

# Characterization of *Acremonium spp* Recovered from Anatomy Laboratory: A Pioneering Study

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## Abstract:

### Background:

*Acremonium* is a genus of fungi has been reported as possible pathogen cause mycoses infections in humans and various domestic animals. **The objective** of the current study was to isolate and identify *Acremonium* species that exist in the tanks of the anatomy department using traditional laboratories techniques including macroscopic and microscopic methods. Moreover, the research compared the antifungal effect of the different concentrations of formalin (2.5, 5, 7.5 and 10 percent) on *Acremonium* molds isolated in the laboratory at the College of veterinary Medicine during June and July. The macroscopic examination of the mold revealed a powdery texture, with a white to pale gray or pale pink color, which turns cottony as the colony grows. Microscopic examination also revealed fungal hyphae using Lactophenol cotton stain with 40 X magnification. The findings showed that the rates of isolation of *Acremonium spp.* were 100%. In addition, the results revealed that formalin had good antifungal effect at the concentration of 10 percent, but the lower concentrations failed to significantly prevent the growth of fungus

### Aims

This study aims to isolate and identify *Acremonium* species present in tanks of the Anatomy Department using macroscopic and microscopic methods.

### Results

*Acremonium spp.* was identified by macroscopic and microscopic techniques, cultural morphology which was confirmed by the powdery texture that was observed. The colony's surface may become cottony as it ages. The surface of the colony was white, pale grey, or pale pink. and microscopically, the hyphae were hyaline and septate.

### Conclusions

*Acremonium spp* fungi can grow in dissection tanks, but their growth is less when the concentration of formalin increases. The primary potential sources of contamination were identified as air, water, and animal specimens. Fungal contamination poses a serious biohazard, potentially causing significant health risks for laboratory personnel and students.

**Keywords:** *Acremonium*, formalin, mycotic, dissection tanks, Antifungal activity.



## Introduction

Embalming is a process that involves the preservation of the body of an animal after death using certain chemical substances. This practice had been used historically to preserve cadavers to be used in anatomy dissection and education (Ajayi et al., 2011). Formaldehyde as a tissue fixative is a cost-effective happen when high concentrations (approximately 10%) are applied. Formalin preservation of animal organs and specimens remains a main method of anatomical research and training. Usually, the tissues are stabilized by formalin concentration levels of 5 to 10 percent and this helps in preventing autolysis and destruction by microbes and a widely used preservation technique that is commonly used in medical and experimental research studies (Thavarajah et al., 2012).

The concentration of the solution used affects changes in the weight and thickness of tissues in formalin (Hořda et al., 2016). The effect of low concentrations of formalin (2% and 4% formalin) is that the tissue weight tends to increase, but the same does not

*Acremonium* spp. is identified as a very ubiquitous genus of saprophytic, parasitic, and endophytic fungi that live in a very diverse ecological range (Hou et al., 2023). In 1809, Link gave the genus *Acremonium* its first definition as a new fungal taxon which produces single spores at the ends of fertile hyphae (Link, 1809). The word *Acremonium* is based on the Greek prefix *acro-*, which implies the top, and *mono-*, which means singularity (Summerbell and Scott, 2015; Hou et al., 2023). Glenn et al. were the first to study *Acremonium* on a large scale in a molecular phylogenetic analysis using partial nuclear ribosomal small subunit (SSU) gene sequences. Subsequent studies showed that *Acremonium* is an extremely polyphyletic genus (Hou et al., 2023). Its taxonomy is still complicated because *Acremonium*-like fungi are currently spread over 63 genera and 14 families (Hou et al., 2023).

Several *Acremonium* species have been identified as opportunistic pathogenic bacteria in both humans and animals (Perez-Cantero and Guarro, 2020; Priyadarshini et al., 2022). These fungi have been known to cause different infections, which include eumycetoma, onychomycosis and hyalohyphomycotic. Diseases related to *Acremonium* spp. are not very common but may show various forms of clinical manifestations of hyalohyphomycosis such as arthritis, osteomyelitis, peritonitis, endocarditis, pneumonia, cerebritis, and cutaneous infections (Fincher et al., 1991). Furthermore, *Acremonium* is associated with post-traumatic diseases, including keratitis and mycetoma, and the presence is reported to increase in bloodstream, central nervous system, sinuses, bones, and joints, as well as in plural and peritoneal cavities and other disseminated infections (Perez-Cantero et al., 2020; Arabatzis et al., 2024).

Several species under this genus are also known to receive bioactive secondary metabolites. As an example, the first antibiotics to be isolated were cephalosporins, which are a part of the b-

lactam group, and they were discovered in *Acremonium strictum*. *Acremonium* genus is a source of biologically active compounds, such as steroids, terpenoids, meroterpenoids, polyketides, alkaloids, peptides, and other miscellaneous compounds (Tian et al., 2017). The spectrum of biological activity of these metabolites is quite wide, including antimicrobial, antitumor, antioxidant, immunosuppressive, and anti-inflammatory effects (Tian et al., 2017). In the current study, it was aimed at and expected to assess the antifungal effect of formalin against *Acremonium* species.

## Materials and Methods

### Collection of samples

Sampling was randomly collected from samples preservative tanks from the Histology and Anatomy Department of veterinary medicine Laboratory at the \University of Diyala, during the months of June and July of the year 2025 (**Figure 1**).



**Figure 1:** *Acremonium* spp in Anatomy tanks with 10% formalin (arrowed)

### Isolation and identification of *Acremonium* spp from formalin tanks

*Acremonium* isolates were obtained by culturing samples on Sabouraud Dextrose Agar (SDA) medium containing 0.05 mg/ml chloramphenicol to inhibit bacterial growth (Kadhim, 2023). The inoculated plates were then incubated at 25°C for a period of seven days. Identification of the fungal isolates was performed using Lactophenol cotton blue staining, which is commonly employed for fungal microscopic examination (Mahmood et al., 2024), along with an assessment of the colony morphology on the culture plates.

### Effect of formalin concentration against *Acremonium*

To examine the antifungal efficacy of formalin at different concentrations against fungal growth, previous studies have evaluated the inhibitory action of commercial disinfectants, including formaldehyde, against saprophytic fungi, demonstrating that formaldehyde solutions can suggestively inhibit fungal growth in culture likened to controls (Abed and Hussein, 2016) . Based

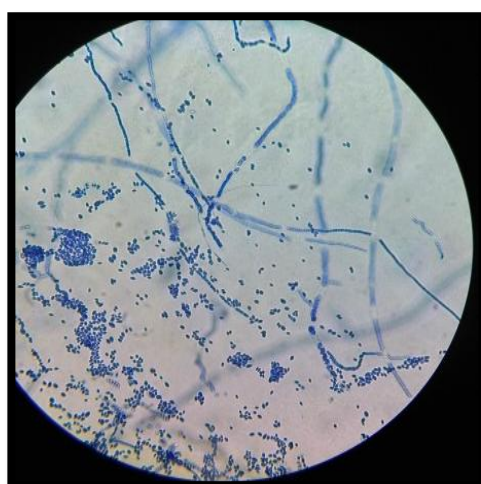
on these findings, the present study evaluated the antifungal activity of formalin at different concentrations. Formalin was prepared at concentrations of 2.5%, 5%, 7.5%, and 10%, and the mixtures were incorporated into culture plates for fungal cultivation and compared with a control group.

## Results

*Acremonium* spp. was identified by macroscopic and microscopic studies (Heras-Martínez et al., 2022; Mahamedi et al., 2025), cultural morphology which was confirmed by the powdery texture that was observed. The colony's surface may become cottony as it ages. The surface of the colony was white, pale grey, or pale pink. and microscopically, the hyphae were hyaline, septate, and typically very fine and narrow, as illustrated in Figure (2) and Macroscopically in Figure (3).

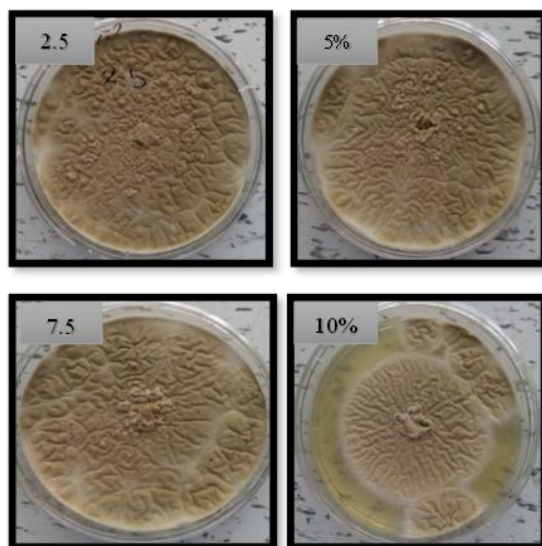


**Figure 2:** Macroscopically appearance of *Acremonium* spp. on SDA after 7 days at 25°C



**Figure 3:** Microscopically appearance of *Acremonium* spp. stained with Lacto phenol Cotton blue (40 X)

The result of antifungal activity of formalin in this study was the best activity of formalin at higher concentration compared to low concentration as shown in 10% concentration while the concentrations of 2.5%, 5%, 7.5% less to for inhibiting *Acremonium* spp. As in Figure (4).



**Figure 4:** Effect different concentration of formalin (2.5%, 5%, 7.5%, and 10%) respectively on *Acremonium* spp. fungus growth

The present study demonstrated that the antifungal potential of formalin increased with higher concentrations (10%), as evidenced by inhibition zone measurements used to determine antifungal activity.

## Discussion

This study was the first to isolate *Acremonium* spp in the College of Veterinary Medicine in Diyala Governorate. Species of *Acremonium* are saprophytic moulds that are found in soil and decomposing vegetation and are extensively spread in nature (Gulzar *et al.*, 2025). The current study based on morphological identification of *Acremonium* spp by both microscopically and macroscopically, macroscopic feature conducted by observation mycelium with irregular dark brown edges, while microscopic characteristics of *Acremonium* spp were investigated by the presence of grouped conidia simulating a head at the tip of the conidiophore (Samson 2004 ; Heras Martínez *et al.*, 2022).The mycelium was constituted mainly by phialides, conidia, and conidiophores. The growth of this mold increased gradually with an increase in humidity level .Humidity had an impact on fungal enzyme activities as it is previously reported that enzyme activities increase as humidity increase (Han *et al.*, 2003). *Acremonium* species are generally recognized for their notable resistance to many antifungal compounds, and according to Guarro *et al.*, (1997), both clinical and environmental isolates of *Acremonium* spp. display reduced

susceptibility to several antifungal agents, particularly azole derivatives, though they often remain sensitive to amphotericin B in in vitro antifungal susceptibility assays .

The present study demonstrated that the antifungal potential of formalin increased with higher concentrations (10%), as evidenced by inhibition zone measurements used to determine antifungal activity. It was suggested that the antimicrobial efficiency of formalin and Dettol® could be attributed to their broad-spectrum antibacterial mechanisms (Ascenzi, 1996; Abed and Hussein, 2016). These agents function by altering the permeability of microbial cell membranes, leading to protein denaturation and enzyme inactivation. Such processes can interfere with oxidative phosphorylation, inhibit active transport, and damage cytoplasmic membranes, ultimately depleting essential metabolites (Aly and Malbach, 1988). Compared with other disinfectants, formalin exhibits a multifaceted mode of action that enhances its antimicrobial performance.

Airborne fungi may also act as potential sources of contamination, particularly in settings where cadavers are preserved using formalin. A previous investigation sought to evaluate the range of fungal species present in the ambient air and on formalin-treated human specimens within the Gross Anatomy Laboratory. The study also aimed to determine and characterize the fungal contaminants at the species level by employing both morphological observations and molecular identification techniques.

Earlier investigations have reported that preserved anatomical cadavers can act as reservoirs for diverse bacterial and fungal contaminants (Ramesh *et al.*, 2017). It has been proposed that the fungal growth detected in such cadavers might arise from environmental or airborne contamination, a possibility previously discussed by Langroudi and Farzanegan (2021).

In Malaysia, Anton *et al.* (2022) observed that cadavers fixed with 5% formalin demonstrated similar microbial findings to fungal colonies in cadavers.

Also (Kalanjati *et al.*, 2012) showed that formalin below 4 percent concentrations are not sufficient enough to contain fungi growth. On the same note, Natekar and Desouza (2007) tested the efficacy of various formalin concentrations in preserving cadavers in humans and discovered that a 15 percent solution of formalin was effective in preventing the growth of bacterial and fungal growth, which in turn prevented the deterioration and discoloration of the tissues [30].

The results of this research were consistent with those of Kalanjati *et al.* (2012) and Anton *et al.* (2022) as it showed that growth of fungi was not suppressed by concentrations lower than 10 percent. Hence, it is advisable that concentrations of formalin of at least 10% be used in order to reduce fungal contamination in anatomical storage tanks.

According to a research project by the College of medicine at the University of Al-Anbar, Iraq, it was found that *Cladosporium* was detected; the first reported case of this fungal genus on human specimens preserved in formalin. The researchers determined that there were various conditions that lead to the occurrence of fungal contamination in laboratories such as room temperature and humidity, large concentrations of fungal spores in the air, and long periods of

exposure that enhance fungal colonization. Normally, 10 percent formalin or 4 percent aqueous formaldehyde is used to prevent the growth of microorganisms. The cadavers in formalin used in academic and medical teaching are usually placed in disinfectants to limit the growth of microorganisms during research and teaching. Nevertheless, the fungal spores that remain suspended in the air in such places are potentially dangerous to the health of those instructors and laboratory workers who have to work with the specimens.

There is a great urgency to develop better fixative formulations with high antifungal activity but which are also safe to the user in order to increase the control of the growth of the fungus. The most frequently used fixatives can be grouped into two main categories, cross-linking agents and organic solvents. In the case of a fixative such as formalin, the fixative reacts with the tissue proteins to create molecular cross-links. Such chemical bonds reinforce the tissue structure and inactivate enzymes, which cause postmortem autolysis. As a result, formalin reacts with the amine functional groups in proteins leading to structural change and dysfunction. It is a non-toxic disinfectant of high efficacy, in addition, formalin contains about 37 percent of formaldehyde gas dissolved in water (Habicht *et al.*, 2018). As a substance, formaldehyde possesses great antimicrobial potential, bactericidal, sporicidal, and veridical properties (Ishii *et al.*, 2006; Anton *et al.*, 2022).

## Conclusions

*Acremonium* spp fungi can grow in dissection tanks, but their growth less when the concentration of formalin increases. The primary potential sources of contamination were identified as air, water, and animal specimens. Fungal contamination poses a serious biohazard, potentially causing significant health risks for laboratory personnel and students. Therefore, it is essential to implement protective measures, ensuring that staff and students handling cadavers wear appropriate personal protective equipment, such as masks and gloves, throughout dissection procedures. Furthermore, *Acremonium* spp must be identified at the molecular level by sequencing the ITS, and detection of genome mutation region, in addition to identify the fungus by conventional methods.

Mycelium was constituted mainly by phialides, conidia, and conidiophores. The growth of *Acremonium* spp increased gradually with an increase in humidity level. Humidity had an impact on fungal enzyme activities as it is previously reported that enzyme activities increase as humidity increase.

## Recommendations:

We recommend strict sanitation and disinfection procedures should be regularly to minimize fungal colonization on laboratory surfaces and tanks.

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### Conflict of interest:

The authors declare no conflict of interest.

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### Authors contribution:

The author was responsible for the experimental work, data analysis and preparation of the manuscript.

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