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Comparison between two Techniques to evaluate the effect of fluconazole on some fungi isolated from laboratory instruments

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ABSTRACT

The purpose of this investigation was to review the antifungal activity of fluconazole on some fungi isolated from laboratory instrument. The study mediated twenty samples taken from laboratories in Al-Karkh University for Science, College of Science, department of microbiology in Baghdad. The sensitivity of fluconazole was tested using disk diffusion procedure and Food poisoning technique for the following molds (*Penicillium*, *Ulocladium*, *Fusarium*, *Aspergillus niger*, *Aspergillus fumigates*, *Botrytis*, and *Rhizopus*). The inhibitory effect of fluconazole using the food poisoning technique was the highest against Fusarium and *Botrytis* in contrast with *Rhizopus* showed resistance to fluconazole. The sensitivity of the isolated mold was tested using disk diffusion procedure for the antifungal drugs (fluconazole 10-µg disks). All the tested molds showed resistance to the fluconazole disk. Fluconazole and other azole medications block the enzyme 14-a-demethvlase, which transforms lanosterol into ergosterol, which is the main building block of fungal cell membranes.

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1. INTRODUCTION

More than 100,000 species of fungi have been discovered through over 100 years of study; the majority of these species are filamentous, saprophytic, yeast-like, or non-pathogenic soil microorganisms. A small number of these species have developed into real pathogens. Public spaces like hospitals and laboratories, are considered as preferred locations for a broad range of opportunistic and harmful fungi to emerge. Different fungi cause infections, some are common infections, whereas some are uncommon, depending on the presence in the environment or the high presence of fungal spores causes infection [2]. Everyone breathes in fungal spore because they are highly concentrated in the air, ranging from one to one hundred conidia per milliliter. Furthermore, it is commonly known that fungi commonly grow in food, tap water, homes, and offices [3], [26].

There are other sources of contamination and spread of microorganisms, such as the water cycle, one source of contamination and transmission, because water is used by a large number of people [6]. The number of antifungal drugs that can be applied to treat mycotic infections is limited but increasing [5]. Effective antifungal drugs often cause harm to membrane sterols or inhibit their production. Polyoxin D and Nikkomycin are two fungal-active antibiotics that target the enzyme chitin synthase [4], [21].

Often used as an antifungal medication, fluconazole inhibits Erg11, a protein involved in 14-demethylation during ergosterol production. As a result, hazardous 14-methylated sterols replace the depleted ergosterol in cellular membranes, increasing membrane fluidity and drug permeability and resulting in decreased biomass formation. Fluconazole-based antifungal prophylaxis has proved effective in lowering fungal colonization and infection [7], [19].

2. METHOD

Sample collection

From period October 2023 to December 2023, twenty samples were taken using sterile transport media swaps from various instruments in laboratories such as (hood, water bath, incubator, refrigerator, oven, autoclave and microscopes) in Al-Karkh University for Science, Collage of Science, department of microbiology in Baghdad.

Identification of fungi

After the incubation period, different fungal colonies were subjected to macroscopic and microscopic examination to observe their growth, and hyphae structure. The samples were identified depending on the morphology characters of fungus on culture medium, then using microscopic examination of pure culture growth of mold colony was examined under magnification for their microscopic structures and cross identified by using mycological keys [25].

Prepare growth medium

Potato dextrose agar was prepared according to the instructions of the manufacturing company. PDA prepared by suspending 39 gm in 1 liter. The media were autoclaved at 121 ºC, 15 Psi for 15 min, after autoclaving, the agar solution was cooled in a water bath for 45 to 50 ºC, then chloramphenicol solution of 250 mg/L was added to the agar solution to inhibit the growth of bacteria.

Preparation of fluconazole suspension

The commercially available sample of antifungal drugs is Fluconazole, each antifungal was prepared as an initial concentration (working stock solution) The suspension was made using (weight 0.30232 gm) of powder drug dissolved in 10 ml of 96% ethanol and then transferred with 80 ml of distal water in sterile screw-capped glass vials to complete the volume, the suspension was filtered with Whitman no.1 filter paper by Buchner funnel. prepared by using the formula [8].

Molarity=wt/mwt*1000/v
Molarity =0.02 fluconazole drug
Molecular weight drug =151.16 g/mol
Volume =100 ml

Estimation of antifungal activity of fluconazole using Food poisoning technique.

To obtain young, actively developing cultures, all isolates were inoculated into SDA plates and cultured at 37°C for three days. The food poisoning technique was then used to measure antimitotic activity.
The solution of fluconazole was mixed with PDA medium (2ml from antifungal solution and 18 ml agar medium at a ratio (1:10) fluconazole: media) and the volume was completed with PDA medium, then a disc of cultivated fungus for 3-5 day–old culture was inoculated in center of Petri dish, in controls sterile distilled water was used instead of antifungal solution. Plates were incubated at 35±2°C, and the diameter of growth was recorded every 24 hours for 2 days [9]. Percentage of inhibition was estimated using this formula [18]:

Percentage of inhibition =

$$growth\ diameter of\ control - growth\ diameter\ of\ fungi\ under\ effect\ of\ fluconazole\ /\ diameter of\ control * 100$$

Estimation of antifungal activity of fluconazole using disk diffusion procedure

The process for identifying the inhibitory zones in fungus was as follows. To test the fungi's susceptibility, an inoculum was made. The fresh colonies were covered with approximately 1ml of sterilized 0.9% saline, and the suspension was prepared by gently probing the colonies with the tip of a transfer pipette. The resulting mixture of conidia and hypha fragments were withdrawn and transferred to a sterile tube. After heavy particles are allowed to settle for 3 to 5 minutes, the upper homogeneous suspension was transferred to a sterile tube, the cap tightened and mixed with a vortex mixer for 15 seconds.

Agar plates were inoculated simultaneously in three directions with a sterile cotton swab dipped in the undiluted mold stock inoculum suspensions. The inoculated agar was allowed to dry for 15 to 30 min, and the mixture was vortexed for 15 seconds after the heavier particles were given three to five minutes to settle.
Three directions of simultaneous inoculation of agar plates were carried out using sterile cotton swabs dipped in the undiluted suspensions of mold stock inoculum. The inoculated agar was allowed to dry for 15 to 30 min [24]. Fluconazole 10-µg disks antifungal contains one cartridge with 50 discs packed in blister and an instruction sheet. The discs are put on to the surface of the culture medium inoculated with the suspension containing conidia of fungi with a pair of forceps.
The plates were incubated at 35±2°C in ambient air. For five days, the diameter of the inhibitory zone was measured every 24 hours [10].

3. RESULTS AND DISCUSSION

3.1 Result

Different colonies of fungi were observed on (PDA) at 37°C for 7 days incubation, the cultural and microscopic examination illustrated according to mycological key (kidd .2016) different colonies of fungi (*Fusarium spp.*, *Botrytis spp, Ulocladium spp.*, *Aspergillus niger*, *Penicillium spp. Aspergillus fumgatus* and *Rhizopus spp*.). all these molds were subjected to sensitivity test to fluconazole.

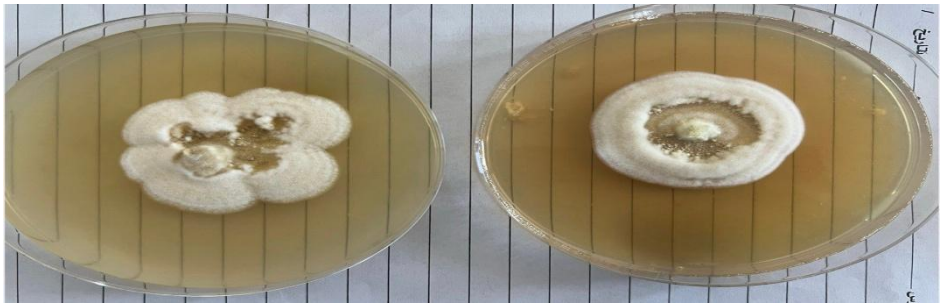
Food poisoning technique

The results showed that the inhibitory effect of fluconazole was the highest against Fusarium and *Botrytis* in a growth diameter of 10 mm in contrast with *Rhizopus* and Penicillium which showed a minimum inhibitory effect *at a* growth diameter of 80mm, and 40 mm respectively in comparison to control which contains distilled water as shown in table 1. Table 1(showed Sensitivity of fungi to fluconazole).

(Table 1) showed Sensitivity of fungi to fluconazole according to food poisoning method.

Fungi	Control	Growth diameter	Percentage of inhibition
Fusarium spp.	50 mm	10 mm	80%
Botrytis spp.	80 mm	10 mm	87.5%
Ulocladium spp.	70 mm	20 mm	71%
Aspergillus niger	80 mm	25 mm	68%
Penicillium spp.	45 mm	40 mm	12.5%
Aspergillus fumgatus	80 mm	40 mm	50%
Rhizopus spp.	80 mm	80 mm	0%

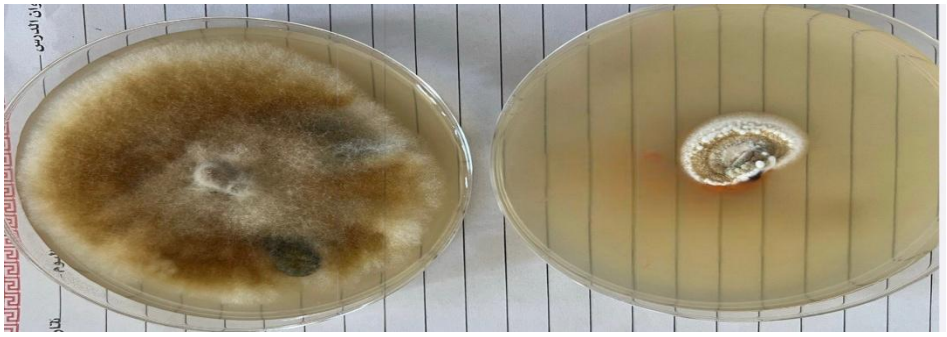
B)



A)

Figure 1: showed a) *Penicillium* control b) *Penicillium* under the effect of fluconazole grow on PDA at 37C after 3-5 day of incubation

C)



D)

Figure 2: showed C)*Ulocladium* control D)*Ulocladium* under the effect of fluconazole **)** grow on PDA at 37^0C after 3-5 day of incubation

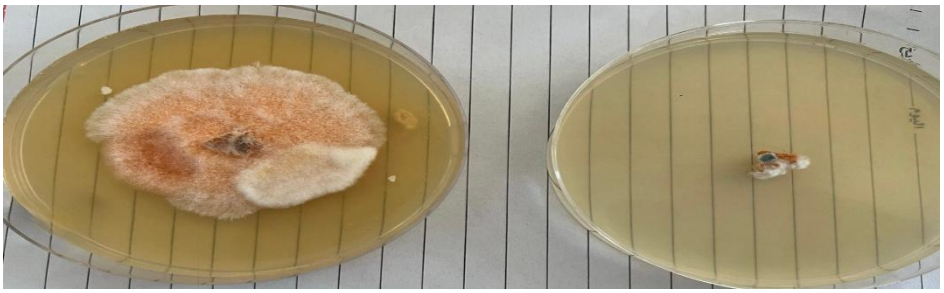
E)  **F)**

Figure 3: showed E) *Fusarium* control F) *Fusarium* under the effect of fluconazole grow on PDA at 37^0C after 3-5 day of incubation.

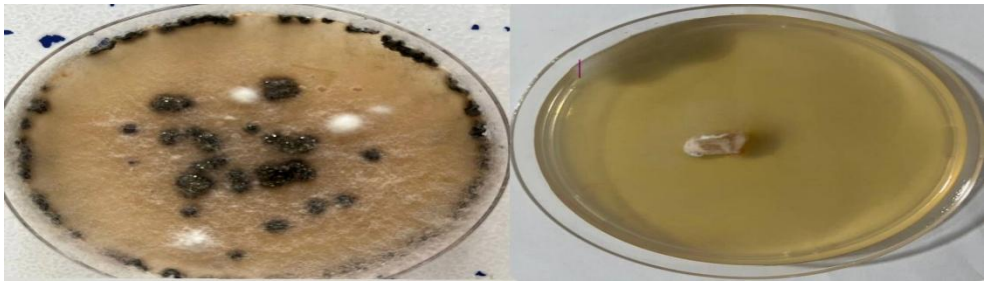
G)  **H)**

Figure 4: showed G) *Botrytis* control H) *Aspergillus niger* under the effect of fluconazole grow on PDA at 37^0C after 3-5 day of incubation

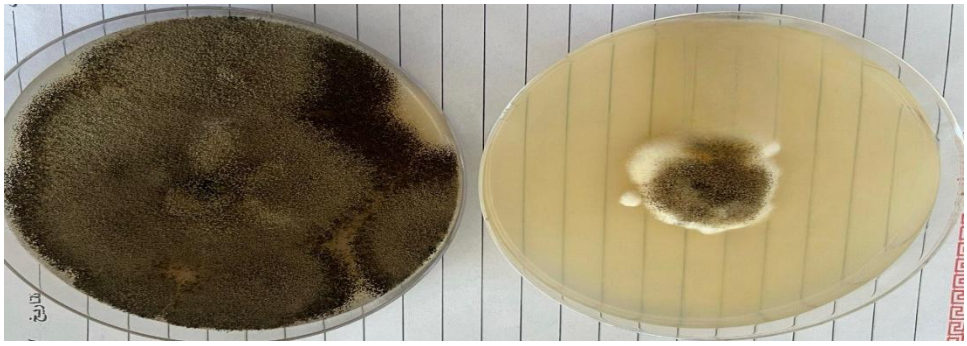
G)  **H)**

Figure 4: showed G) *Botrytis* control H) *Aspergillus niger* under the effect of fluconazole grow on PDA at 37^0C after 3-5 day of incubation

I)  **J)**

Figure 5: showed G) *Aspergillus niger* control H) *Aspergillus niger* under the effect of fluconazole ***grow*** on PDA at 37^0C after 3-5 day of incubation

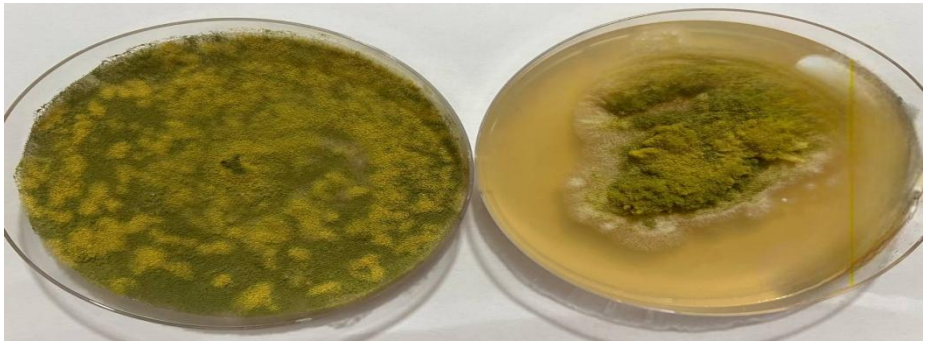
K)  K)

Figure 6: showed I) *Rhizopus* control J) *Rhizopus* under the effect of fluconazole ***grow*** on PDA at 37^0C after 3-5 day of incubation

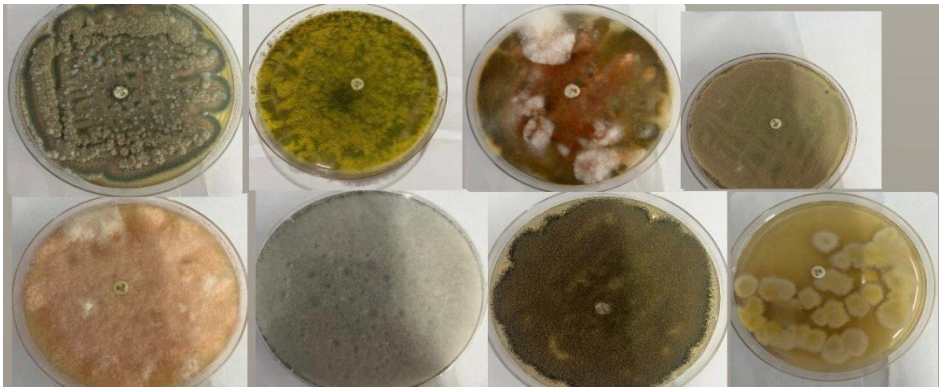


Figure 7: showed G) *Aspergillus fumgatus* control H) *Aspergillus fumgatus* under the effect of fluconazole grow on PDA at 37^0C after 3-5 day of incubation

Disk diffusion procedure

The sensitivity of the isolated mold was tested using disk diffusion procedure for the antifungal drugs (fluconazole 10-µg disks) antifungal contains one cartridge with 50 discs packed in blister and an instruction sheet. All the tested molds (*Penicillium, Ulocladium, Fusarium, Aspergillus niger, Aspergillus fumgatus* and *Rhizopus*) showed resistance to fluconazole disk. Fig. 6 shows disk diffusion procedure on (*Penicillium, Ulocladium, Fusarium, Aspergillus niger, Aspergillus fumgatus* and *Rhizopus*).

Discussion

The present study evaluated fluconazole's fungicidal effect on some fungi isolated from laboratory devices Fluconazole and other azole medications block 14-a-demethvlase, an enzyme that changes lanosterol into ergosterol, which is the main ingredient in fungal cell membranes [1], [19].its broad antifungal spectrum, its low toxicity and its excellent pharmacokinetic properties [20].

Initially, it was thought that the organisms would have reduced survival after long-term incubation when exposed to fluconazole in growth conditions (fluconazole + culture media), compared to untreated organisms. In certain situations, this impact may bear resemblance to the previously attributed post-antifungal effect of fluconazole [12]-[14] According to these investigations, fluconazole might impede some ongoing processes that are necessary for the organisms to survive for extended periods incubated. Undoubtedly, some metabolism continues even when the microbes are kept in settings that prevent them from proliferating. In overextended cultures, the fungal cell membranes will probably start to degrade and require repair. In overextended cultures, the fungal cell membranes will probably start to degrade and require repair. The decrease in viability observed throughout the prolonged in vitro incubations in these experiments may have been due to fluconazole's suppression of this particular form of upkeep. Alternatively, it's possible that even in the lack of nutrients, In the distilled water medium, there was low levels of proliferation; in this instance, fluconazole may have inhibited the process and prevented the development of new progeny with longer lifespans. Finally, azoles may have additional effects on the fungal cells they target [15]; the reduction of ergosterol production is responsible for growth inhibition under normal conditions. However, prolonged exposure may also lead to a decrease in viability, which could be one of the impacts.

The differential impact of fluconazole on organism survival at 37°C compared to 25°C suggests that these effects may have been modulated by the metabolic rate of fungal cells [16]. Recent studies highlight an increasing issue with fluconazole resistance, particularly among Candida species. Recent Research has documented rising resistance rates, which could affect treatment outcomes. Monitoring resistance patterns and adjusting treatment protocols are crucial in managing these infections [22]-[23].In summary, fluconazole plays an important part in the management of various fungal infections by inhibiting the production of ergosterol, disrupting fungal cell membranes, and ultimately leading to fungal cell death. Its broad spectrum of activity, clinical efficacy, and relatively favorable safety profile make it a valuable therapeutic option in the control of fungus-related infections [17].

4. CONCLUSION

Many fungi isolate from laboratory instruments this result indicated the contamination with fungal spores, fluconazole broad spectrum antifungal effective in lowering fungal colonization and infection. It can be used as antifungal medication in treating fungal infections.

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