated catheter ( $1.96 \pm 0.25$ ,  $p < 0.05$ ). Nonetheless, the mean

of A595 in voriconazole-coated catheter ( $3.23 \pm 0.90$ ) was remarkably increased in comparison with that in the uncoated catheter (Figure 3B,  $p < 0.05$ ). Therefore, the results proposed that TOH can be utilized as a coating agent to reduce the biofilm formation of *S. apiospermum* CBS 117410.

Biofilm metabolic activity on the luminal surface of the catheter segments was further examined with the use of XTT reduction assay. At 12 h post-inoculation, the mean of A490 in TOH-coated catheter (100  $\mu$ M) ( $0.18 \pm 0.01$ ) was remarkably lessened in comparison with the uncoated catheter ( $0.44 \pm 0.16$ ,  $p < 0.05$ ) but not voriconazole-coated-catheter (100  $\mu$ M) (Figure 3C,  $0.21 \pm 0.02$ ,  $p > 0.05$ ). At 48 h post-inoculation, however, the means of A490 in uncoated catheter, voriconazole-coated-catheter, and TOH-coated-catheter were not statistically significant (Figure 3C,  $0.31 \pm 0.09$ ,  $0.36 \pm 0.11$ , and  $0.29 \pm 0.02$ , respectively,  $p > 0.05$ ). Therefore, the results proposed that TOH can be utilized as a coating agent to reduce the biofilm formation of *S. apiospermum* CBS 117410.

## Survival Time

Following *S. apiospermum* CBS 117410 inoculation in the catheter lumen for 6 h, experimental catheterized rats were



**Figure 3** Biofilm of *Scedosporium apiospermum* CBS 11741 ( $10^8$  conidia/mL) in the luminal surface of tryptophol (TOH)-coated catheter after 12 and 48 h post-inoculation as examined by (A) H-score, (B) crystal violet, and (C) XTT assays. Results are presented as mean  $\pm$  standard deviation (SD). \*,#Indicated as significant difference at  $p < 0.05$  by 2-way ANOVA.

further monitored within 12 to 48 h. Severe neurological symptoms, moribund, and death were seen in *S. apiospermum*-infected rats. However, rat mortality rate of 100% was noted in the uncoated catheter group, while TOH-coated and voriconazole-coated catheter groups have shown a mortality rate of 40% and 50%, respectively (data not shown).

## Histopathological Changes of the Brain and Lungs

*Scedosporium apiospermum* CBS 11741 infection in rats with an uncoated catheter prompted severe pathological changes in the brain in comparison with TOH-coated catheter groups as presented in Figure 4. Following 48

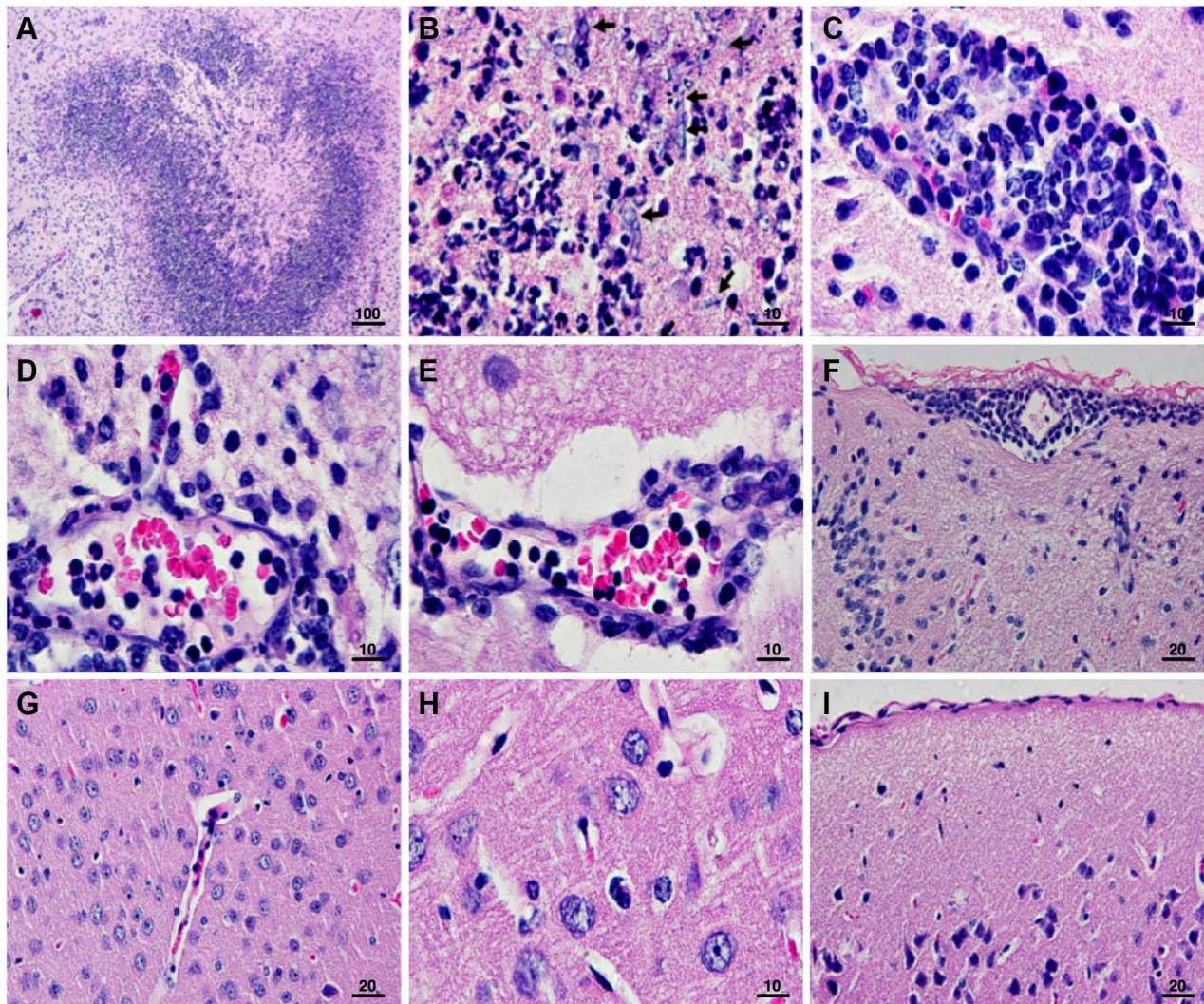
h post-inoculation, histopathological studies of brains from the uncoated catheter group showed meningitis, hemorrhage, brain abscess surrounded with edema, and cerebritis with no encapsulation (Figure 4A–F). The histological tissues had *S. apiospermum* hyphae (Figure 4B) and perivascular cuffing principally with neutrophil and mononuclear infiltrates around the site of infection (Figure 4B–E) and in the meninges (Figure 4F). The infected brain lesions have also shown a central necrotic area surrounded with inflammatory cells, unlike the brain with no abscess (Figure 4G–I). Additionally, the number of brain abscesses was examined in the cerebral cortex, midbrain, hippocampus, and hypothalamus. Nevertheless, olfactory bulb, diencephalon, and cerebellum did not present any abscess.

The lungs of *S. apiospermum* CBS 11741-infected rats with an uncoated catheter also prompted severe pathological changes in comparison with TOH-coated catheter groups as presented in Figure 5. Invasive pulmonary scedosporiosis was characterized by severe hemorrhage (hemothorax) in experimental rat from the uncoated catheter group (Figure 5A and B). Furthermore, large inflammatory zones, fibrosis, and granules were noted in uncoated catheter groups (data not shown). The infected lung lesions in the uncoated catheter groups have also shown the presence of inflammatory cells such as lymphocytes and macrophages unlike the TOH-coated catheter groups (Figure 5C and D). Histopathological studies of brains and lungs from voriconazole-coated catheter groups displayed similar to TOH-coated catheter groups (data not shown), proposing that TOH can be utilized as a coating agent, which exhibited antifungal effects similar to voriconazole. The presence of *S. apiospermum* hyphae in brains and lungs from uncoated catheter groups was also validated with the use of GMS staining (Supplementary Figure 1).

## Effect of *S. apiospermum*-Infected Rat Serum on the Survival of *G. mellonella*

Colonies of *S. apiospermum* CBS 11741 from infected rat sera in TOH-coated catheter, voriconazole-coated catheter, and uncoated catheter groups were observed following 48 h post-infection on SDA. The colony diameter of *S. apiospermum* from rat sera of the uncoated catheter group increased from day 2 to day 6 (Table 1). It is interesting to note that the colony diameter of *S. apiospermum* from rat sera of TOH-coated catheter





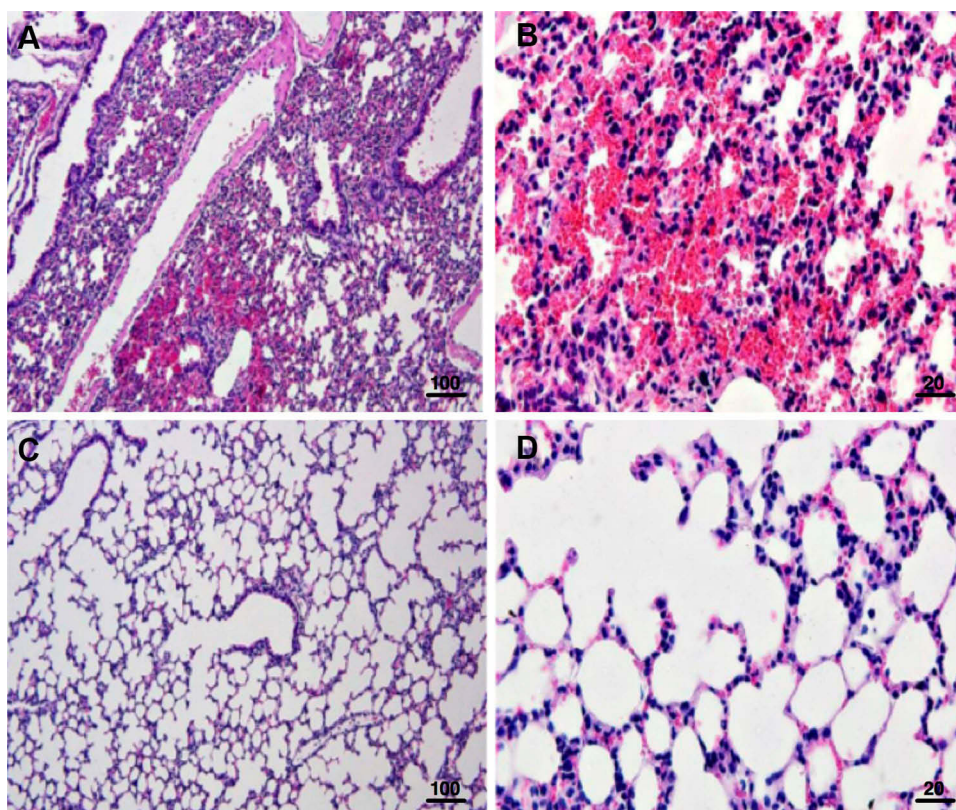
**Figure 4** Brain histopathological studies in catheterization rats with *Scedosporium apiospermum* CBS 11741 infections after 48 h post-inoculation. In uncoated catheter groups, (A) brain abscess was developed without encapsulation. (B–E) The perivascular cuffing mainly with neutrophil infiltrates were found around the site of infection with the presence of *S. apiospermum* hyphae (as indicated by the arrows in B) and (F) in meninges. (G–I) Unlike the brain without abscess in tryptophol (TOH)-coated catheter groups, absent of necrotic area surrounded with inflammatory cells were noted compared to infected brains. Scale bar = 100, 20, and 10  $\mu$ m.

(100  $\mu$ M) group was remarkably smaller than the uncoated catheter group ( $p < 0.05$ ) similar to the voriconazole-coated catheter (100  $\mu$ M) group from day 2 to day 6 (Table 1).

The ability of TOH to lessen the *S. apiospermum* infection was further determined in *G. mellonella*. *Scedosporium apiospermum*-infected rat sera from TOH-coated, voriconazole-coated, and uncoated catheter groups were injected in *G. mellonella* larvae, and the survival rate was determined. Normal saline (0.9%) was injected in *G. mellonella* as a control. Following 12 h post-injection, the mean lifespan of normal saline treatment (144 h) was remarkably highest among other treated groups (Table 2).

However, rat sera from TOH-coated catheter (100  $\mu$ M) remarkably increased the mean lifespan ( $106.40 \pm 22.45$  h, 35.34%,  $p < 0.0001$ ) in comparison to sera from voriconazole-coated catheter (100  $\mu$ M) ( $84.00 \pm 25.79$  h, 22.09%,  $p < 0.05$ ) or uncoated catheter ( $68.80 \pm 20.65$  h) (Figure 6A, Table 2). Following 48 h post-injection, the mean lifespan of normal saline treatment ( $132.00 \pm 16.97$  h) was remarkably highest among other treated groups (Table 2). However, rat sera from TOH-coated catheter remarkably increased the mean lifespan ( $94.40 \pm 29.52$  h, 131.37%,  $p < 0.0001$ ) in comparison to sera from voriconazole-coated catheter ( $72.00 \pm 25.21$  h, 76.47%,  $p < 0.0001$ ) or uncoated catheter ( $40.80 \pm 15.63$  h)





**Figure 5** Lung histopathological studies in catheterization rats with *Scedosporium apiospermum* CBS 11741 infections after 48 h post-inoculation. In uncoated catheter groups, **(A)** severe hemorrhage (haemothorax) and **(B)** the presence of inflammatory cells including lymphocytes and macrophages were seen. In tryptophol (TOH)-coated catheter groups, **(C and D)** absent of severe hemorrhage and inflammatory cells were noted. Scale bar = 100 and 20 µm.

(Figure 6B, Table 2). These findings indicated that TOH-coated catheter lessens the pathogenicity of *S. apiospermum* infection in *G. mellonella*.

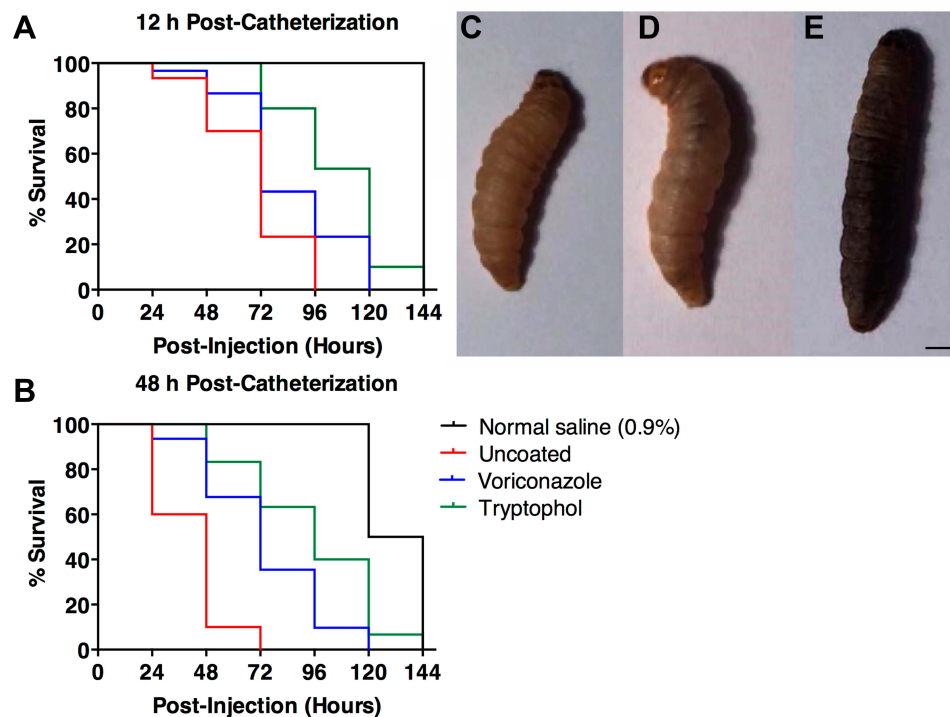
The *S. apiospermum* infection was also correlated with the skin melanization of *G. mellonella* larvae. The cuticle color of *G. mellonella* that is injected with *S. apiospermum*-infected rat sera from the uncoated-catheter group was stated to change from lightly

colored to dark within 2–8 h post-injection. An accumulation of melanin was initially seen following 1–2 h post-infection (Figure 6C and D), and it became totally black near the time of death (Figure 6E). Consistent with the mortality results, however, larvae injected with *S. apiospermum*-infected rat sera from TOH-coated catheter group showed delay melanization (12–24 h) in comparison with voriconazole-coated catheter group (6–12 h).

**Table 2** Effect of *S. apiospermum*-Infected Rat Serum on the Survival of *G. mellonella*

| Experimental Group   | Mean   | SD    | SE    | Median | Max    | No. | p-value |      | %      |
|--|--------|-------|-------|--------|--------|-----|---------|------|--------|
| Normal saline (0.9%) <sup>a</sup>                                | 144.00 | 0.00  | 0.00  | 144.00 | 144.00 | 30  | <0.0001 | **** |        |
| Rat sera from uncoated catheter <sup>a</sup>                     | 68.80  | 20.65 | 3.77  | 72.00  | 96.00  | 30  |         |      |        |
| Rat sera from voriconazole-coated catheter (100µM) <sup>a</sup>  | 84.00  | 25.79 | 4.71  | 72.00  | 120.00 | 30  | 0.0309  | *    | 22.09  |
| Rat sera from TOH-coated catheter (100 µM) <sup>a</sup>          | 106.40 | 22.45 | 4.09  | 120.00 | 144.00 | 30  | <0.0001 | **** | 35.34  |
| Normal saline (0.9%) <sup>b</sup>                                | 132.00 | 16.97 | 12.00 | 132.00 | 144.00 | 30  | <0.0001 | **** |        |
| Rat sera from uncoated catheter <sup>b</sup>                     | 40.80  | 15.63 | 2.85  | 48.00  | 72.00  | 30  |         |      |        |
| Rat sera from voriconazole-coated catheter (100 µM) <sup>b</sup> | 72.00  | 25.21 | 4.60  | 72.00  | 120.00 | 30  | <0.0001 | **** | 76.47  |
| Rat sera from TOH-coated catheter (100µM) <sup>b</sup>           | 94.40  | 29.52 | 5.39  | 96.00  | 144.00 | 30  | <0.0001 | **** | 131.37 |

**Notes:** Means, standard deviation (SD), standard error (SE), median, and maximum of lifespan were shown in hours. Total worms were represented as the summation of worms in triplicates (with censored worms excluded). The lifespan data were assessed using the Log-rank test, and the p-values for each individual experiment were shown, which are compared to the corresponding control as \*p < 0.05 and \*\*\*\*p < 0.0001 and not significant (ns). <sup>a</sup>Results presented in Figure 6A. <sup>b</sup>Results presented in Figure 6B.



**Figure 6** *S. apiospermum* infected rat sera from TOH-coated catheter extended *G. mellonella* lifespan. Data represented percentages of survival of *G. mellonella* larvae treated with *S. apiospermum* infected rat sera after (A) 12 h post-injection and (B) 48 h post-injection. A percentage of survival in each condition was also compared to normal saline (0.9%). (C) *G. mellonella* larvae showed melanized in 1–2 h post-injection. (D) Melanization of *G. mellonella* larvae cuticle was darker and (E) completely black near the time of death. Scale bar = 1 mm. Lifespan was analyzed using Kaplan–Meier analysis and *p*-values were calculated using Log-rank test. Statistical details of lifespan are summarized in Table 2.

## Discussion

The nosocomial outbreak caused by *Scedosporium* spp. in immunocompromised hosts and in transplant recipients has been continually increased over the past decade.<sup>6,30</sup> In spite of the antifungal therapy, mortality rates of patients with *Scedosporium* infections are still incredibly high as 50–70%.<sup>31</sup> Several studies have proposed that *S. apiospermum* infections are linked to intravenous catheters, injections, or blood collection.<sup>13,16,32–35</sup> The disease pathogenesis of catheter-related *Scedosporium* spp. infections is complex. Various factors have been highlighted in the disease pathogenesis such as the duration of catheter placement, catheter materials, and the pathogen vs host properties.<sup>36</sup> Thus, the strategy to remove fungal biofilm on the catheter lumen is urgently needed to prevent or cure catheter-related fungal infections.

Here, we reported that a fungal QSM known as tryptophol (TOH) hampered the growth of *S. apiospermum* CBS 117410 in a concentration-dependent manner. A moderate concentration (100  $\mu$ M) of TOH remarkably hampered the growth of *S. apiospermum*. Furthermore, TOH appeared to be as effective as voriconazole, a standard antifungal drug for *Scedosporium* spp. infection.<sup>8,37</sup> Our current study

showed that *S. apiospermum* manifested an ability to form biofilms on the surface of catheter lumen, which is similar to prior reports on the biofilm formation covering polystyrene surfaces.<sup>17</sup> *Scedosporium apiospermum* biofilm formation in the catheter was remarkably lessened when the catheter was pre-coated with TOH rather than voriconazole. Quantifications of *S. apiospermum* biofilms were performed using H-scores, CV staining, and XTT reduction assays. The results demonstrated that TOH appeared to be effective in controlling *S. apiospermum* biofilm formation than known antifungal agents of voriconazole. CV staining and reduction of XTT are used to explain all the cells in the biofilm community and determine cell viability as the reduction of formazan by mitochondrial dehydrogenase in living cells.<sup>27,28</sup> Our results showed that biofilm bulk and level of XTT metabolic activity in TOH-coated catheter were lower than in voriconazole-coated catheter following 48 h post-inoculation, proposing that cell viability in the biofilm community was lessened when coating catheter with TOH. Furthermore, TOH has been reported to lessen filamentation in *Aspergillus* spp. and in *C. albicans*.<sup>23,38</sup> Therefore, our study gives the use of QSM of TOH for surface coating

to prevent biofilm formation in the catheter lumen. However, the molecular mechanisms of anti-biofilm in TOH against *S. apiospermum* infection are still to be further studied in depth.

Degree severity of *Scedosporium* infection has been thoroughly studied, showing a wide range from mild lymphocytotoxic (LC) infections to severe life-threatening disseminated infections involving the central nervous system (CNS).<sup>39</sup> Invasive *S. apiospermum* infection in distant organs including the brain and lungs was noted in the experimental rat with uncoated catheter in our study. Invasive CNS infections were characterized by cellular damages in various areas of the meninges, cerebral cortex, midbrain, hippocampus, and hypothalamus. We also found that pulmonary infection by *S. apiospermum* was observed consistently with CNS infection in the uncoated catheter group. Several inflammatory areas, hemothorax, fibrosis, and granules in pleural cavity and lung tissues, characterized as invasive pulmonary scedosporiosis, were also observed in the experimental rat in the uncoated catheter group and were resolved in the TOH-coated catheter group. Additionally, a mortality rate of 100% in the uncoated control group was lessened to 50% in the TOH-coated catheter group. The catheter from catheterization further determined the presence of *S. apiospermum*. The growth of *S. apiospermum* from TOH-coated catheter was lessened in comparison to that from the voriconazole-coated catheter. Furthermore, the similar phenomenon of *S. apiospermum* growth inhibition was noted in the culture of rat sera acquired from the TOH-coated catheter group.

It is possible that conidia of *S. apiospermum* on the catheter lumen may enter the brain and lungs through the bloodstream.<sup>6</sup> These conidia are germinated, causing hyphal invasion in the target organs. Prior study has shown that *S. apiospermum* generates a 33-kDa extracellular serine protease peptidase, which degrades human fibrinogen, proposing that it has a role in lung invasion and inflammation.<sup>40</sup> Apart from that, *S. apiospermum* has been reported to display a siderophore activity, making it iron dependent.<sup>41</sup> Therefore, it may also explain *S. apiospermum* neurotropism because the CNS has free iron unlike the serum. *Scedosporium* spp. can tolerate temperature between 37°C and 42°C and easily adapt themselves to a wide variety of environmental stresses such as osmotic stress, halo-tolerance, and low-oxygen environments.<sup>39,42</sup> Furthermore, *Scedosporium* frequently shows resistance in antifungal therapy, elucidating their rapid hematogenous dissemination, which affected very distant organs and render among high percentages of blood cultures.<sup>34</sup> Even though the precise mechanism of how

*Scedosporium* passes the blood-brain-barrier still is unknown, one hypothesis illuminated that alveolar macrophages infected with *Scedosporium* conidia may be the principal cause of disease pathogenesis. *Scedosporium*-infected macrophages can pass the blood-brain-barrier, and then conidia succeedingly germinate and damage brain tissue. This hypothesis was well recognized, as macrophages are generally known vehicles for eukaryotic pathogens such as *Leishmania* and *Toxoplasma* that can pass through the blood-brain-barrier.<sup>43</sup> Our recent study also showed that *S. apiospermum* infection induced severe cerebral edema correlated with abscess in immunocompromised mice. Elevation of tumor necrotic factor- $\alpha$  and downregulation of aquaporin-4 and nuclear factor erythroid-2 related factor in neuronal cells and myelin degeneration induced by *S. apiospermum* were found in non-treated mice, proposing the cause of water imbalance and oxidative stress which lead to cerebral edema and abscess.<sup>29</sup> Thus, our results proposed that a pre-coating catheter with TOH lessens the risk of cerebral and pulmonary complications due to *S. apiospermum* catheter-related infection.

Biofilm formation and fungal germination are considered to be important risk factors in patients with a life-threatening systemic infection, especially in the long-term use of inserted devices including *S. apiospermum*-caused catheter-related infections.<sup>13</sup> Contaminated catheter and infected sera with *S. apiospermum* can foster fungal growth and therefore create a continuous cycle of reinfection, permitting a great potential to cause opportunistic infection in healthcare units. By pre-coating the catheter with TOH, our results indicated that *S. apiospermum*-infected rat sera appeared to be less virulent in vivo than voriconazole-coated catheter group. In fact, injection of rat sera from the uncoated catheter group generated immediate melanization in *G. mellonella*, leading to a high mortality rate in comparison with the TOH-coated catheter group. Melanization and nodulation are considered to be the chief immune responses of *G. mellonella* against pathogenic fungal infection.<sup>23</sup> Enhanced survival rate of *G. mellonella* after being injected with *S. apiospermum*-infected rat sera was also noted in longer post-inoculation periods in the TOH-coated catheter group (48 h post-inoculation). Our results propose that delayed mortality of *G. mellonella* injected with rat sera from TOH-coated catheter group is consistent with reduced *S. apiospermum* virulence. We formerly reported that TOH mediates the reduction of pathogenicity and induction of cellular apoptosis in *C. albicans*.<sup>23</sup> We showed that TOH treatment remarkably increases *CARD-9* and *Noxa* and reduces *Bcl-2* of



*C. albicans* mRNA expressions. Generally, CARD-9 is correlated with Bcl-10 to positively regulate the apoptosis by activating the nuclear factor-kappa B (NF- $\kappa$ B) stress pathway.<sup>44</sup> Furthermore, Noxa (a pro-apoptotic protein of Bcl-2) is directly cooperated with anti-apoptotic Bcl-2 protein to regulate oxidative stress-induced apoptosis.<sup>45</sup> Therefore, it is possible that TOH can prompt the cellular apoptosis and lessen the virulence of *S. apiospermum* similar to *C. albicans*. Thus, our results propose that TOH can be utilized as a coating agent in medical devices, which may help lessen the risk of opportunistic infections especially the virulence of *S. apiospermum*. Additionally, we plan to examine the molecular mechanisms of TOH-mediated reduction in *S. apiospermum* pathogenicity in the future.

## Conclusion

Our current study shows that a QSM known as tryptophol (TOH) can be coated on the luminal surface of the CVC to lessen catheter-related cerebral and pulmonary infections by *S. apiospermum*. Decreased growth and virulence of *S. apiospermum* were related to the decreased biofilm formation of *S. apiospermum* and increased mortality rate of experimented animals in TOH-coated catheter group. The current study shows that TOH can be more effective in controlling *S. apiospermum* colonization on catheter lumen than voriconazole, as an antifungal agent. Nonetheless, further extensive studies are required to determine the mechanisms of TOH involved in the antifungal susceptibility of *S. apiospermum* biofilms and to use TOH as an alternative catheter coating agent for antifungal property.

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## Disclosure

The authors declare that they have no conflicts of interest in this work.

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