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# Developing a modified mycological culture medium for *Malassezia furfur* by supplementing sabouraud dextrose agar with extracted lipid oil from egg yolk

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**Abstract**

*Malassezia furfur (M. furfur)* is a lipophilic yeast that is associated with a variety of superficial and systemic dermatological conditions, including pityriasis versicolor, seborrheic dermatitis, and atopic eczema. Though it's a clinically important etiological agent, *in vitro* research has been insufficient due to a lack of convenient culture media to isolate and subculture the organism. This study was aimed at developing a modified mycological culture medium for *M. furfur* by supplementing sabouraud dextrose agar (SDA) with extracted lipid oil from egg yolk. *Malassezia furfur* was isolated from Pityriasis versicolor patients attending Teaching Hospital Jaffna, in SDA with ghee (10%) media, and its presence was confirmed using microscopic, macroscopic, and biochemical examinations. Egg yolk oil was extracted by the solvent extraction method. SDA culture plates with different volumes of egg yolk oil (2x, x, x/2, x/4, x/8, and x/16; x refers to egg yolk oil extracted from one egg yolk) were streaked with confirmed colonies of *M. furfur* and incubated at 32 °C. Additionally, the optimal growth temperature and the impact of additives such as $Na_2HPO_4$, NaCl, and $MgSO_4$ were evaluated. Growth level (5-point scale), isolation, and isolated colony size (mm) were taken on the 3rd and 5th days of incubation. Furthermore, the growth of *M. furfur* in the modified culture medium, incorporating all optimized conditions, was assessed and compared with traditional agar formulations like SDA and SDA supplemented with ghee. The isolated colony size was reported as the mean and standard deviation (SD), and the data were subjected to examination by analysis of variance (ANOVA) ($P<0.05$) followed by Tukey's test ($\alpha = 0.05$) by using software, SPSS Statistics version 21.0. The volume of oil extracted from one egg yolk and the yield in percentage of oil were 2 mL and 33.33 % respectively. Among the tested concentrations of egg yolk lipid oil, the x/8 was selected as the minimal volume required for optimal growth of *M. furfur*. The organism exhibited optimal growth at 32 °C. Furthermore, supplementation with $Na_2HPO_4$, NaCl, and $MgSO_4$ significantly enhanced growth. In the comparative study, the modified culture medium demonstrated better performance compared to the other existing culture media.

**Keywords** – Egg yolk oil – Lipophilic yeast – *Malassezia furfur* – Modified mycological culture media –Pityriasis versicolor – Solvent extraction

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## Introduction

For more than a century, yeasts of the genus *Malassezia* have been known to be a part of the normal flora of human skin and other warm-blooded animals (Kindo et al. 2004). *Malassezia* spp. is classified into 14 species; among which *M. furfur* is considered clinically significant (Gaitanis et al. 2013, Spatz et al. 2020). *Malassezia furfur* belongs to a monophyletic genus of lipid dependent, dimorphic commensal yeasts commonly residing on the skin of humans and animals (Vest et al. 2023). These organisms typically comprise more than 80% of the total fungal population on human skin and are frequently isolated from both healthy individuals and those with dermatological conditions (Saunte et al. 2020, Hadrich et al. 2022). *Malassezia furfur* is implicated in a range of skin conditions, including pityriasis versicolor, seborrheic dermatitis, head and neck dermatitis, and Malassezia folliculitis (Saunte et al. 2020). Although this yeast was initially considered to cause only superficial infections, it has since been associated with systemic infections in immunocompromised patients, especially those receiving parenteral nutrition (Rathnapriya et al. 2016). Despite its clinical significance, the interactions between *M. furfur* and its human hosts remain poorly understood, largely due to the restricted availability of specialized lipid enriched culture media. This limitation, coupled with insufficient research attention, may obscure its potential associations with other important human diseases (Chua et al. 2005).

*Malassezia furfur* requires medium with long chain fatty acids (C12 to C24) for *in vitro* growth due to its lipid dependency (Buick et al. 1997). This lipid dependency arises from the lack of fatty acid synthase genes, which renders *M. furfur* unable to synthesize these essential fatty acids de novo, thereby necessitating reliance on exogenous lipid sources (Saunte et al. 2020). Nevertheless, *M. furfur* exhibits lipid assimilation versatility and can utilize a variety of lipid substrates, including butter, lecithin, glycerol, olive oil, PEG 7 glycerol monocaprylate (Cetiol HE), PEG glyceryl stearate (Tagat 82), PEG 3,5 castor oil (Cremophor EL), and PEG 9 stearate (Cremophor 89) (Buick et al. 1997).

Over the years, a diverse range of mycological culture media has been developed based on information published on nutrients and trace components that would encourage the growth of this yeast (Manna et al. 2015). Dixon and Leeming Notman agar and their modified versions are effective for *M. furfur* (Ali et al. 2021). Conventionally, sabouraud dextrose agar (SDA) supplemented with olive oil is widely used for culturing *M. furfur*; however, it presents several challenges, including altered colony morphology, inconsistent growth due to variations in oil quality, volatility hazards, limited oxygen availability, and frequent contamination or overgrowth by other fungi and bacteria (Buick et al. 1997). Modified Dixon's Agar (mDA) is a highly effective and versatile culture medium for *M. furfur*; nevertheless, its cost is high, its ingredient availability is restricted, and its deep hue results from the incorporation of many markers (Manna et al. 2015). The inadequate accessibility and feasibility of other agar mediums, such as Christensen’s urea agar, bromocresol purple milk agar, and Egg yolk agar in many developing countries and resource limited settings, contribute to a lack of enthusiasm for *in vitro* research on *M. furfur* (Wanigasekara et al. 2019).

Previous studies in Sri Lanka have explored the supplementation of SDA with various lipid sources, including butter, ghee, margarine, milk powder, virgin coconut oil, castor oil, olive oil, and sesame oil, to support the growth of *M. furfur*. In this study, egg yolk lipid oil was chosen as the lipid supplement, marking the first time it has been used for this purpose. This decision was based on its biological relevance and its close compositional resemblance to the lipids found in human skin. Although prior research has demonstrated the successful use of egg yolk agar for culturing *M. furfur*, particularly for assessing phospholipase activity, the use of egg yolk oil specifically introduces a novel approach to investigating the biochemical properties of this species (Jain et al. 2017).

Egg yolk lipids offer several advantages: they are easily affordable, widely accessible, and serve as a suitable alternative lipid supplement. However, the direct use of whole liquid egg yolk in agar media is not always appropriate, since egg yolk contains not only lipids but also proteins, water, minerals, and pigments (Abeyrathne et al. 2022). These non lipid components may promote the growth of undesired microorganisms. Therefore, extracting oil from egg yolk provides a more purified and targeted supplementation, enhancing medium specificity and reducing the risk of

contamination. Additionally, egg yolk oil has a longer shelf life compared to whole egg yolk, facilitating the preparation and storage of supplemented media for extended use in long-term research.

This study aimed to develop and evaluate a cost-effective culture medium, sabouraud dextrose agar supplemented with egg yolk oil, for the isolation and subculture of *M. furfur*. Furthermore, the study aimed to determine the minimum oil volume required for optimal growth, incubation temperature, evaluate the effectiveness of selected additives in enhancing fungal growth, and compare the performance of the modified medium with standard agar formulations.

## Materials & Methods

### Study population and Sample size.

Isolated colonies of *M. furfur* were obtained from the primary culture of skin swabs collected from patients diagnosed with pityriasis versicolor who attended the Dermatology Unit of the Teaching Hospital, Jaffna. This laboratory based experimental study involved collecting skin swab samples from 25 patients for isolation of the organism. A total of 26 culture plates were prepared for each media type for comparative analysis, including SDA, SDA supplemented with ghee (10%), and the modified media.

### Inclusion and Exclusion Criteria

All male and female patients clinically diagnosed with pityriasis versicolor were included. Patients who had received antifungal therapy within the previous seven days were excluded.

### Extraction of oil from egg yolk

Fresh eggs of *Gallus domesticus* were obtained from local breeders in Jaffna District, Northern Province, Sri Lanka. Oil extraction followed the protocol of Kovalcuks & Duma (2014) with minor modifications. Egg yolk oil was extracted using a 2-propanol: hexane solvent mixture (30:70, v/v) at room temperature (25 °C). Liquid egg yolk was added to the solvent in a 1:2 ratio (yolk: solvent) and vigorously stirred in an ice bath at 21 °C for 30 minutes. The resulting mixture was filtered sequentially through filter paper and sterile cotton cloth, and the filtrate was collected in a sterile, labeled container. The egg yolk oil was recovered by solvent evaporation under vacuum at 80 °C using a rotary evaporator.

Preliminary extraction used three egg yolks, and the mean yolk volume was determined using the formulas described by Han & Lee (1992):

$$\text{Mean yolk Volume} = \frac{\text{Total Volume of 03 Egg yolks (mL)}}{03} \quad (1)$$

$$\text{The mean volume of extracted lipid oil from an egg yolk (x)} = \frac{\text{Volume of oil (mL)}}{\text{Number of Egg yolks used}} \quad (2)$$

Where x is the volume of extracted oil from one egg yolk. The extracted oil was stored at 4 °C until further usage. The presence of lipids in the extracted oil from egg yolk was tested by using a Sudan III reagent.

### Fungal isolates collection

### Data collection and sample collection

Ethical approval was obtained from the Ethical Review Committee, Faculty of Medicine, University of Jaffna, in the 157$^{th}$ ERC meeting held on 28$^{th}$ March 2024. Informed consent was obtained from participants or guardians attending the Dermatology Unit, Teaching Hospital, Jaffna, Northern Province, Sri Lanka. Sample collection was approved by the Hospital Director and Dermatologist. Pityriasis versicolor was confirmed by a Consultant Dermatologist/ Medical Officer/ Resident house officer, and both tape and skin swab samples were collected.

Suspected patients were examined, and sample sites confirmed. Skin swabs and cellophane tape samples were collected by Consultant Dermatologist/ Medical Officer/ Resident house officer using standard procedures. Samples were labeled, sealed, and transported within 15 minutes at room temperature to the Medical Laboratory Sciences Laboratory, University of Jaffna, following guidelines and precautionary measures.

**Primary culturing of *M. furfur***

**Preparation of Sabouraud Dextrose Agar (SDA) media supplemented with ghee (10%) (positive control culture medium) and SDA (negative control culture medium)**

An amount of 32.5 g of the SDA base, as specified by the manufacturer, was suspended in 500 milliliters of distilled water, then 50 mL of melted ghee was added. Then it was sterilized by autoclaving at 121℃ for 15 minutes. After autoclaving, 10 mL of gentamicin IV solution (80 mg/2 mL) was added. Then the base was cooled to 50 ℃. Finally, the media was rotated gently and poured (20 mL per plate) into Petri dishes. The sterility of the prepared media was checked by incubating at 32 ℃ for 7 days. This agar medium was considered a positive control (Wanigasekara et al. 2019). The negative control culture medium was prepared using standard sabouraud dextrose agar (SDA) with gentamicin, without the addition of ghee or any lipid supplementation (Thliza et al. 2020).

**Inoculation of skin swab samples**

One set of plates was used for each positive and negative culture inoculation. Normal bacteriological streaking (isolation streaking) method was performed. Skin swab samples were inoculated onto SDA media supplemented with ghee and incubated at 32 ℃ in an incubator for 7 days.

**Confirmation of *M. furfur***

The presence of *M. furfur* was determined through microscopic, macroscopic, and biochemical examinations. For direct microscopic observation, a cellophane tape preparation was examined under the microscope, revealing the characteristic "spaghetti and meatball" morphology, consisting of hyphae and spores, which served as the preliminary indicator of *M. furfur* in the clinical specimen.

For macroscopic examination, swab cultures obtained from the same patient were incubated, and colony growth was observed on the third day on the primary culture plate. The colonies appeared shiny, pasty, and white to cream in color, later becoming dull and beige upon maturation. Biochemical confirmation was performed using the catalase test. In this test, 3% hydrogen peroxide (10 vol) was added to a Khan tube, and an isolated colony was immersed using a sterile wooden applicator. The production of gas bubbles was recorded as a positive catalase reaction, confirming the presence of *M. furfur*. All isolates were identified based on concordant findings from microscopic, macroscopic, and biochemical analyses (Kindo et al. 2004).

**Assessment of optimal growth of *M. furfur***

A semi-quantitative method was used to identify the growth of *M. furfur*. Quantification of growth from swabs was evaluated on a 5-point scale, and scores were provided as mentioned below in Table 1 (Wanigasekara et al. 2019). In addition to growth level, the presence of isolated colonies and isolated colony size were also recorded. The assessment was performed independently by five observers, who were blinded to the experimental conditions.

**Preparation of modified culture media (SDA supplemented with lipid oil extracted from egg yolk)**

**Sterility checks for the extracted lipid oil from egg yolk**

Two sets of SDA plates were prepared. One plate was streaked with extracted oil from egg yolk. The remaining plate was not streaked with egg yolk oil. Plates were incubated at 32 ℃ for 7 days. After 7 days plates were examined for sterility. The experiment was replicated to ensure reproducibility.

**Table 1** Assessment of optimal growth

| | |
|---|---|
| 0 | No growth / NIL |
| 1+ | If the growth is limited to the well |
| 2+ | If the growth up to the first set of streak lines |
| 3+ | If the growth up to the second set of streak lines |
| 4+ | If the growth up to the limits of streaks |

**Determination of the minimal volume-to-volume ratio of extracted oil from egg yolk for the optimal growth of the *M. furfur***

An amount of 27.3 g of SDA base, as specified by the manufacturer, was suspended in 420 mL of distilled water. As shown in Table 2, SDA media with different oil concentrations were prepared. One flask was left without added oil, while the remaining flasks were supplemented with varying volumes of extracted egg yolk oil 2x, x, x/2, x/4, x/8, and x/16. The media were sterilized by autoclaving at 121 ℃ for 15 minutes. After autoclaving, 1.2 mL of gentamicin IV solution (80 mg/2 mL) was added to each sterilized medium. The media were cooled to 50 ℃, gently mixed, and poured (20 mL per plate) into Petri dishes. Triplicate sets of plates were prepared for each oil concentration. Isolation streaking was performed by streaking the yeast colonies from primary culture plates (SDA supplemented with ghee) onto SDA plates containing different volumes of egg yolk oil. Plates were incubated at 32 ℃ for 7 days. After incubation, growth was evaluated using a semi quantitative method (Table 1), and the minimum volume of extracted oil supporting optimal growth was selected for further investigations.

**Table 2** Preparation of different egg oil concentrations

| Vol. of Oil (mL) | 2x | x | x/2 | x/4 | x/8 | x/16 |
|---|---|---|---|---|---|---|
| Vol. of Agar (mL) | 20 | 20 | 20 | 20 | 20 | 20 |

*x- Standard volume of oil extracted from one egg yolk.

**Optimization of the temperature with the predetermined optimal minimal volume-to-volume ratio of extracted oil from egg yolk for the optimal growth of the *M. furfur***

SDA medium was prepared and supplemented with the previously determined optimal concentration of extracted egg yolk oil, following standard microbiological protocols. The sterilized medium was cooled and poured into Petri dishes. *Malassezia furfur* isolates from primary cultures were inoculated using the isolation streaking method. Triplicate sets were incubated at 32 °C and 37 °C for 7 days. These temperatures were selected based on published literature. Growth was assessed semi quantitatively (Table 1), and the temperature supporting optimal growth was selected for further investigations.

**Evaluation of optimal growth of *M. furfur* with and without additional components present in egg yolk agar, with predetermined optimal conditions**

SDA medium was prepared and supplemented with the previously determined optimal concentration of extracted egg yolk oil, following standard microbiological protocols. The medium was divided into two portions: one was further supplemented with $Na_2HPO_4$, NaCl, and $MgSO_4$, while the other was not supplemented and maintained as a control. The additives were selected based on published literature to enhance growth (Buick et al. 1997). After sterilization and cooling, the medium was poured into Petri dishes, with triplicate dishes prepared for each condition. All plates were incubated at the predetermined optimal temperature for 7 days, and growth was assessed semi-quantitatively (Table 1) to determine conditions supporting optimal growth.

**Preparation of modified culture media with all the predetermined optimal conditions**

Modified SDA culture media were prepared by using optimal conditions, such as a minimal volume-to-volume ratio of extracted oil from egg yolk, temperature, and additional substances selected from the study, and it was incorporated and evaluated. Isolates from previous fungal cultures were inoculated into modified culture media. Triplicate sets were prepared. After seven days of incubation, optimal growth was evaluated by using a semi quantitative method shown in Table 1.

The initial formulation of the medium did not include specific pH adjustment. However, *M. furfur* is known to grow optimally at a mildly acidic pH (5.0–6.0). For future optimization, maintaining the pH at 5.5 ± 0.1 before sterilization is recommended to enhance consistency and reproducibility.

**Comparative study for *M. furfur* with previously described formulations - SDA and SDA supplemented with ghee (10%) media and modified culture media**

The performance of the modified mycological culture medium supplemented with extracted egg yolk oil was compared with previously described formulations, including SDA and SDA supplemented with ghee (10%), for the primary isolation of *M. furfur*. Parameters assessed included growth level, presence of isolated colonies, and isolated colony size. A total of 26 culture plates were prepared for each media type for comparative analysis.

**Data analysis**

Semi-quantitative method shown in Table 1 was used to identify the growth level of the *M. furfur*. In cases where growth levels were equivalent, the presence of isolated colonies and colony size were also measured to distinguish potential differences. Growth assessment was undertaken in triplicate for each SDA medium supplemented with different volume-to-volume ratios of extracted oil based on a semi-quantitative grading method. Scores were given, and the highest average growth scores of the triplicate cultures were used to determine the minimal volume of oil required for optimal growth by excluding the other volumes of oil supplemented SDA medium. Subsequently, using the selected minimal optimal volume of egg oil-supplemented medium, the temperature was optimized. Growth was assessed at 32 ° C and 37 °C temperatures, and the temperature that yielded the highest mean growth score from the triplicate cultures was chosen as optimal by excluding the alternative temperature conditions. Under the optimized lipid volume and temperature, the efficacy of adding additional substances was evaluated. Substances were assessed based on whether they enhanced growth, determined by the highest mean growth scores.

A comparative study for the primary isolation of *M. furfur* with previously described formulations (SDA, SDA supplemented with ghee (10%)), and modified SDA medium was conducted. Isolated colony size was used as an indicator of culture performance. The isolated colony size was reported as the mean and standard deviation (SD), and the data were subjected to examination by analysis of variance (ANOVA) ($P<0.05$) followed by Tukey's test ($\alpha = 0.05$) by using software, SPSS 21.0 for the Windows version (Fabros et al. 2024).

**Results**

**Determination of the volume of extracted oil from egg yolk and Determination of the yield in percentage of oil from egg yolk volume**

The volume of oil extracted from one egg yolk was determined as 2 mL. The yield in percentage of the total extracted oil from 23 egg yolks was calculated using the following equation 3.

$$\text{Yield in percentage} = \frac{V_1}{V_2} \times 100\ \% \quad (3)$$

Where $v_1$ (mL) is the extracted oil from 23 egg yolks and $v_2$ (mL) is the total volume of 23 egg yolks. The yield in percentage of extracted oil from egg yolk was 33.33% (Fig. 1).

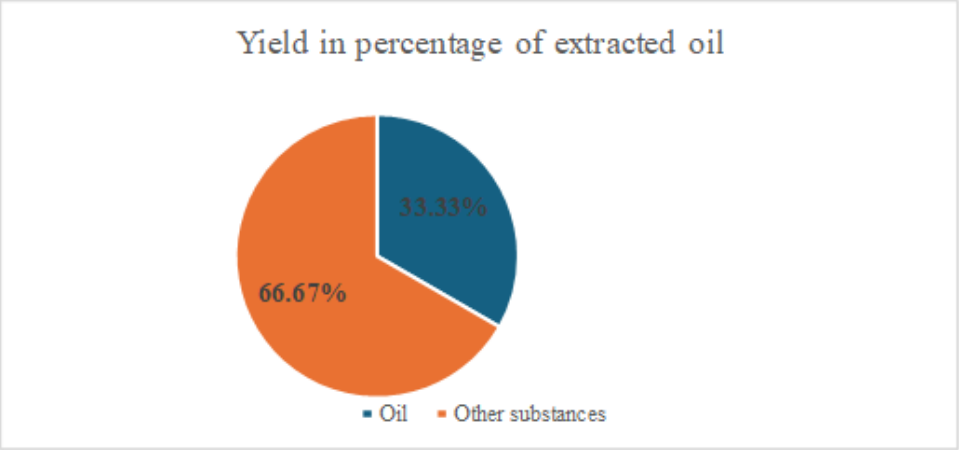


**Fig. 1** – Yield in percentage of extracted egg yolk oil.

The presence of lipids in the extracted egg yolk oil was confirmed using Sudan III reagent. Upon addition of the reagent, a distinct red coloration was observed, indicating lipid presence. This result was consistent with the positive control, whereas the negative control showed no color change**.**

**Confirmation of *M. furfur***

The presence of *M. furfur* was confirmed by using microscopic, macroscopic, and biochemical examinations. Direct microscopic examination of the cellophane tape sample exhibits the presence of a "Spaghetti and meatball" (spores and hyphae) appearance (Fig. 2), which was considered the preliminary identification for the presence of *M. furfur* in the clinical specimen.

The appearance of colonies obtained from the swab culture of the same patient was evident from the 3$^{rd}$ day of incubation in the primary culture plate. The colonies of *M. furfur* initially appeared shiny and pasty white to cream in color, gradually transitioning to a dull, beige appearance over time.

A catalase test was done on the colonies obtained from the swab culture as a biochemical confirmation. Positive reaction indicated by the release of gas bubbles confirmed the presence of *M. furfur*. All the confirmatory tests were examined by experts. After the confirmation, the isolated colonies of *M. furfur* were used for further subculturing. These confirmatory tests were done for each batch of isolated colonies of *M. furfur*.

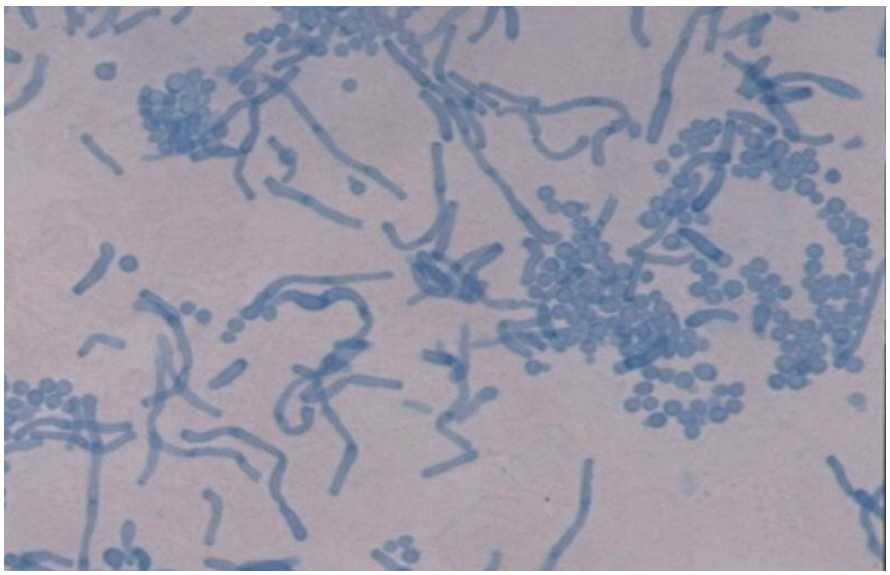

**Fig. 2** – Exhibiting spaghetti and meatball appearance under microscope (x40).

**Determination of the minimal optimal volume of extracted oil from egg yolk required for optimal growth of *M. furfur***

The appearance of colonies was evident from the 3rd day onwards on all the SDA culture plates supplemented with different volumes of extracted lipid oil. Corresponding readings, such as level of growth (5-point scale), the presence of isolation, and size of isolated colonies (mm), were taken on the 3rd and 5th days of incubation. These results were tabulated below in Table 3.

**Table 3** Mean Level of growth, isolation, and colony size against different volumes of extracted egg yolk oil on the 3rd and 5th day

| Volume of Oil | Mean Values on the 3rd Day | | | Mean Values on 5th Day | | |
|---|---|---|---|---|---|---|
| | Mean Growth (Day 3) | Isolation | Colony Size (mm) ±SD | Mean Growth (Day 5) | Isolation | Colony Size (mm) ±SD |
| 2x | 4 | P | 1.5 ± 0.1 | 4 | D | 0 |
| X | 4 | P | 1.5 ± 0.1 | 4 | D | 0 |
| x/2 | 4 | P | 1.5 ± 0.1 | 4 | P | 2.0 ± 0.1 |
| x/4 | 4 | P | 1.5 ± 0.1 | 4 | P | 2.0 ± 0.1 |
| x/8 | 4 | P | 1.5 ± 0.1 | 4 | P | 2.0 ± 0.1 |
| x/16 | 4 | N | 0 | 4 | N | 0 |

(P – present; N – absent; D – Diffused)

Mean growth, isolation, and colony size of *M. furfur* on days 3 and 5 at different volumes of extracted egg yolk oil were recorded. Triplicate measurements were performed for each volume, and the minimal optimal volume supporting maximal growth is indicated. Based on the observations of the 3rd and 5th day of incubation, x/2, x/4, and x/8 exhibited similar Growth level (4), Isolation (P), and colony size (2.0 ± 0.1 mm). Among them, x/8 was selected as the minimal optimal volume for optimal growth of *M. furfur* for further study.

**Determination of the preferable growth temperature, efficacy of adding additive substances ($Na_2HPO_4$, NaCl, $MgSO_4$), and evaluation of the growth of *M. furfur* in the modified culture media with all the predetermined optimal conditions**

The appearance of colonies was evident from the 3rd day onwards on all the SDA culture plates incubated under different conditions. Corresponding readings, such as level of growth (5-point scale), the presence of isolation, and size of isolated colonies (mm), were taken on the 3rd and 5th days of incubation. These results are tabulated below in Table 4.

**Table 4** Mean Level of growth, isolation, and colony size against temperature, additives, and modified culture media on the 3rd and 5th day.

| Conditions | Mean Values on the 3rd Day | | | Mean Values on the 5th Day | | |
|---|---|---|---|---|---|---|
| | Mean Growth (Day 3) | Isolation | Colony Size (mm) ±SD | Mean Growth (Day 5) | Isolation | Colony Size (mm) ±SD |
| 32 °C | 4 | P | 1.5 ± 0.1 | 4 | P | 2.0 ± 0.1 |
| 37 °C | 4 | N | 0 | 4 | N | 0 |
| 32 °C with Additives | 4 | P | 1.9 ± 0.1 | 4 | P | 2.7 ± 0.1 |
| 32 °C without Additives | 4 | P | 1.5 ± 0.1 | 4 | P | 2.0 ± 0.1 |
| Modified culture media | 4 | P | 2.0 ± 0.1 | 4 | P | 2.7 ± 0.1 |

(P – present; N – absent)

Considering the above observations on the 3rd and 5th day of incubation, at 32 °C, colonies exhibited Growth level (4), Isolation (P), and colony size (2.0 ± 0.1 mm). Therefore, 32 °C was selected as the preferred optimal temperature for the growth of *M. furfur* as it promotes the formation of isolated colonies. These findings were used for further studies.

With additives after the 3rd and 5th day of incubation, colonies exhibited Growth level (4), Isolation (P), and colony size (2.7 ± 0.1 mm). As indicated by the observations, the addition of additives such as $Na_2HPO_4$, NaCl, and $MgSO_4$ is enhancing the efficacy of the growth of *M. furfur*. Therefore, these findings were used for further investigations. Under all the optimized conditions in modified culture media, after 3rd and 5th day of incubation, colonies exhibited Growth level (4), Isolation (P), and colony size (2.7 ± 0.1 mm).

**Evaluation of the growth of *M. furfur* in the SDA and SDA supplemented with ghee (10%) media**

Corresponding readings, such as level of growth (5-point scale), the presence of isolation, and size of isolated colonies (mm), were taken on the 3rd and 5th days of incubation. These results were tabulated below in Table 5. Considering the observations on the 3rd and 5th day of incubation, SDA supplemented with Ghee medium, colonies exhibited Growth level (4), Isolation (P), and colony size (1.8 ±0.1 mm), and in SDA medium, colonies exhibited Growth level (1); none of the plates formed isolated colonies.

**Table 5** Mean Level of growth, isolation, and colony size in SDA+Ghee and SDA culture media on the 3rd and 5th day.

| | Mean Values on the 3rd Day | | | Mean Values on 5th Day | | |
|---|---|---|---|---|---|---|
| | Mean Growth (Day 3) | Isolation | Colony Size (mm) ±SD | Mean Growth (Day 5) | Isolation | Colony Size (mm) ±SD |
| SDA+ Ghee culture media | 4 | P | 0.8 ± 0.2 | 4 | P | 1.8 ± 0.1 |
| SDA culture media | 1 | N | 0 | 1 | N | 0 |

(P – present; N – absent)

**Comparative study for *M. furfur* with previously described formulation SDA and SDA supplemented with ghee media (10%) and modified culture media**

This comparison was designed to demonstrate that the performance of our modified medium was statistically significant in enhancing the primary isolation of *M. furfur*. Corresponding readings, such as level of growth (5-point scale), the presence of isolation, and size of isolated colonies (mm), were taken on the 3rd and 5th days of incubation. These results were tabulated below in Table 6.

**Table 6** Comparative study for *M. furfur* with previously described formulation, SDA, and SDA supplemented with ghee media and modified culture media for evaluation of growth level, isolation, and colony size on the 3rd and 5th day.

| Media Type | Mean Values on 3rd Day | | | Mean Values on 5th Day | | |
|---|---|---|---|---|---|---|
| | Mean Growth (Day 3) | Isolation | Colony Size (mm) ±SD | Mean Growth (Day 5) | Isolation | Colony Size (mm) ±SD |
| SDA+ Ghee | 4 | P | 0.8 ± 0.1 | 4 | P – 81%<br>D - 19% | 1.4 ± 0.7 |
| Modified | 4 | P | 2.0 ± 0.1 | 4 | P | 2.7 ± 0.2 |
| SDA | 1 | N | 0 | 1 | N | 0 |

(P – present; N – absent; D – Diffused)

On day 3, both SDA supplemented ghee and the modified medium achieved growth level 4, producing isolated colonies with mean sizes of 0.8 ± 0.1 mm and 2.0 ± 0.1 mm, respectively, whereas

SDA showed limited growth (50% no growth; 50% level 1). By day 5, ghee-supplemented SDA maintained growth level 4, with 19% diffuse and 81% isolated colonies (mean size 1.4 ± 0.7 mm), while the modified medium showed superior performance with all plates at growth level 4 and larger colonies (2.7 ± 0.2 mm). In contrast, SDA alone was restricted to growth level 1 with no isolated colonies (Fig. 3).

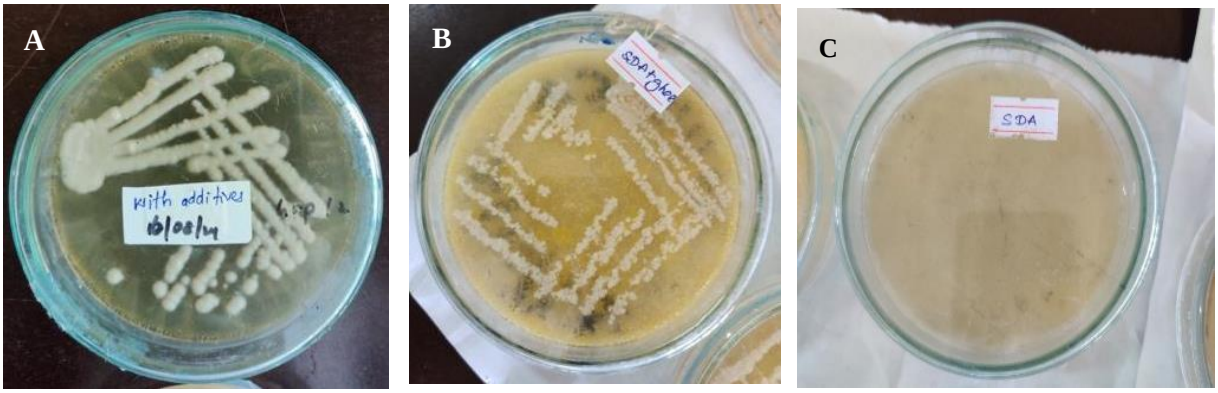


**Fig. 3** – Growth of *M. furfur* on different culture media. A- Modified culture media. B- SDA+Ghee. C- SDA.

One-way ANOVA results were tabulated below in Table 7. The colony size increases in the following order on the 3rd and 5th days, respectively: SDA (0 mm) < SDA supplemented with Ghee (0.8 ± 0.1 mm) < modified culture media (2.0 ± 0.1 mm) for day 3, and SDA (0 mm) < SDA supplemented with Ghee (1.4 ± 0.7 mm) < modified culture media (2.7 ± 0.2 mm) for day 5.

**Table 7** Mean colony size of *M. furfur* on the 3rd and 5th day of incubation

| | **SDA** | **SDA + GHEE** | **MODIFIED** |
|---|---|---|---|
| Colony Size in mm on the 3rd day (Mean ± SD) | $0^{c}$ | $0.8 \pm 0.1^{b}$ | $2.0 \pm 0.1^{a}$ |
| Colony Size in mm on the 5th day (Mean ± SD) | $0^{c}$ | $1.4 \pm 0.7^{b}$ | $2.7 \pm 0.2^{a}$ |

The values represented as mean standard deviation values with different superscripts, in the same row, differ significantly ($p < 0.05$).

**Discussion**

In our study, egg yolk oil was extracted following the protocol established by Kovalcuks & Duma (2014), utilizing a solvent extraction method with 2-propanol and hexane as solvents. The yield of egg yolk oil obtained was 33.33%, in contrast to the 28.90% yield reported in their study. Despite employing the same extraction methodology, the variation in yield can likely be attributed to differences in the composition and volume of the egg yolk, influenced by factors such as the diet of the source animal and geographical location.

In the present study, a modified mycological culture medium was developed for *M. furfur* by supplementing oil extracted from egg yolk. This is the first reported study to utilize egg yolk oil as a lipid supplement for *M. furfur*. The optimal growth conditions were determined as a minimal lipid volume of x/8 (250 µL) and an incubation temperature of 32 °C. Supplementation with $Na_2HPO_4$, NaCl, and $MgSO_4$ further enhanced growth, producing a maximum growth level of 4 and a mean colony size of 2.7 ± 0.1 mm, which falls within the typical 2–3 mm range for *M. furfur*.

These findings are consistent with those of Manna et al. (2015), who reported similar growth levels on modified Dixon agar (mDA). However, colonies grown on coconut milk agar (CMA) in

their study were smaller (1–2 mm), suggesting that CMA may lack certain essential nutrients present in mDA. Similarly, Vijayakumar et al. (2006) observed optimal growth on SDA supplemented with butter, reaching a growth level of 4, while Wanigasekara et al. (2019) reported luxuriant growth on SDA and PDA supplemented with butter or ghee, but poor or absent growth with margarine, olive oil, or virgin coconut oil. However, their study assessed only the presence or absence of a colony, limiting direct comparison.

Atsü et al. (2022) demonstrated that FastFung agar performed well for *M. furfur* isolation, achieving growth levels of 1 in 69–73% of samples, comparable to mDA. The comparatively higher growth observed in the present study may be attributed to the complex lipid composition of egg yolk oil. Suzuki et al. (2022) developed artificial sebum containing mLNA, designed to replicate the physical properties and HLB of natural human sebum; unfortunately, it was very costly. In contrast, while our modified culture medium also supports the robust growth of *M. furfur*, it offers a significant advantage in terms of cost effectiveness, making it a practical alternative for routine laboratory use.

In our study, the optimal growth temperature for *M. furfur* was found at 32 °C, accompanied by a mean colony sized 2 mm. This finding contradicts some previous studies. Vijayakumar et al. (2006) identified 30 ± 2 °C as the optimal growth temperature for *M. furfur*, while Dhanablan et al. (2022) found that temperatures between 30 °C and 37 °C supported growth, with minimal growth below 30 °C. The lack of isolated colonies at 37 °C in our study could be due to factors like the modified media composition, lipid source, or yeast strain. Additionally, variations in experimental conditions, such as humidity, pH, or incubation time, may have influenced the results, indicating a need for further research to optimize growth conditions.

The study conducted by Manna et al. (2015) indicated that *M. furfur* exhibits optimal growth at a pH range of 5.5 to 7.5. Similarly, Vijayakumar et al. (2006) identified an optimal pH range of 7 to 9 for the growth of *M. furfur*. These findings suggest that *M. furfur* has specific pH preferences, and deviations from these ranges can significantly hinder growth due to potential disruptions in metabolic processes or enzyme activities. Although pH was not controlled in the present study, previous literature indicates that the growth of *M. furfur* is influenced by the medium's acidity. Studies have reported optimal growth at pH 5.0–6.0, corresponding to the natural skin environment. Therefore, future investigations should include pH standardization to confirm its impact on lipid utilization and colony morphology.

Traditional culturing techniques employing SDA with an olive oil overlay have several limitations, including contamination and inconsistent streaking (Chua et al. 2005; Manna et al. 2015; Rathnapriya et al. 2016). The modified medium developed here overcomes these issues by incorporating essential lipids directly into the aqueous phase, eliminating the need for an oil overlay. This prevents contamination, improves streak formation, and enhances colony morphology for accurate identification. Additionally, incorporating gentamicin before autoclaving effectively reduced bacterial contamination, aligning with methods used by Buick (1997) and Chua et al. (2005).

Comparative analysis revealed that colony sizes increased in the order SDA < SDA + Ghee < Modified Medium. ANOVA results confirmed significant differences among the media on both the 3$^{rd}$ and 5$^{th}$ incubation days, with the modified medium yielding the largest colonies. Chua et al. (2005) previously compared IMU-Mf and Leeming Notman agar, reporting superior performance over Dixon's agar, while SDA with oil overlay failed to support discrete colonies. Their study also faced higher contamination rates, which were minimized in the present study by using subcultured isolates and antibiotic supplementation. The present study, however, faced two major limitations: standardized commercial media such as Leeming Notman agar were not locally available, and a standard *M. furfur* reference strain was unavailable in Sri Lanka. Consequently, SDA and SDA + Ghee were employed as reference formulations. The observation of colony formation on SDA suggests that *M. furfur* may utilize alternative nutrients in SDA through non-lipolytic enzyme activity, consistent with the findings of Vijayakumar et al. (2006). In SDA + Ghee plates, colonies observed on day three became diffused by day five, possibly due to variations in ghee quality, whereas colonies on the modified medium remained morphologically stable. Future research should

focus on incorporating reference strains and standardized media, as well as developing lipid enriched agar formulations using freeze dried egg yolk extract.

Overall, this modified culture medium stands out as a novel approach and significantly contribute to a deeper understanding of dermatological conditions caused by *M. furfur*, suitable for laboratories worldwide, including resource limited settings.

## Conclusions

A modified mycological culture medium was developed for *M. furfur* by incorporating oil extracted from egg yolk. The optimized conditions, including minimal lipid volume (250 µL), incubation at 32 °C, and supplementation with $Na_2HPO_4$, NaCl, and $MgSO_4$, supported reproducible and enhanced fungal growth. This approach provides a biologically compatible, cost-effective, and scalable alternative to conventional media, highlighting the potential of nature-based formulations for mycological research. Future studies should address variability in egg yolk composition and pH effects to further standardize this medium for broader applications.

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## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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