Jurnal Info Kesehatan
Vol. 24, No. 1, March 2026, pp. 1-14
P-ISSN 0216-504X, E-ISSN 2620-536X
DOI: *10.31965/infokes.Vol24.Iss1.2239*
Journal homepage: *https://jurnal.poltekkeskupang.ac.id/index.php/infokes*

RESEARCH Open Access

Air Conditioner Condensate and Mineral Water as Alternative Solvents for Potato Dextrose Agar: Comparative Growth of *Trichophyton rubrum*

M. Atik Martsiningsih[1a*], Yusmiarti[1b], Anik Nuryati[1c], Siti Nur Azizah[1d], Menik Kasiyati[1e], Budi Setiawan[1,2f]

[1] Department of Medical Laboratory Technology, Poltekkes Kemenkes Yogyakarta, Yogyakarta, Indonesia
[2] Biomedical Engineering, School of Engineering, University of Glasgow, Scotland, United Kingdom

[a] Email address: atik.martsiningsih@poltekkesjogja.ac.id
[b] Email address: yusmiartiekaputrii@gmail.com
[c] Email address: nuryati.anik@gmail.com
[d] Email address: sitinurazizah@gmail.com
[e] Email address: rifani.2010@gmail.com
[f] Email address: b.setiawan.1@research.gla.ac.uk

Received: 16 October 2025 Revised: 16 December 2025 Accepted: 20 December 2025

Abstract
Trichophyton rubrum is the predominant cause of dermatophytosis worldwide, particularly in tropical countries such as Indonesia. Potato Dextrose Agar (PDA) is a standard medium for fungal culture, conventionally prepared with distilled water; however, its limited availability and high cost in resource-constrained laboratories necessitate alternative solvents. This study aimed to evaluate the growth of T. rubrum on PDA prepared with distilled water, air conditioner (AC) condensate, and bottled mineral water by comparing colony diameter and morphology. A quasi-experimental study with a post-test-only non-equivalent control group design was conducted. PDA was prepared using the three solvents, inoculated by the single-dot method, and incubated at a fixed temperature of 28°C for 7 days. Colony diameter and macroscopic and microscopic morphology were assessed. Statistical analyses included the Shapiro–Wilk test, One-Way ANOVA, and Independent Sample T-test ($p < 0.05$). All media supported typical T. rubrum growth. Colony diameters obtained using AC condensate were not significantly different from those obtained using distilled water, whereas mineral water resulted in significantly smaller colonies. These findings indicate that AC condensate can serve as an effective and economical substitute for distilled water in PDA preparation, while the use of mineral water should be limited due to variability in composition and lack of standardization.

Keywords: *Trichophyton rubrum,* Potato Dextrose Agar, AC Condensate, Mineral Water, Dermatophytosis.

Corresponding Author:

M. Atik Martsiningsih
Department of Medical Laboratory Technology, Poltekkes Kemenkes Yogyakarta, Yogyakarta, Indonesia
Email: atik.martsiningsih@poltekkesjogja.ac.id

1. INTRODUCTION

Dermatophytosis is among the most common superficial fungal infections, affecting nearly 25% of the global population (Kumar et al., 2023). It is caused by keratinophilic fungi of the genera *Trichophyton*, *Microsporum*, and *Epidermophyton* (Sanguino, Jarros, & Negri, 2019). Among them, *Trichophyton rubrum* is the most prevalent species, responsible for chronic infections such as tinea pedis, tinea cruris, and onychomycosis (Nigam, Syed, & Saleh, 2025). The prevalence of fungal skin disorders in Indonesia varies from 2.93% to 27% and has risen by 65% from 2010 to 2014 (Prameswari et al., 2023). In 2016 and 2020, the incidence of fungal infections among dermatological patients was 12.3%. Pityriasis versicolor (PV) is the most prevalent fungal dermatosis, accounting for 4.1% of cases, followed by tinea capitis (2.2%), tinea cruris (1.6%), and tinea corporis (1.4%) (Prakoeswa, Pramuningtyas, & Dimawan, 2022). These conditions collectively render dermatophytosis a considerable public health issue (Khan, & Saunte, 2025).

Laboratory culture is the gold standard for diagnosing dermatophyte infections (Anton et al., 2023). Potato Dextrose Agar (PDA) is widely used due to its nutrient composition and acidic pH, which favours fungal growth and suppresses bacterial contaminants (Ribeiro *et al.*, 2025). Conventionally, PDA is prepared with distilled water (Smit et al., 2011). However, in some laboratories, especially in lower-middle-income countries, distilled water is expensive or not always readily available. Therefore, alternative solvents with similar physicochemical properties are needed (Bains et al., 2021). Air conditioner (AC) condensate, produced as a by-product of cooling systems, is essentially condensed atmospheric moisture with minimal dissolved minerals, resembling distilled water (Ahmed, 2025). Mineral water, although inexpensive and widely available, contains varying levels of ions such as Ca^{2+}, Mg^{2+}, and Na^{+}, which may influence fungal growth (Macêdo et al., 2019).

Previous research has suggested the potential of AC condensate as a substitute for distilled water in culturing fungi including *Candida albicans* and *Trichophyton mentagrophytes* (Hamada, & Fujita, 2002). Previous studies evaluated AC condensate using different fungal species. However, *T. rubrum* was chosen since it is the most prevalent dermatophyte worldwide and the principal causative agent of chronic dermatophytosis (Smijs et al., 2007). *T. rubrum* exhibits growth requirements akin to other dermatophytes, rendering it an effective and physiologically relevant model for evaluating various solvents in PDA formulation (Täuber, & Müller-Goymann, 2014). However, studies specifically comparing its effectiveness in PDA preparation for *T. rubrum* remain limited (Zheng et al., 2025). Therefore, this study aimed to compare the growth of T. rubrum on PDA prepared with AC condensate, mineral water, and distilled water, focusing on colony diameter and morphology. The diameter of a colony is a common quantitative indicator of fungal growth, whereas both macroscopic and microscopic morphology reveal sporulation patterns and physiological reactions to cultural conditions, as noted in previous dermatophyte studies (Bitew et al., 2018).

This study addresses the lack of species-specific validation of air conditioner condensate as an alternative solvent for Potato Dextrose Agar in culturing *Trichophyton rubrum*, the most clinically relevant dermatophyte. By comparatively evaluating colony growth and morphology under standardized conditions, this study provides practical evidence for the feasibility of alternative solvents in resource-limited laboratory settings.

2. RESEARCH METHOD

This study applied a quasi-experimental design with a post-test-only non-equivalent control group. Experiments were conducted between February and March 2025 at the Mycology and Parasitology Laboratories, Poltekkes Kemenkes Yogyakarta, Indonesia. The test organism was a laboratory stock isolate of Trichophyton rubrum sourced from the Microbiology

Laboratory, Universitas Gadjah Mada (Yogyakarta, Indonesia). Species identity had been established by the provider and was re-verified in our laboratory based on characteristic macroscopic features (white to yellowish-white, cottony colonies with a dark-red reverse) and microscopic morphology on LPCB staining (septate hyphae, pencil-shaped macroconidia, and tear-shaped microconidia). The isolate was maintained on fungal nutrient agar and subculture onto fresh PDA to ensure active growth prior to experimentation; only fresh, single-species cultures without visible contamination were used.

Three types of Potato Dextrose Agar (PDA) media were prepared using different solvents. The first was prepared with distilled water, which served as the control. The second used air conditioner (AC) condensate that had been collected from air conditioning units in our university, filtered through sterile gauze to remove particulate matter, and sterilized prior to use. The third type employed commercially available bottled mineral water, representing an accessible and low-cost alternative. For each preparation, 3.9 g of PDA powder (Oxoid™, UK) was dissolved in 100 mL of the respective solvent (Samukha et al., 2024). The mixtures were homogenized and sterilized by autoclaving at 121°C under 15 psi pressure for 15 minutes (Chiewchan, Phungamngoen, & Siriwattanayothin, 2006). After sterilization, the molten medium was cooled to approximately 45–50°C before being aseptically poured into sterile Petri dishes, with about 10 mL of medium dispensed per plate (Terrones-Fernandez et al., 2023). The plates were allowed to solidify at room temperature and subsequently stored at 4°C until further use (Sanders, 2012).

The fungal suspension of T. rubrum was standardized to a turbidity equivalent to 0.5 McFarland (approximately 1×10^6 CFU/mL) to ensure uniform inoculum density (Romulo et al., 2018). Using a sterile micropipette, 10 µL of the suspension was applied as a single drop at the center of each PDA plate (single-dot inoculation method). The plates were then allowed to absorb the inoculum under aseptic conditions before being incubated in an upright position at a controlled temperature 28°C for seven consecutive days (Matthews, 2003). Colony growth was monitored daily, and final observations were recorded after the 7-day incubation period.

Macroscopic observations included an assessment of colony color, surface appearance, texture, and reverse pigmentation. Microscopic characteristics were examined using Lactophenol Cotton Blue (LPCB) staining, focusing on the presence of septate hyphae, macroconidia, and microconidia. Colony growth was evaluated by measuring the diameter of colonies in millimetres using a digital calliper.

Normality was assessed using the Shapiro–Wilk test, while group differences were analyzed with One-Way ANOVA for three-group comparisons and the Independent Sample T-test for two-group comparisons. A p-value of <0.05 was considered statistically significant. A total of nine replications per group were performed, resulting in 27 data points across the experimental and control groups, consistent with common practice in exploratory in vitro fungal growth studies. The absence of formal power analysis is acknowledged as a methodological limitation.

3. RESULTS AND DISCUSSION

The growth of *Trichophyton rubrum* on PDA media prepared with distilled water, AC condensate, and bottled water after 7 days of incubation is shown in Figure 1. Colonies generally appeared circular with irregular margins, white to yellowish white in color, cotton-like in texture, and produced dark red pigmentation on the reverse side. Detailed macroscopic characteristics are presented in Table 1.

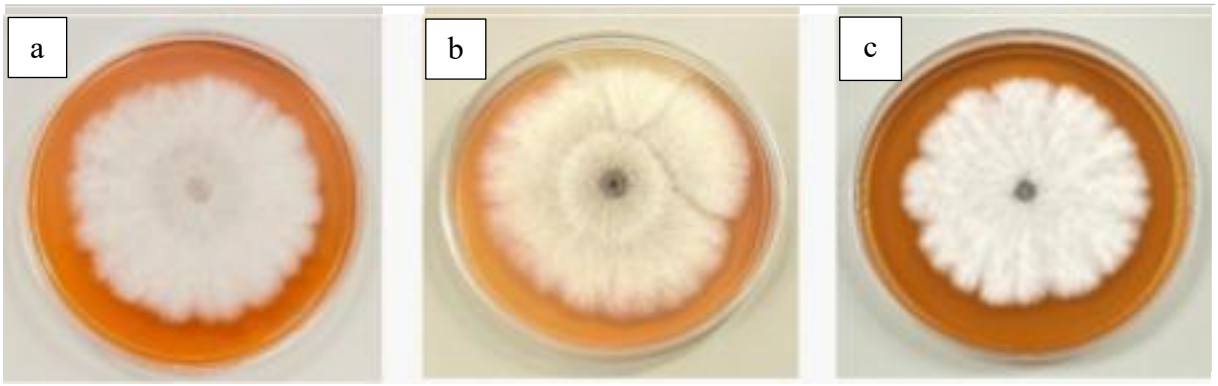

Figure 1. Growth of *Trichophyton rubrum* colonies after 7×24 hours of incubation on: (a) PDA prepared with distilled water, (b) PDA prepared with AC condensate, and (c) PDA prepared with mineral water.

Figure 1 illustrates the representative colony morphology of *T. rubrum* grown on PDA prepared with different solvents. All media supported typical dermatophyte growth, while differences were primarily observed in pigmentation intensity and colony texture rather than overall morphology. The observed alterations in colony pigmentation may result from interactions between minerals and fungi that influence the fungi's pigment synthesis and degradation processes. Fungal pigments, including melanins, are secondary metabolites whose production is affected by environmental factors and metal availability. Multiple investigations have demonstrated that divalent metal ions such as Ca^{2+} and Mg^{2+} can establish coordination interactions with functional groups in fungal cell walls and pigment molecules. This may alter the stability, location, and expression of pigments (Fogarty, & Tobin, 1996; Maia et al., 2025). In dermatophytes, alterations in the mineral composition of the growth media may influence enzymatic pathways related to pigment synthesis or affect the binding of pigments to cell wall constituents, resulting in discernible changes in colony coloration (Lin & Xu, 2022). Furthermore, interactions between fungi and minerals have been shown to induce physiological alterations without hindering growth, thus clarifying the preserved hyphal structure observed in our study despite changes in pigmentation (Lin & Xu, 2022; Maia et al., 2025).

In contrast, the colony on PDA formed with AC condensate (Figure 1b) shows cottony white mycelial growth, but it is less red than the control. The observed discrepancies in colony colors and growth patterns may be attributed to changes in the chemical composition of the water sources employed to prepare PDA (Westphal et al., 2021). It is commonly asserted that air conditioner condensate has few dissolved minerals, as it originates from the condensation of atmospheric moisture (Mahvi, 2013). Mineral water contains varying concentrations of ions such as calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}), contingent upon its source (Rosborg et al., 2005). Metal ions have been shown to interact with organic compounds in the fungal cell wall and pigment molecules through coordination bonds, hence influencing pigment synthesis, stability, and expression (Singh et al., 2025). Previous studies indicate that fungal pigments, including melanins, react to mineral availability and may function as adaptive strategies to environmental and chemical fluctuations (Cordero, & Casadevall, 2017). This study did not analytically evaluate the specific mineral composition of distilled water, AC condensate, and mineral water, nonetheless, the observed pigmentation variations correspond with established fungal–mineral interactions that affect metabolic and physiological responses without impeding hyphal growth. Thus, the variance in colony architecture reported in this study likely reflects variable mineral-mediated effects on fungal metabolism rather than inherent differences in growth ability.

Besides that, the colony looks bright white and thick on PDA with mineral water (Figure 1c), with little to no reddish hue. The alteration in pigmentation may result from dissolved minerals, such as calcium and magnesium, which can affect fungal secondary metabolism, including pigment biosynthesis. In the PDA prepared with mineral water, the colonies appeared dazzling white and dense, with minimal to no reddish hue. These observations suggest that the differences in pigmentation reflect phenotypic variation rather than growth inhibition. As no biochemical or molecular analyses were performed, potential mechanisms related to mineral–pigment interactions are discussed as hypothetical interpretations supported by previous literature.

Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were specifically mentioned since they are common divalent cations in mineral water and significantly affect fungal physiology (Trofimova, Walker, & Rapoport, 2010). Both ions can establish coordination interactions with negatively charged functional groups in the components of fungal cell walls and pigment-associated compounds. This may alter the stability, positioning, or biosynthetic regulation of pigments. Prior studies have shown that fungal pigments, including melanins and similar compounds, can chelate metal ions, potentially modifying pigment properties or suppressing visible pigmentation while maintaining hyphal development (Robinson, Isikhuemhen, & Anike, 2021). The reduced reddish color seen in mineral water–based PDA may suggest that minerals alter pigment expression pathways instead than directly inhibiting fungus growth. Even though the total diameter of the colonies was the same for all treatments, differences in texture and color imply that the nature of the water source affects metabolic activity.

All three media supported the growth of *T. rubrum,* demonstrating that PDA is a suitable culture medium regardless of the water source utilized. The changes in colony color and texture show that the physicochemical properties of water, such as pH, ionic composition, and osmolarity, may affect how fungi appearance and how they make secondary metabolites (Zehner et al., 2023). The variations in pH, ionic composition, and osmolarity of the water sources employed in PDA production may collectively influence fungal morphology and the production of secondary metabolites (Wadhwa et al., 2024). Variations in pH can alter enzyme functionality and pigment synthesis, whereas fluctuations in ionic composition and osmolarity can affect ion permeability across cell membranes, ion mobility, and intercellular signaling (Felle, 2001; Gao et al., 2004). This study observed physicochemical variables as changes in colony pigmentation, texture, and thickness, whereas hyphal structure and radial growth were mostly preserved. The observations suggest that the fungal cells underwent metabolic and physiological adaptations instead of growth inhibition, leading to modified expression or localization of secondary metabolites, such as pigments. These adaptive responses correspond with previous research indicating that environmental and chemical stimuli can affect fungal morphology and metabolism without jeopardizing overall viability or hyphal development (Shree, Pal, & Verma, 2024). Distilled water, being a chemically neutral solvent, facilitates uniform growth and pigment synthesis, while mineral rich or demineralized alternatives may somewhat influence fungal metabolism without compromising viability (Bueno and Gallardo, 1998; Gmoser et al., 2017).

Table 1. Macroscopic observation of *T. rubrum* on PDA media with three different solvents

Characteristic	Distilled Water	AC Condensate	Mineral Water
Shape	Circular with slightly irregular/wavy edges	Circular with slightly irregular/wavy edges	Circular with slightly irregular/wavy edges
Color	White on the surface, red on the reverse	Yellowish-white on the surface, red on the reverse	White on the surface, red on the reverse
Surface	Flat	Flat	Flat
Texture	Cotton-like, slightly hairy	Cotton-like, slightly hairy	Cotton-like, slightly hairy

Table 1 shows the macroscopic parameters of *Trichophyton rubrum* colonies grown on PDA media using three different water sources. These results are consistent with the colony morphology shown in Figure 1. All treatments produced colonies with a circular shape characterized by somewhat uneven or undulating margins, demonstrating persistent radial development regardless of the solvent used. The flat, even surface on all of the samples shows that the hyphal spreading pattern is the same and that there are no differences in elevation between the media. However, there were differences in color amongst the treatments. Colonies on PDA made with distilled water had a typical *T. rubrum* shape, with white surface mycelia and a reddish reverse. These results highlight that the pigment synthesis was quite good. In contrast, PDA made with AC condensate produced colonies that were yellowish-white and had less pigmentation than the control. This suggests that the condensate's low mineral or ion content may have caused a subtle change in pigment production. Colonies grown on mineral water-based PDA had a white surface and a reddish underside. However, the reddish tint was less noticeable in Figure 1c, which suggests that dissolved minerals like calcium or magnesium may have affected how pigments spread.

All colonies exhibited a cottony, slightly hairy texture typical of *T. rubrum* morphology, indicating that fungal survival and initial growth were not significantly affected by the different solvents. The results, supported by macroscopic observation (Table 1) and visual documentation (Figure 1), demonstrate that *T. rubrum* may reliably multiply on PDA media prepared with various water sources. However, minor differences in color intensity and surface properties indicate that the water's chemical makeup may subtly influence secondary metabolite expression, particularly pigment synthesis, without changing the overall growth pattern.

Microscopic examination using Lactophenol Cotton Blue (LPCB) staining revealed septate hyphae, smooth-walled cylindrical macroconidia, and tear-shaped microconidia. The findings are shown in Figure 2 and summarized in Table 2.

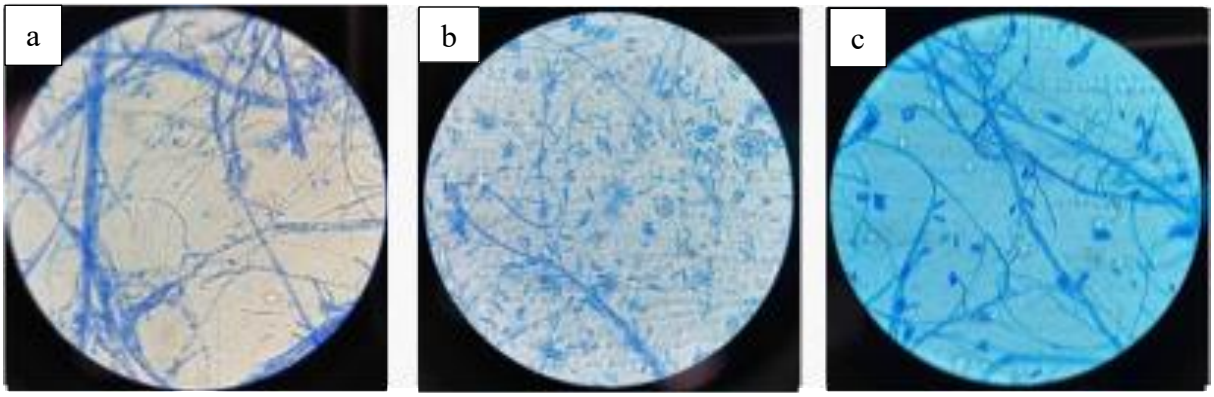

Figure 2. Microscopic morphology of *T. rubrum* on PDA prepared with distilled water (a), AC condensate (b), and mineral water (c), observed at 40× magnification with LPCB staining.

In Figure 2a (PDA with distilled water), *T. rubrum* exhibits distinctive morphology, characterized by septate, branching hyphae and many microconidia located along the hyphal sides (sessile or in small clusters), in addition to sporadic slender, elongated, thin-walled macroconidia. This appearance indicates strong fungal growth and active sporulation, which is consistent with the macroscopic features shown in Figure 1a and Table 1, where the optimal colony color and texture were also clear. Figure 2b (PDA with AC condensate) shows that the hyphae are well-developed but not as thick as in the control. The number of conidia is also much lower than in the control. This could mean that sporulation activity is a little less active, which could be due to changes in the ionic or mineral makeup of the AC condensate. However,

the hyphal structure remains intact, with clear patterns of septation and branching, showing that *T. rubrum* can still grow well under these conditions. In Figure 2c (PDA with mineral water), the microscopic field shows thick networks of hyphae and a lot of microconidia, just like in the control group. The staining intensity appears to be considerably elevated, possibly indicating variations in cell wall permeability or LPCB absorption driven by minerals such as calcium and magnesium in the growth media. The morphology is typical of *T. rubrum*, and no structural problems were found. Microscopic examination confirms macroscopic findings, illustrating that *T. rubrum* may develop its distinctive reproductive and vegetative structures on PDA derived from many water sources. There are some small differences in the density of conidia and the intensity of staining, but these differences do not affect the structure of the fungus. This suggests that the composition of the water source has a bigger effect on pigmentation and sporulation rate than on hyphal growth.

Table 2. Microscopic observation of *T. rubrum* on PDA media with three different solvents.

Characteristic	Distilled Water	AC Condensate	Mineral Water
Hyphae	Septate, branched, thin, hyaline	Septate, branched, thin, hyaline	Septate, branched, thin, hyaline
Macroconidia	Few, cylindrical, smooth-walled, with 3 septa	Numerous, cylindrical, smooth-walled, with 3–5 septa	Few, cylindrical, smooth-walled, with 2–4 septa
Microconidia	Numerous, distributed along hyphae, tear-shaped	Very few, almost absent	Few, distributed along hyphae, tear-shaped
Formation	Microconidia predominated and spread along hyphae	Macroconidia formed singly from hyphae	Both microconidia and macroconidia formed singly

Table 2 shows the microscopic features of *Trichophyton rubrum* that were grown on PDA media made with three different types of water. The results correspond with the microscopic images in Figure 2, providing accurate information about the shapes of hyphae and conidial structures. In all treatments, *T. rubrum* had septate, branching, thin, and hyaline hyphae, which showed that the plant was growing normally, no matter what solvent was used. The consistency in hyphal morphology suggests that variations in the content of water sources did not interfere with essential hyphal development or structural integrity, corroborating the uniform macroscopic growth patterns depicted in Figure 1.

Nonetheless, differences were seen in the development and incidence of conidia. *T. rubrum* had a lot of microconidia along the hyphae and a small number of macroconidia with smooth, cylindrical walls and about three septa on PDA made with pure water. The high number of microconidia shows that the organism is actively reproducing and that the colonies in Figure 1a are dense and well-pigmented, and that the spores are spreading far, as shown in Figure 2a. In contrast, when AC condensate was added to PDA, the fungus made very few microconidia, which were almost none, but many macroconidia grew on their own along the hyphae. The macroconidia were cylindrical and had smooth walls. They had 3 to 5 septa. The reduced frequency of microconidia signifies a modification in conidial growth, presumably influenced by the low mineral or ionic concentration in the AC condensate, leading to suppressed micro-conidiation and a concomitant increase in macroconidia production, as illustrated in Figure 2b.

On PDA augmented with mineral water, both microconidia and macroconidia were noted in considerable amounts, with macroconidia displaying 2–4 septa. Microconidia were distributed along the hyphae in tear-shaped arrangements, similar to the control group, but in diminished abundance. The balanced formation pattern shown in Figure 2c is due to dissolved minerals (such Ca^{2+} and Mg^{2+}) that change the signalling pathways for sporulation. This leads to a conidial density that is in between the other two treatments.

The microscopic findings indicate that while the hyphal structure remains unchanged, sporulation patterns, specifically the ratio of microconidia to macroconidia are influenced by the chemical composition of the water source. Distilled water promotes excellent micro-conidiation, AC condensate fosters macro-conidial development, and mineral water yields a mixed profile, highlighting the intricate influence of water quality on fungal reproductive morphology.

Measurement of colony diameters after 7 days of incubation revealed differences among solvents (Table 3).

Table 3. Average colony diameter of *T. rubrum* on PDA media with three solvents after 7 days of incubation.

Solvent	**Average Diameter (mm)**
Distilled Water	71.41
AC Condensate	75.98
Mineral Water	67.22

After 7 days of incubation, Table 3 shows the average colony diameter of *Trichophyton rubrum* grown on PDA media made with three different water sources. The data show that there were big disparities in radial growth, which suggests that the type of solvent used to make the media had an effect on how the fungal colonies grew overall.

The largest colony diameter (75.98 mm) was found on PDA with AC condensate added. Although the mean colony diameter on PDA prepared with AC condensate appeared numerically larger, statistical analysis demonstrated no significant difference between AC condensate and distilled water. Therefore, AC condensate supports fungal growth at a level comparable to distilled water rather than enhancing growth beyond the control condition.

The colony diameter reached 71.41 mm on PDA supplemented with distilled water, demonstrating continuous and homogeneous growth. This result is consistent with the macroscopic and microscopic characteristics illustrated in Figures 1a and 2a, where colonies exhibited distinctive coloring, a dense texture, and abundant microconidia formation. These studies confirm that distilled water is an optimal and standardized solvent for fungal culture, providing a neutral environment that facilitates both vegetative and reproductive growth.

In contrast, PDA prepared with mineral water produced colonies with the smallest diameter (67.22 mm), which means that radial expansion was only slightly slowed down. This decline may be attributed to dissolved minerals, such as calcium and magnesium, which could alter osmotic balance or hyphal permeability, thereby hindering nutrient absorption. The smaller size of the colony is linked to the less pigmentation and moderate sporulation seen both macroscopically (Figure 1c) and microscopically (Figure 2c).

These data show that all three water sources can help *T. rubrum* develop, but the pace of growth and the shape of the colonies depend on the chemical makeup of the solvent. Statistical analysis demonstrated that colony diameters obtained using AC condensate were not significantly different from those obtained using distilled water. Therefore, AC condensate supports fungal growth at a level comparable to distilled water rather than enhancing growth beyond the control condition. These results highlight to choose the right solvent when standardizing fungal cultures.

Table 4 shows a summary of the results of the statistical tests on the colony sizes of *Trichophyton rubrum* grown on PDA media with different water sources. The statistical analysis sought to determine whether the changes in colony size observed among treatments were statistically significant. The Shapiro–Wilk test showed that all of the datasets PDA made with distilled water ($p = 0.996$), AC condensate ($p = 0.612$), and mineral water ($p = 0.943$) had a normal distribution ($p > 0.05$). This confirms that the normality assumption was met for all

groups, allowing the use of parametric tests in further investigation. The homogeneity test produced a non-significant outcome ($p = 0.128$), indicating that the variances among the three groups were homogeneous ($p > 0.05$). The data satisfied the requirements for normality and homogeneity, thereby validating the use of One-Way ANOVA for comparison.

The One-Way ANOVA revealed a significant difference ($p = 0.000$) in colony diameters among the three media formulations, distilled water, AC condensate, and mineral water. This result shows that the type of water used to make the medium had a significant effect on how the fungus grew. The difference in colony diameter matches the information in Table 3, which shows that the highest growth happened with AC condensate, followed by distilled water, and the least growth happened with mineral water.

However, a subsequent pairwise analysis using an Independent T-Test between distilled water and AC condensate yielded a non-significant result ($p = 0.634$). This indicates that while both solvents promoted similar levels of colony expansion, the significant variation shown in the ANOVA is likely affected by the reduced growth recorded in the mineral water group.

This statistical analysis confirms that the solvent type influences the growth performance of T. *rubrum*. While distilled water and AC condensate produced comparable colony sizes, mineral water resulted in significantly smaller colonies, corroborating previous morphological observations. These results emphasize the importance of choosing the right water supply for maintaining stable fungal culture conditions.

Table 4. Statistical test results of *T. rubrum* colony diameters on PDA media.

Test	Solvent	p-value	Interpretation
Shapiro–Wilk	Distilled Water	0.996	Normal
	AC Condensate	0.612	Normal
	Mineral Water	0.943	Normal
Homogeneity	All groups	0.128	Homogeneous
One-Way ANOVA	Distilled Water vs AC Condensate vs Bottled Water	0.000	Significant difference
Independent T-Test	Distilled Water vs AC Condensate	0.634	No significant difference

This study investigated the influence of three different water sources on the growth performance of *Trichophyton rubrum*, revealing comparable outcomes between AC condensate, mineral water and distilled water. The absence of significant variations in colony sizes between these two groups (AC condensate and distilled water) underscores their analogous physicochemical characteristics. AC condensate is just distilled water that comes from the condensation of humid air, it has very few minerals and a low conductivity (≤5 µS/cm) (Scalize et al., 2018). These characteristics reduce osmotic differences and make sure that *T. rubrum's* metabolic needs are met.

Previous research show the pH of AC condensate is between 6.5 to 6.7 (Khan, 2023), which is the same as the best pH for PDA growth is neutral (5.6) (Fogle et al., 2008). The current results corroborate previous research, demonstrating that condensate not only facilitates fungal survival but also preserves characteristic colony shape, encompassing cottony white growth, reddish reverse pigmentation, and unique conidial development. This statistical similarity demonstrates that any trace elements or volatiles present in AC condensate do not impose significant physiological stress on *T. rubrum*, hence confirming its suitability as a substitute for distilled water.

The practical consequences are substantial. Laboratories can safely use AC condensate without affecting the accuracy of their tests (Scalize et al., 2018; Jahne et al., 2023; Matarneh et al., 2024). This method could change microbiological techniques by cutting costs while still making sure that tests are accurate. This is especially useful in places where distilled water is hard to come by.

In contrast, mineral water produced colonies with diameters that were much smaller than those of AC condensate and distilled water. Even if the general shape of the fungi stays the same, the fact that they don't grow as much is probably because of dissolved minerals like Ca^{2+}, Mg^{2+}, Na^{2+}, and bicarbonates (Sengupta, 2013; Lakshmanan, Kannan & Senthil Kumar, 2003; Collins et al., 2022; Dehnavi, Sarikhani, & Nagaraju, 2011). These ions can mess up the metabolism of fungi in a number of ways, such as changing the speed of enzymes, stopping the absorption of food, and causing osmotic regulation imbalance (Fiorentini et al., 2021). Another research have found that environments with a lot of minerals can stop fungi from making spores (Muksy, Kolo & Gorony, 2023; Jiang et al., 2022; Seidel et al., 2024; Dong et al., 2022; Semenov et al., 2022).

Based on this research mineral water can be utilized as an emergency solvent in laboratory settings, but its use comes with important considerations (Dicks, 2009; Risbandini, 2020; Hailes, 2007). Still, the different mineral concentration in different brands leads to different results in fungal development (Rosling et al., 2004). However, some research mention mineral water should not be used as a primary or emergency solvent in laboratories because it contains dissolved minerals and ions such sodium, calcium, and potassium, these can change the pH of solutions, contaminate them, and break fragile equipment, which can make experiment less reliable (Dicks, 2009).

The current results support earlier studies using AC condensate as a solvent for culture media. Tominik and Haiti (2020) demonstrated its effectiveness in facilitating the growth of Candida albicans on Sabouraud Dextrose Agar (Tominik, & Haiti, 2020), although Sophia et al. (2023) reported comparable colony counts between condensate and aquabidest for C. albicans (Sophia, & Suraini, 2023). This research enhances the current knowledge regarding dermatophytes grown on PDA, demonstrating that AC condensate can substitute distilled water without compromising morphological integrity or growth parameters. This research demonstrate that AC condensate is a dependable, cost-effective, and sustainable choice among several fungal species. The validation in *T. rubrum*, an important dermatophyte in medicine, makes it more useful in diagnostic and teaching labs. In schools, where students need a lot of media for practical sessions, using condensate can cut down on operating costs by a large amount. In clinical contexts, it serves as a fast substitute amid supply shortages. This method aligns with sustainable scientific principles by converting a by-product commonly considered waste into a valuable laboratory asset.

This study was limited to one dermatophyte species (*T. rubrum*) and one culture media (PDA). Furthermore, while colony morphology and diameter were evaluated, sporulation efficiency and biochemical reactions were not investigated. The chemical profile of AC condensate was not performed simultaneously with fungal tests, hence constraining the interpretation of any detectable contaminants. Future research must include comprehensive physicochemical characterization of condensate and examine its impacts on diverse fungal taxa and culture circumstances, hence enhancing its utility in broader mycological frameworks.

4. CONCLUSION

The findings of this study indicate that air conditioner condensate can serve as a feasible alternative to distilled water for culturing T. rubrum under controlled laboratory conditions. However, broader application to clinical diagnostics, teaching laboratories, or sustainability initiatives should be interpreted cautiously, given the study's methodological limitations.

REFERENCES

Ahmed, H. M. (2024). Air Conditioning Condensate: A Potential Water Source for Irrigation. *In Business Sustainability with Artificial Intelligence (AI): Challenges and Opportunities*.

Cham: Springer Nature Switzerland (Studies in Systems, Decision and Control), pp. 1213–1223. https://doi.org/10.1007/978-3-031-71526-6_105

Anton, A., Plinet, M., Peyret, T., Cazaudarré, T., Pesant, S., Rouquet, Y., ... & François, J. M. (2023). Rapid and Accurate Diagnosis of Dermatophyte Infections Using the DendrisCHIP® Technology. *Diagnostics,* 13(22), 3430. https://doi.org/10.3390/diagnostics13223430

Bains, W., Petkowski, J. J., Zhan, Z., & Seager, S. (2021). Evaluating alternatives to water as solvents for life: The example of sulfuric acid. *Life,* 11(5), 400. https://doi.org/10.3390/life11050400

Bitew, A. (2018). Dermatophytosis: Prevalence of dermatophytes and non-dermatophyte fungi from patients attending Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia. *Dermatology research and practice,* 2018(1), 1–6. https://doi.org/10.1155/2018/8164757

Bueno, L., & Gallardo, R. (1998). Preservación de hongos filamentosos en agua destilada estéril. *Rev Iberoam Micol*, 15(3), 166-168.

Chiewchan, N., Phungamngoen, C., & Siriwattanayothin, S. (2006). Effect of homogenizing pressure and sterilizing condition on quality of canned high fat coconut milk. *Journal of food engineering*, 73(1), 38-44. https://doi.org/10.1016/j.jfoodeng.2005.01.003

Collins, M. T., Marcucci, G., Anders, H. J., Beltrami, G., Cauley, J. A., Ebeling, P. R., ... & Thakker, R. V. (2022). Skeletal and extraskeletal disorders of biomineralization. *Nature Reviews Endocrinology*, 18(8), 473-489. https://doi.org/10.1038/s41574-022-00682-7

Cordero, R. J., & Casadevall, A. (2017). Functions of fungal melanin beyond virulence. *Fungal Biology Reviews*, 31(2), 99-112. https://doi.org/10.1016/j.fbr.2016.12.003

Dehnavi, A. G., Sarikhani, R., & Nagaraju, D. (2011). Hydro geochemical and rock water interaction studies in East of Kurdistan, NW of Iran. *Int J Environ Sci Res*, 1(1), 16-22.

Dicks, A. P. (2009). A review of aqueous organic reactions for the undergraduate teaching laboratory. *Green Chemistry Letters and Reviews*, 2(1), 9-21. https://doi.org/10.1080/17518250902820182

Dong, H., Huang, L., Zhao, L., Zeng, Q., Liu, X., Sheng, Y., ... & Chen, H. (2022). A critical review of mineral–microbe interaction and co-evolution: mechanisms and applications. *National science review,* 9(10), 1-21. https://doi.org/10.1093/nsr/nwac128

Felle, H. H. (2001). pH: signal and messenger in plant cells. *Plant biology*, 3(06), 577-591. https://doi.org/10.1055/s-2001-19372

Fiorentini, D., Cappadone, C., Farruggia, G., & Prata, C. (2021). Magnesium: biochemistry, nutrition, detection, and social impact of diseases linked to its deficiency. *Nutrients,* 13(4), 1136. https://doi.org/10.3390/nu13041136

Fogarty, R. V., & Tobin, J. M. (1996). Fungal melanins and their interactions with metals. *Enzyme and microbial technology*, 19(4), 311-317. https://doi.org/10.1016/0141-0229(96)00002-6

Fogle, M. R., Douglas, D. R., Jumper, C. A., & Straus, D. C. (2008). Growth and mycotoxin production by Chaetomium globosum is favored in a neutral pH. International journal of molecular sciences, 9(12), 2357-2365. https://doi.org/10.3390/ijms9122357

Gao, D., Knight, M. R., Trewavas, A. J., Sattelmacher, B., & Plieth, C. (2004). Self-reporting Arabidopsis expressing pH and [Ca2+] indicators unveil ion dynamics in the cytoplasm and in the apoplast under abiotic stress. *Plant physiology*, 134(3), 898-908. https://doi.org/10.1104/pp.103.032508

Gmoser, R., Ferreira, J. A., Lennartsson, P. R., & Taherzadeh, M. J. (2017). Filamentous ascomycetes fungi as a source of natural pigments. *Fungal biology and biotechnology*, 4(4), 1-25. https://doi.org/10.1186/s40694-017-0033-2

Hailes, H. C. (2007). Reaction solvent selection: the potential of water as a solvent for organic transformations. *Organic process research & development,* 11(1), 114-120. https://doi.org/10.1021/op060157x

Hamada, N., & Fujita, T. (2002). Effect of air-conditioner on fungal contamination. *Atmospheric Environment*, 36(35), 5443-5448. https://doi.org/10.1016/S1352-2310(02)00661-1

Jahne, M., King, D., Kovalcik, K., Donohue, M., Pfaller, S., Helmick, K., Eades, G., Pait, M., Nye, M., Thimons, S., & J. Garland. (2023). *Water Quality Assessment of AC Condensate for Onsite Collection and Use.* United States Enviromental Protection Agency.

Jiang, L., Pettitt, T. R., Buenfeld, N., & Smith, S. R. (2022). A critical review of the physiological, ecological, physical and chemical factors influencing the microbial degradation of concrete by fungi. *Building and Environment,* 214, 108925. https://doi.org/10.1016/j.buildenv.2022.108925

Khan, M. O. (2023). Quality Assessment of Condensate Water Generating from Air Conditioning Units. https://doi.org/10.20944/preprints202310.1639.v1

Khan, S. S., & Saunte, D. M. L. (2025). Dermatophyte Treatment Failure: A Rapid Global Response to an Emerging Global Health Issue. *Acta dermato-venereologica,* 105, adv42948. https://doi.org/10.2340/actadv.v105.42948

Kumar, P., Ramachandran, S., Das, S., Bhattacharya, S. N., & Taneja, B. (2023). Insights into changing dermatophyte spectrum in India through analysis of cumulative 161,245 cases between 1939 and 2021. *Mycopathologia,* 188(3), 183-202. https://doi.org/10.1007/s11046-023-00720-6

Lakshmanan, E., Kannan, R., & Kumar, M. S. (2003). Major ion chemistry and identification of hydrogeochemical processes of ground water in a part of Kancheepuram district, Tamil Nadu, India. *Environmental geosciences,* 10(4), 157-166. https://doi.org/10.1306/eg100403011

Lin, L., & Xu, J. (2022). Production of fungal pigments: molecular processes and their applications. *Journal of Fungi,* 9(1), 44. https://doi.org/10.3390/jof9010044

Macêdo, W. V., Sakamoto, I. K., Azevedo, E. B., & Damianovic, M. H. R. (2019). The effect of cations (Na+, Mg2+, and Ca2+) on the activity and structure of nitrifying and denitrifying bacterial communities. *Science of the Total Environment*, 679, 279-287. https://doi.org/10.1016/j.scitotenv.2019.04.397

Mahvi, A. H., Alipour, V., & Rezaei, L. (2013). Atmospheric moisture condensation to water recovery by home air conditioners. *American Journal of Applied Sciences,* 10(8), 917. – 923. https://doi.org/10.3844/ajassp.2013.917.923

Maia, V. R. T., Sampaio, E. B. T., de Oliveira, A. J., da Silva Santos, L., da Silva, M. K., de Queiroz, A. C., ... & Duarte, A. W. F. (2025). *Pigment Production by Fungi from Polar Environments: From Cold to Industry. In Polar Microbes and Climate Change: A Molecular Understanding for Sustainable Future*. Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-032-07096-8_13

Matarneh, S., AlQaraleh, L., Alkhrissat, T., & Abdel-Jaber, M. (2024). Assessing water production from air conditioning systems as an unconventional supply source: A focus on water quality and social acceptance perspectives. *Case Studies in Chemical and Environmental Engineering,* 9, 100585. https://doi.org/10.1016/j.cscee.2023.100585

Matthews, C. (2003). *Soft Drinks* | Production. *Encyclopedia of Food Sciences and Nutrition (Second Edition),* 2023, 5352–5358. https://doi.org/10.1016/B0-12-227055-X/01102-0

Muksy, R., Kolo, K., & Abdullah, S. M. (2023). Bacterial and fungal-mineral interactions and their application in bioremediation–A review. *Journal of Ecological Engineering*, 24(5), 1-13. https://doi.org/10.12911/22998993/157567

Nigam, P.K., Syed, H.A. and Saleh, D. (2025). *Tinea Pedis.* Treasure Island (FL): StatPearls Publishing. Retrieved from: https://www.ncbi.nlm.nih.gov/books/NBK470421/

Prakoeswa, F. R. S., Pramuningtyas, R., & Dimawan, R. S. A. (2022). The Epidemiologic and Sociodemographic Features of Superficial Fungal Infection Among Children in East Java Suburban Public Hospital. *Berkala Ilmu Kesehatan Kulit dan Kelamin,* 34(2), 120-124. https://doi.org/10.20473/bikk.V34.2.2022.120-124

Prameswari, F. U., Muharram, F. R., Setyaningrum, T., & Prakoeswa, C. R. S. (2023). Burden of skin and subcutaneous diseases in Indonesia 1990 to 2019. *Acta Dermato-Venereologica*, 103, 18291. https://doi.org/10.2340/actadv.v103.18291

Ribeiro, E. C., Araújo, E. K. N., Penha, M. S. C., Nascimento, A. S. D., da Silva, D. F., & Miranda, R. D. C. M. D. (2025). Optimisation of Potato Dextrose Agar Culture Medium for Actinobacteria Growth. *Microorganisms*, 13(3), 654. https://doi.org/10.3390/microorganisms13030654

Risbandini, C. (2020). Utilization of Unused Autoclaves to Produce Aquades (Aquabits) in the Laboratory of Biosciences and Plant Technology, Department of Biology, Faculty of Science ITS. *Jurnal Temapela*, 3(1), 19–24. https://doi.org/10.25077/temapela.3.1.19-24.2020

Robinson, J. R., Isikhuemhen, O. S., & Anike, F. N. (2021). Fungal–metal interactions: a review of toxicity and homeostasis. *Journal of Fungi,* 7(3), 225. https://doi.org/10.3390/jof7030225

Romulo, A., Zuhud, E. A., Rondevaldova, J., & Kokoska, L. (2018). Screening of in vitro antimicrobial activity of plants used in traditional Indonesian medicine. *Pharmaceutical biology*, 56(1), 287-293. https://doi.org/10.1080/13880209.2018.1462834

Rosborg, I., Nihlgård, B., Gerhardsson, L., Gernersson, M. L., Ohlin, R., & Olsson, T. (2005). Concentrations of inorganic elements in bottled waters on the Swedish market. *Environmental Geochemistry and Health*, 27(3), 217-227. https://doi.org/10.1007/s10653-004-1612-8

Rosling, A., Lindahl, B. D., Taylor, A. F., & Finlay, R. D. (2004). Mycelial growth and substrate acidification of ectomycorrhizal fungi in response to different minerals. *FEMS microbiology ecology*, 47(1), 31-37. https://doi.org/10.1016/S0168-6496(03)00222-8

Samukha, V., Fantasma, F., D'Urso, G., Caprari, C., De Felice, V., Saviano, G., ... & Iorizzi, M. (2024). NMR metabolomics and chemometrics of commercial varieties of Phaseolus vulgaris L. seeds from Italy and in vitro antioxidant and antifungal activity. *Plants*, 13(2), 227. https://doi.org/10.3390/plants13020227

Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *Journal of Visualized Experiments (JoVE),* (63), e3064. https://doi.org/10.3791/3064

Sanguino, T. C., Jarros, I. C., & Negri, M. (2019). Occurrence of dermatophytoses in patients from the Sistema Único de Saúde. *Anais brasileiros de dermatologia*, 94(3), 293-297. https://doi.org/10.1590/abd1806-4841.20197491

Scalize, P. S., Soares, S. S., Alves, A. C. F., Marques, T. A., Mesquita, G. G. M., Ballaminut, N., & Albuquerque, A. C. J. (2018). Use of condensed water from air conditioning systems. *Open Engineering,* 8(1), 284-292. https://doi.org/10.1515/eng-2018-0031

Seidel, D., Wurster, S., Jenks, J. D., Sati, H., Gangneux, J. P., Egger, M., ... & Kontoyiannis, D. P. (2024). Impact of climate change and natural disasters on fungal infections. *The Lancet Microbe*, 5(6), e594-e605. https://doi.org/10.1016/S2666-5247(24)00039-9

Semenov, M. V., Krasnov, G. S., Semenov, V. M., & van Bruggen, A. (2022). Mineral and organic fertilizers distinctly affect fungal communities in the crop rhizosphere. *Journal of Fungi*, 8(3), 251. https://doi.org/10.3390/jof8030251

Sengupta, P. (2013). Potential health impacts of hard water. *International journal of preventive medicine*, 4(8), 866–875. Retrieved from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3775162/

Shree, A., Pal, S., & Verma, P. K. (2024). Structural diversification of fungal cell wall in response to the stress signaling and remodeling during fungal pathogenesis. *Physiology*

and Molecular Biology of Plants, 30(5), 733-747. https://doi.org/10.1007/s12298-024-01453-6

Singh, D., Chaturvedi, V., & Verma, P. (2025). The amazing world of biological pigments: A review on microbial melanins. *Dyes and Pigments,* 237, 112711. https://doi.org/10.1016/j.dyepig.2025.112711

Smijs, T. G., Bouwstra, J. A., Schuitmaker, H. J., Talebi, M., & Pavel, S. (2007). A novel ex vivo skin model to study the susceptibility of the dermatophyte Trichophyton rubrum to photodynamic treatment in different growth phases. *Journal of Antimicrobial Chemotherapy,* 59(3), 433-440. https://doi.org/10.1093/jac/dkl490

Smit, Y., Cameron, M., Venter, P., & Witthuhn, R. C. (2011). Alicyclobacillus spoilage and isolation–a review. *Food Microbiology,* 28(3), 331-349. https://doi.org/10.1016/j.fm.2010.11.008

Sophia, A., & Suraini. (2023). The effectiveness of aquabidest and AC water as a solution of SDA media for the growth of Candida albicans. *Bioma : Jurnal Biologi Makassar*, 8(1), 16–22. Retrieved from: https://www.cabidigitallibrary.org/doi/full/10.5555/20230031340

Täuber, A., & Müller-Goymann, C. C. (2014). Comparison of the antifungal efficacy of terbinafine hydrochloride and ciclopirox olamine containing formulations against the dermatophyte Trichophyton rubrum in an infected nail plate model. *Molecular pharmaceutics,* 11(7), 1991-1996. https://doi.org/10.1021/mp400711q.

Terrones-Fernandez, I., Casino, P., López, A., Peiró, S., Ríos, S., Nardi-Ricart, A., ... & Piqué, N. (2023). Improvement of the pour plate method by separate sterilization of agar and other medium components and reduction of the agar concentration. *Microbiology spectrum,* 11(1), e0316122. Retrieved from: https://doi.org/10.1128/spectrum.03161-22

Tominik, V. I., & Haiti, M. (2020). Limbah air AC sebagai pelarut media sabouraud dextrose agar (SDA) pada jamur Candida albicans. *Masker Medika Jurnal STIKes Muhamadiyah Palembang*, 8(1), 15-20. https://doi.org/10.52523/maskermedika.v8i1.368.

Trofimova, Y., Walker, G., & Rapoport, A. (2010). Anhydrobiosis in yeast: influence of calcium and magnesium ions on yeast resistance to dehydration–rehydration. FEMS microbiology letters, 308(1), 55-61. https://doi.org/10.1111/j.1574-6968.2010.01989.x.

Wadhwa, K., Kapoor, N., Kaur, H., Abu-Seer, E. A., Tariq, M., Siddiqui, S., ... & Alghamdi, S. (2024). A comprehensive review of the diversity of fungal secondary metabolites and their emerging applications in healthcare and environment. Mycobiology, 52(6), 335-387. https://doi.org/10.1080/12298093.2024.2416736

Westphal, K. R., Heidelbach, S., Zeuner, E. J., Riisgaard-Jensen, M., Nielsen, M. E., Vestergaard, S. Z., ... & Sondergaard, T. E. (2021). The effects of different potato dextrose agar media on secondary metabolite production in Fusarium. International Journal of Food Microbiology, 347, 109171. https://doi.org/10.1016/j.ijfoodmicro.2021.109171.

Zehner, L.M., Krespach, M.K.C., Stroe, M.C., Rosin, M., Schroeckh, V., & Brakhage, A.A.. (2023). *Activation of Secondary Metabolite Production in Fungi. In: Pöggeler, S., James, T. (eds) Evolution of Fungi and Fungal-Like Organisms.* Cham: Springer. https://doi.org/10.1007/978-3-031-29199-9_11

Zheng, H., Xie, W., Li, D., Mei, H., Li, X., & Liu, W. (2025). A New Medium That Promotes Mass Sporulation of Trichophyton rubrum In Vitro. Mycoses, 68(3), e70039. https://doi.org/10.1111/myc.70039