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Effect of Low-Intensity Ultrasound – Continuous Mode on Candida Parapsilosis

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Abstract

The opportunistic fungus Candida parapsilosis is the cause of both invasive and superficial illnesses. Fungal growth may be impacted by low-intensity ultrasound through mechanical and thermal effects. The study aimed to assess the effects of continuous low-intensity ultrasound at different exposure times on the colony diameter and growth of Candida parapsilosis. Ninety samples (skin swabs) were taken between 20 -50 years old patients who had been clinically diagnosed as having candidiasis and referred to Kirkuk and Azadi Teaching Hospital. Fungal isolates were identified using both macroscopic and microscopic traits after being cultivated on Sabouraud Dextrose Agar (SDA). Continuous ultrasound at 40 kHz and 0.35 W/cm² intensity was used for culture irradiation with varying exposure time(1-6min). Fungal colony diameter and dry weight were measured. Fungal growth progressively reduced at each increasing CLIU exposure time. The mean of colony diameter decreased significantly from ($p < 0.001$) from (4.20 to 1.70cm) at each increasing in exposure time. The inhibition in Growth recorded 59.52%, relative growth 40.48%, and dry weight decreased from (89 mg to58 mg) after 6 minutes, representing a reduction of 34.83%. Continuous low-intensity ultrasound at 40 kHz and 0.35 W/cm² inhibited the growth of Candida parapsilosis in a time-dependent manner; after six minutes, the inhibition reached 59.52%. These results suggest that it could be used as a physical strategy to inhibit fungus growth.

Keywords

Candida parapsilosis,
Continuous Low Intensity Ultrasound,
Growth and
Relative growth.

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1.Introduction

A form of therapeutic ultrasound called low-intensity ultrasound (LIUS) delivers continuous acoustic waves throughout the exposure time. Continuous ultrasound applies acoustic energy to the exposed medium continuously, in contrast to pulsed ultrasound, which alternates between ultrasonic emission and rest. The biological effects of the target medium are influenced by its physical characteristics, frequency, intensity, and duration of exposure [1]. Thermal and non-thermal biological effects can be produced by ultrasound [2].

The absorption of acoustic energy produces thermal effects, which could lead to a slow rise in temperature. The majority of non-thermal effects are related to mechanical interactions, such as fluid streaming, cavitation, and acoustic radiation force [3]. The physical surroundings of biological cells and membrane permeability may be impacted by these mechanisms [4]. Acoustic cavitation is a significant ultrasonic mechanism that involves the creation, oscillation, and potential collapse of tiny gas bubbles in a liquid media. Shear stress, microstreaming, and localized mechanical stresses could result from these actions, which could have an impact on cellular membranes and the surrounding environment [5] The applied acoustic settings and exposure conditions affect the biological reaction to continuous low-intensity ultrasound. As a result, it is incorrect to believe that its effects are only stimulatory or inhibitory. Clarifying the possible impact of ultrasound on microbial growth and viability may be possible by examining these effects in controlled laboratory settings. [6]

A significant human pathogen, especially in infections linked to healthcare, is the opportunistic, yeast-like fungus Candida parapsilosis [7, 8]. It belongs to the species complex of Candida parapsilosis, which also contains Candida orthopsilosis, Candida metapsilosis, and Candida parapsilosis [9]. This yeast is frequently linked to the human skin and can spread through contaminated the medical equipment and direct touch. It is especially crucial for newborns, hospitalized patients, and people with compromised immune systems [10, 11]. Under the right circumstances, it can cause invasive and superficial infections even though it may exist as a commensal bacterium [12].

C. parapsilosis's pathogenicity is linked to a number of virulence factors, such as biofilm development, adherence to host tissues and medical devices, and the synthesis of extracellular hydrolytic enzymes like phospholipases and proteinases. Biofilm formation causes adhesion to medical surfaces, which may restrict antifungal drug susceptibility [13]. C. parapsilosis is a crucial organism in clinical microbiology because it has shown the capacity to acquire antifungal resistance, especially to azole and echinocandin medications [14]. Because of its growth and cellular properties, it is an appropriate subject for laboratory experiments looking at how physical treatments affect fungal survival and proliferation [15]. The study aimed to assess the effects of Continuous Low-Intensity Ultrasound in different exposure times on the colony diameter and growth of Candida parapsilosis.

2.Materials & methods

2.1. Yeast Collection

ninety samples (skin swabs) were taken between 20 - 50 years old patients who had been clinically diagnosed as having candidiasis and referred to Kirkuk and Azadi Teaching Hospital. The specimens were collected between January and September 2026. All swabs were transferred to the microbiology laboratory aseptically for culture set up.

2.2. Candida parapsilosis Separation and Identification

Sabouraud Dextrose Agar (SDA), a selective medium for fungi, was streaked with all skin swabs and cultured for 48 hours at 37 °C. After incubation, fungal colonies were first separated based on physical characteristics such diameter, color, hyphae structure, and colony shape. The smears were examined under a light microscope to confirm the

species of Candida, and Gram staining was also used.The isolates Candida identified by using VITEK2 YEST system manufactured by bio Merieux,France. The species level identified according to the manufacture instructions.

2.3. Ultrasound exposure

A low-frequency ultrasound transducer operating at 40 kHz was used to expose Candida parapsilosis cultures at a target intensity of 0.35 W/cm² in continuous mode. The transducer was positioned beneath the SDA culture plate at a distance of zero cm. A thin layer of ultrasonic coupling gel was applied between the transducer and the external bottom surface of the plate to ensure effective acoustic coupling and minimize air gaps. The exposure durations were 1, 3, 6, 9, and 12 minutes, while the control group received no ultrasound treatment. To minimize thermal effects, the culture plates were pre-equilibrated at 37°C. Medium temperature was measured immediately before and after exposure using a K-type thermocouple (±0.1°C). All experimental groups were evaluated in triplicate.

2.4. After -Irradiation Analysis

2.4.1. Colony Diameter Measurement

A 0.5-cm-diameter agar disk containing fungal growth that had been CLIU-treated was placed on brand-new, sterilized SDA. A similar disk of a culture that had not been exposed to radiation served as the control. Each treatment in the experiment contained three repetitions, and the plates were incubated for four days at 37 °C.

The fungal colonies' diameter was measured, and the inhibition percentage was computed using:

$$Inhibition\ Ratio\ (\%) = (MDCC - MDTC\ /\ MDCC) \times 100$$

MDCC: (Mean diameter of control Colonies).

MDTC: (Mean diameter of treated colonies).

2.4.2. Determination Of Dry weight:

The freshly produced culture (1% V/V) was added to sterile 100 ml SD broth in 150 ml glass flasks. Yeast inoculation: Four disks of irradiation yeasts (1, 2, 3, 4, 5, 6, exposure) per flask were combined with three duplicates and unfractionated control inoculums to create a composite yeast solution. For four days, cultures were cultivated at 37 °C. Fungal mycelia were filtered using pre-weighted sterile filter paper following incubation. The dry weight of the fungal biomass was then measured after the filter sheets were dried for 24 hours at 80 °C in an oven.

2.5. Statistical Analysis

For statistical comparisons, one-way ANOVA and T-tests were employed, with a significance threshold of $P < 0.05$ and a 95% confidence level. SPSS software was used for statistical analyzes.

3. Results

3.1. Colony Diameter:

At 40 kHz frequency, 0.35 W/cm2 acoustic intensity with continues mode and thermal control. Largest mean colony diameter was determined in the control group (4.20 cm) at (4.20 ± 0.14) in±0.1°C. The colony diameter reduced significantly ($p < 0.001$) from (4.20 to 1.70cm) at each increasing in exposure time (4.20 ± 0.14, 3.90 ± 0.14, 3.50± 0.13, 3.05 ± 0.12, 2.50± 0.10, 2± 0.09, 1.70± 0.06) respectively with a slight increasing recorded in temperature Figure (1).

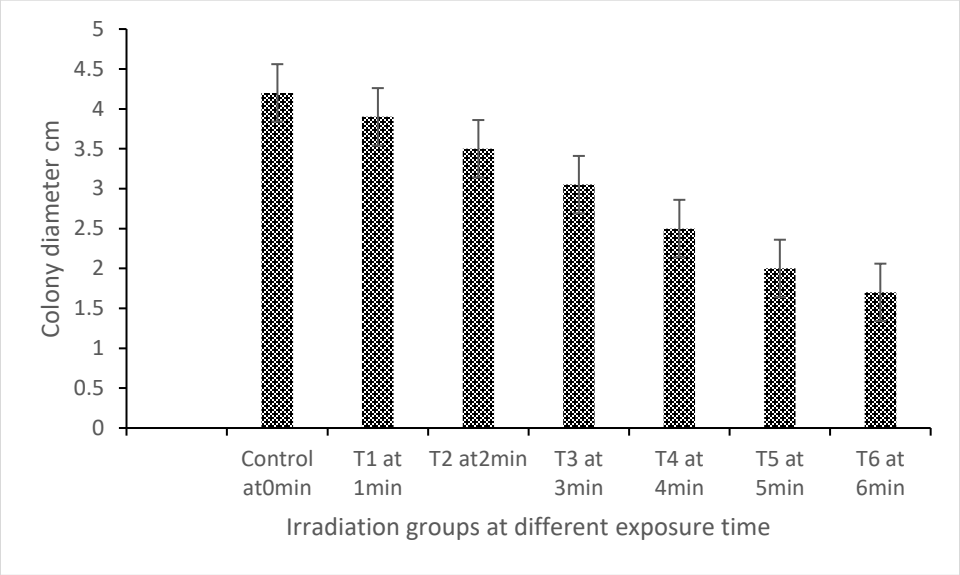


Figure1: The mean of Colony Diameter after CLIU irradiation at different exposure times with ***P < 0.001.

3.2. Inhibition Percentage of Colony Growth:

A significant inhibition colony growth found in group irradiated by low intensity ultra sound at 40 kHz frequency, 0.35 W/cm2 acoustic intensity with continues mode for six minutes (59.52%) in compared with control (0.0%) and other irradiated groups (7.14%, 16.67%, 27.38%, 40.48%, 52.38%) respectively Figure (2).

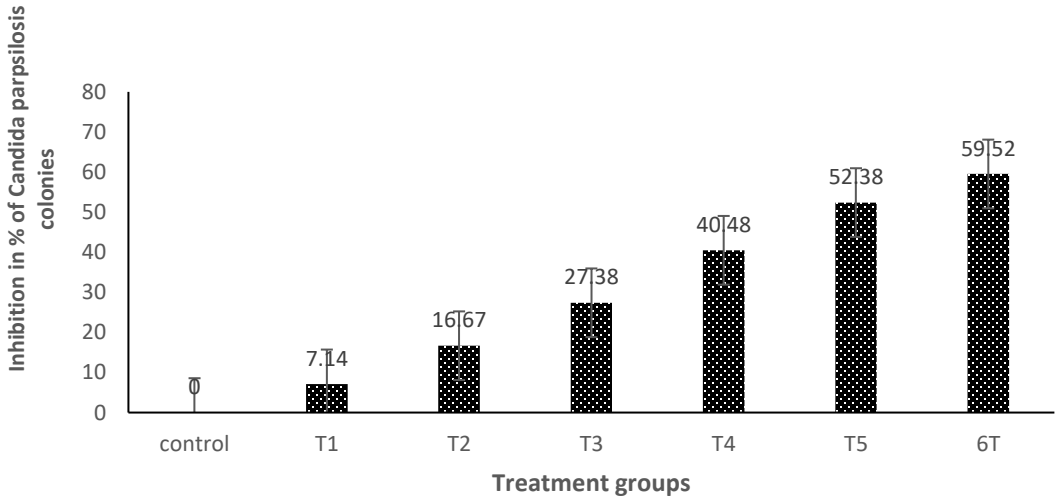


Figure2: Precentage of Candida parapsilosis inhibition after CLIU exposure with different times at*P < 0.001.**

Fungal Biomass Dry Weight:

In comparing the mean dry weight control group(89mg) with treatment groups the results showed the mean dry weight decreased at each increasing exposure time with $p < 0.001$ in group treated with 6min (58 mg) and (85,80, 75, 71,65 mg) respectively with other treatment groups Figure (3).

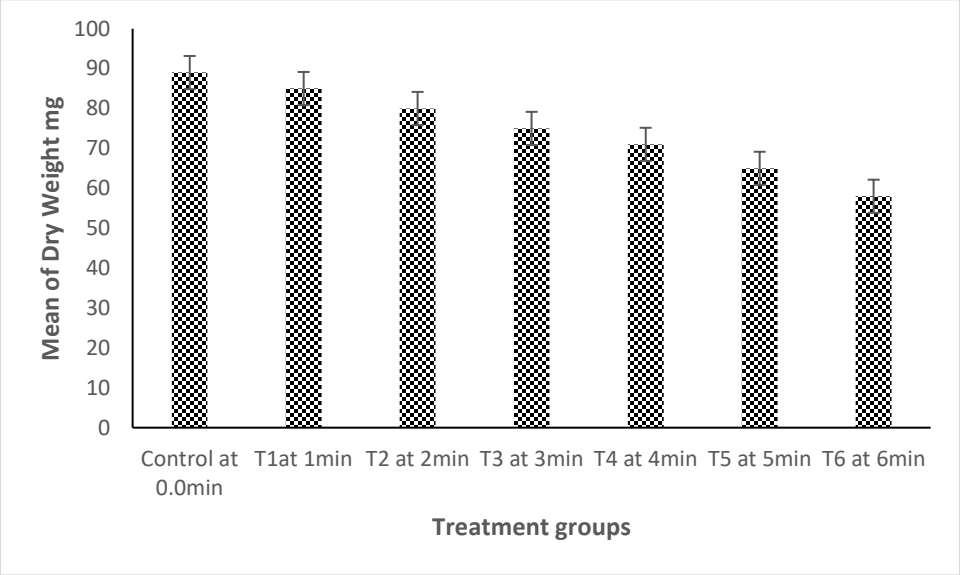


Figure3: Mean of dry weight after CLIU irradiation at ***P < 0.001.

As the ultrasonic exposure time increased at constant intensity of 0.35 W/cm², the results demonstrated a progressive decrease in Candida parapsilosis development. In the control group, the mean colony diameter dropped from 4.20 cm to 1.70 cm after six min of exposure. Similarly, growth inhibition rose from 7.14% to 59.52%, but dry weight dropped from 89 mg to 58 mg. These results point to a time-dependent inhibition of fungal growth by ultrasound Table (1).

Table 1: The Effect of continues low intensity ultra sound irradiation on inhibition dry weight, colony diameter, recovery percentage, and daily growth rate of *C. parapsilosis*

Treatment Groups	Irradiation time (sec)	Mean± SD Colony Diameter	Inhibition (%)	Mean± SD Dry Weight	DGR Cm\day	Relative growth (%)
Control	0	4.20 ± 0.14	0.00	89 ± 3	105	100
T1	1	3.90 ± 0.14	7.14	85 ± 3	0.98	92.86
T2	2	3.50± 0.13	16.67	80 ± 3	0.88	83.33
T3	3	3.05 ± 0.12	27.38	75 ± 2.2	0.76	72.62
T4	4	2.50± 0.10	40.48	71± 2.0	0.63	59.52
T5	5	2± 0.09	52.38	65± 1.6	0.50	47.62
T6	6	1.70± 0.06	59.52	5.8± 1.1	0.43	40.48

4. Discussion

The study showed that continuous low-intensity ultrasound exposure resulted in a progressive, time-dependent decrease in Candida parapsilosis proliferation Fig (1) The average colony diameter reduced from 4.20 cm to 1.70 cm after 6 minutes of exposure. As a result, the growth inhibition increased from 7.14% to 59.52%, and relative growth reduced from 92.86% to 40.48%. The findings suggest that longer exposure durations were linked with stronger suppression of a fungus colony formation. Fungal dry weight showed a similar pattern, decreasing from 89 mg to 58 mg after 6 min, representing approximately 34.83%.

The ultrasonic exposure observed decreasing both colony expansion and fungal biomass buildup is supported by an agreement both colony diameter and dry-weight measurements. These effects could be related to mechanical phenomena caused by ultrasound, including acoustic streaming and cavitation. Fluid movement and bubble oscillation may cause mechanical stress, compromise the integrity of fungal cell membranes, and obstruct the transfer of nutrients and cellular metabolism [16]. The current results are consistent with earlier research. According to Yang et al. (2018), cavitation and elevated intracellular reactive oxygen species were suggested as potential mechanisms by which 42-kHz ultrasound at 0.30 W/cm² improved the antifungal efficacy of amphotericin B-loaded nanoparticles against Candida albicans. Their work indicates the possible involvement of ultrasound in influencing fungal cells even if it involved a combination therapy. Their results indicate that low- intensity ultrasound may have an impact on fungal cells, even though they were treated with an antifungal medication.

According to Soro et al. (2021), ultrasound at 20 kHz caused noticeable alterations in yeast cell structures and decreased Saccharomyces cerevisiae counts by 1.3 log CFU/mL. These results confirm that ultrasound can affect yeast development, even though a different variety of yeast was examined. The idea of ultrasonic-associated growth inhibition is supported by a recent study by Liu et al. (2026) that found delayed C. albicans growth after exposure to low-intensity pulsed ultrasound. The decreased relative growth and dry weight as well as the higher inhibition percentage indicate that, under the circumstances of this experiment, longer ultrasonic exposure was linked to a greater suppression of C. parapsilosis growth.

Conclusions

Continuous low-intensity ultrasound at 40 kHz and 0.35 W/cm² inhibited the growth of Candida parapsilosis in a time-dependent manner; after six minutes, the inhibition reached 59.52%. These results suggest that it could be used as a physical strategy to inhibit fungal growth.

Limitations

The experiments in this study were done in vitro with fungal isolates. Nonetheless, the findings may serve as the base for future research investigating the possible use of ultrasound in the treatment of fungal infections in vivo.

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