Activity of Esomeprazole and Fluconazole against Aspergillus Fumigatus and Candida Albicans: An in Vitro Study

Dr. Sachin T Jose

Assistant professor, department of pharmacology, institute of medical sciences and SUM hospital, Siksha O Anusandhan (deemed to be university), Bhubaneswar, India.

DOI: https://doi.org/10.68050/JAMS.2026.486

Received: 14 Sept 2026; Accepted: 15 Sept 2026; Published: 18 September 2026

ABSTRACT

Background: Fungal infections caused by Aspergillus fumigatus and Candida albicans are a critical clinical challenge in immunocompromised individuals, and drug repurposing policies may improve the antifungal efficacy and overcome resistance.

Aim: This study assessed the interaction of esomeprazole and fluconazole in an in vitro experimental setting against eight clinical isolates of A. fumigatus and four clinical isolates of C. albicans.

Methods: Nineteen sputum samples were collected from tuberculosis patients, of which 12 yielded fungal growth (eight A. fumigatus and four C. albicans), using morphological and molecular identification techniques. Antifungal susceptibility was measured by broth microdilution to determine the median inhibitory concentration (IC50) and minimum fungicidal concentration (MFC) of fluconazole with and without esomeprazole (30 µg/mL). Statistical analyses were performed using an independent samples t-test.

Results: The incorporation of esomeprazole with fluconazole markedly reduced ($p < 0.05$) the IC50 of the latter against all isolates of both fungal species. The average IC50 of fluconazole against A. fumigatus decreased from 120.96 µg/mL to 17.02 µg/mL when esomeprazole was added, while it decreased from 7.45 µg/mL to 3.90 µg/mL against C. albicans. Also, a marked reduction in MFC values was recorded for most A. fumigatus isolates, with a decrease in the overall mean from 736.00 µg/mL to 166.51 µg/mL ($p < 0.01$). However, the reduced MFC values for C. albicans were not statistically significant. Conclusion: Esomeprazole markedly enhances fluconazole's antifungal activity in vitro, demonstrating a positive interaction, especially against A. fumigatus.

Keywords: Proton pump inhibitors, Aspergillus fumigatus, Candida albicans, in vitro investigation, Broth microdilution bioassay

INTRODUCTION

Fungal infections caused by Aspergillus fumigatus and Candida albicans are a critical challenge in human and veterinary medicine (Langfeldt et al., 2022; Brown et al., 2024). These fungi are among the most clinically important pathogens capable of causing a wide range of superficial and systemic mycoses in animals, especially when the host's immune defenses are compromised.

Aspergillus fumigatus is a ubiquitous saprophytic mold that commonly occurs in soil, water, decaying vegetation, dust, and feed materials, facilitating constant contact with animals and humans (Nji et al., 2023; Zakaria, 2024). It is the most clinically significant species and the primary causative agent of aspergillosis (van de Veerdonk et al., 2025). In veterinary medicine, aspergillosis has been reported in companion animals (de Jong et al., 2025), avian species (Stilz et al., 2025), and livestock (Dieste-Pérez et al., 2025), causing respiratory and systemic disease. The opportunistic nature of A. fumigatus makes it capable of causing severe infections, particularly in immunocompromised hosts. However, it can also cause severe disease in apparently immunocompetent animals under stress (Liu et al., 2024).

Candida albicans is also an opportunistic yeast that inhabits the mucosa of the gastrointestinal, genitourinary, and respiratory systems in many animal species (Pohlman and Chengappa, 2022). Animal candidiasis is often associated with immunosuppressive therapy, metabolic disorders, stress, and prolonged antibiotic therapy (Haroun et al., 2026).

The therapeutic management of animal fungal infections remains challenging. Presently available systemic antifungal agents are confined to three principal classes: azoles, polyenes, and echinocandins (Saini et al., 2025), of which azoles are often used due to their favorable pharmacokinetic properties and oral bioavailability (Eschenauer, 2024). Fluconazole is a triazole antifungal that acts by suppressing fungal cytochrome P450-dependent lanosterol 14α- demethylase, resulting in the inhibition of ergosterol production and altering cell membrane integrity (Carmo et al., 2023). Despite the widespread use of fluconazole, therapy outcomes are often suboptimal, partly because of the intrinsic reduced susceptibility of fungal pathogens and the emergence of acquired resistance to azole compounds (Halliday et al., 2025; Van Rhijn and White, 2025). Candidiasis in livestock negatively impacts animal health and productivity, and it can lead to chronic infections in companion animals, requiring prolonged antifungal therapy (Simões et al., 2023).

Resistance to azoles among Candida and Aspergillus species isolates has become a growing concern in both clinical and environmental settings (Rivelli Zea and Toyotome, 2022; Sen et al., 2022; Daneshnia et al., 2023), and the underlying mechanisms are multifactorial, including mutations in the cyp51A gene, overexpression of target enzymes, biofilm-associated tolerance, and increased activity of efflux pumps that expel antifungal agents from fungal cells (Kashyap et al., 2023; Eknure et al., 2025). Efflux-mediated resistance, involving ATP-binding cassette (ABC) transporters and major facilitator superfamily (MFS) transporters, reduces intracellular drug accumulation and contributes to therapeutic failure (Yang et al., 2024; Zhang et al., 2025). These resistance mechanisms underscore the urgent need for novel strategies to reestablish antifungal susceptibility or enhance the activity of existing agents, rather than relying solely on the development of new antifungal classes, which is costly and time-consuming.

Drug repurposing and combination therapy have emerged as promising strategies to enhance antifungal effectiveness and delay the development of resistance. Proton pump inhibitors (PPIs), commonly used to inhibit gastric acid secretion, have attracted interest for their potential off-target antimicrobial effects (Glajzner et al., 2024). Esomeprazole, the S-isomer of omeprazole, suppresses the gastric H+/K+-ATPase in parietal cells, leading to sustained inhibition of gastric acid secretion (Wołowiec et al., 2025). Besides their indications for reducing gastric acid secretion, evidence suggests that PPIs may interfere with microbial metabolism, including modulation of membrane transport systems and suppression of efflux pumps. PPIs can act synergistically with fluconazole against resistant Candida species, thereby increasing fluconazole accumulation within fungal cells. The underlying mechanisms include inhibition of efflux pump activity and the suppression of extracellular enzyme production (Gao et al., 2024). Although these findings support the potential synergistic interaction between esomeprazole and fluconazole against A. fumigatus and C. albicans, data on this interaction remain scarce, especially for veterinary-relevant isolates.

The increasing incidence of azole resistance, the therapeutic challenges of fungal infections, and the limited availability of approved antifungal agents in veterinary and human practices make exploring combination therapy that potentiates antifungal effectiveness clinically relevant. Hence, this study aimed to assess the in vitro activity of esomeprazole and fluconazole combination against A. fumigatus and C. albicans using standardized broth microdilution assays. Particular emphasis was on assessing whether esomeprazole could enhance fluconazole activity, potentially by modulating resistance-associated mechanisms, such as efflux pump suppression. Although A. fumigatus is generally considered intrinsically resistant to fluconazole and the drug is not routinely used for the treatment of aspergillosis, fluconazole remains a useful experimental model for investigating resistance-modifying strategies. Demonstrating restored susceptibility or enhanced activity against a fungus with well-recognized intrinsic resistance may provide insight into the ability of adjunctive agents, such as proton pump inhibitors, to interfere with resistance-associated pathways and improve azole efficacy. The study's findings may contribute to the development of adjunctive therapeutic schemes for the management of aspergillosis in veterinary medicine.

MATERIALS AND METHODS

Fungal samples, drugs, and chemicals

Nineteen sputum samples from patients infected with tuberculosis were kindly provided by The Center for Chest and Respiratory Diseases in Sulaimani City, Sulaymaniyah, Kurdistan Region, Iraq. Samples were collected from deep, productive cough sputum of patients using sterile swabs, which were then transferred into a transfer medium for later inoculation and fungal identification. The samples were from patients aged 65–75 years with tuberculosis and co-infection with fungal pathogens. The samples were collected from deep, productive cough sputum of patients; sterile swabs were used to transfer the sputum into a transfer medium for later culture.

Esomeprazole and fluconazole were kindly provided by Pioneer Pharmaceutical Company in Sulaymaniyah, Iraq. Sabouraud dextrose agar (SDA) was purchased from Merck Millipore.

Inoculation of collected samples and identification of fungal species

Sabouraud dextrose agar (SDA) was used to cultivate the collected samples; after sterilization, the samples were inoculated onto SDA medium in a biosafety cabinet. After inoculation, plates were incubated for 96 hours at different temperatures: 28°C for Aspergillus species, 37°C for Candida albicans. After incubation, plates were examined macroscopically and microscopically to assess growth rate and detect contamination, if present.

Aspergillus fumigatus appears macroscopically as blue-green or greenish-gray with a powdery to velvety texture, often with a white border, while Candida albicans is characterized by round, moist, white, creamy colonies. Microscopically, Aspergillus spp are characterized by long branching hyphae with a sac-like structure of oval-shaped vesicles at the end of the hyphae, from which dispersed spores radiate. Candida albicnas appears as polymorphic, budding yeasts and elongated pseudohyphae (Fatima et al., 2025).

Molecular identification of fungal species

Fungal isolates were first cultured on appropriate solid media and incubated under optimal growth conditions until sufficient mycelial growth was obtained. Genomic DNA was extracted directly from freshly grown fungal mycelia using the ADD-Bio DNA Extraction Kit (ADD-Bio, Korea) according to the manufacturer's instructions. Briefly, approximately 100 mg of fungal mycelium was scraped from the culture plate and transferred into a sterile microcentrifuge tube containing 200 µL of lysis buffer. The samples were thoroughly mixed and incubated at 60°C for 10 minutes to ensure complete cell lysis. Following the addition of binding and washing buffers. Purified DNA samples were stored at -20°C until use in polymerase chain reaction (PCR) amplification.

The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using the universal primer pair ITS-1 (5′-TCC GTA GGT GAA CCT GCGG-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (Hardy et al., 2023). The expected size of the amplified fragment was approximately 600 base pairs (bp). Each PCR reaction was performed in a total volume of 25 µL, containing the following components:10 µL of 2× PCR Master Mix (Genet Bio, Korea), 1 µL of ITS-1 primer (10 pmol/µL),1 µL of ITS-4 primer (10 pmol/µL), 5 µL of DNA template, and 3 µL of nuclease-free water. PCR amplification was carried out in a thermal cycler (e.g., Bio-Rad, USA) under the following cycling conditions: Initial denaturation at 95°C for 5 minutes, 40 cycles of: Denaturation at 95°C for 30 seconds, Annealing at 59°C for 30 seconds, Extension at 72°C for 35 seconds, and final extension at 72°C for 5 minutes. The amplified PCR products were held at 4°C until further analysis.

PCR products were analyzed by agarose gel electrophoresis using a 1.5% agarose gel prepared in 1× TAE buffer. For visualization, a safe nucleic acid dye (SYBR Safe DNA Gel Stain, Thermo Fisher Scientific, USA) was used instead of ethidium bromide to ensure user and environmental safety. A 100 bp DNA ladder (Genet Bio, Korea) was included as a molecular size marker. Electrophoresis was performed at 150 V for 40 minutes.

After electrophoresis, gels were visualized under UV transillumination, and digital images of the amplified bands were captured for documentation.

Antifungal susceptibility testing

The broth microdilution bioassay was used to determine the median inhibitory concentration (IC50) of esomeprazole and fluconazole. For this, 96-well flat-bottom plates were used, dispensing 90 µL of RPMI 1640 (prod. no. R8758, Sigma-Aldrich), containing 33 µg/mL esomeprazole into each well. 45 µL of the stock drugs was added to the first well. Then, two-fold serial dilutions were performed in consecutive wells by adding 90 µL of RPMI 1640 medium containing 256 µg/mL to the second well, mixing the contents, then transferring 90 µL from the second well to the next well. The procedure continued until the last well, then 90 µL from the last well was discarded, leaving all wells containing 90 µL after the serial dilution. Fluconazole concentrations in the wells ranged from 256 µg/mL to 1 µg/mL, while the final concentration of esomeprazole in each well was 30 µg/mL (Shin and Bae, 2023). Aliquots of 10 µL of the inoculum containing 2.5 × 104 colony forming unit (CFU)/mL were dispensed into each well for each fungal isolate, resulting in a final volume of 100 µL per well containing 2.5 × 103 CFU/mL. Negative control wells were included on each plate and contained 100 µL RPMI 1640 medium. Also, wells containing RPMI 1640 medium and 2.5 × 103 CFU/mL of the fungal isolate were included on each plate to confirm cell viability (viability control). The same procedure was repeated on separate plates, omitting esomeprazole from the wells, to investigate the interactive effect of esomeprazole with fluconazole.

To determine IC50 values, the colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS), a water-soluble salt, was used as described by the supplier (Promega Corporation). After 24 hours of incubation with the drug, 20 µL of a solution containing MTS and phenazine methosulfate (PMS; product no. P9625, Sigma-Aldrich) was added to each well (final concentrations of 333 mg/mL MTS and 25 mM PMS). Incubations were continued for another 4 hours at 35°C, and the plates were read at 490 nm. The endpoints were read with a microplate reader (Al-Shahrani et al., 2017). According to CLSI criteria, 100% growth inhibition is defined as clear wells. Therefore, the optical density (OD) readings similar to those of the negative control without Aspergillus were also considered the minimum fungicidal concentration (MFC). The concentration that inhibited 50% of visual growth was recorded and interpreted as the IC50. All tests were conducted in triplicate.

After determining the IC50, the two higher inhibitory concentrations and the positive controls were subcultured in triplicate on SDA plates. After 24 h of incubation at 35 ±2°C, MFC readings were performed relative to the growth controls. The MFC was defined as the lowest drug concentration that prevented visible growth of the subculture (i.e., no fungal growth on the solid medium used) (Al-Shahrani et al., 2017). The IC50 was defined as the lowest drug concentration that inhibited fungal growth by 50% (Lu et al., 2020).

Dose Reduction Index (DRI)

The DRI is a pharmacological measure that quantifies how many fold a drug's dose can be reduced when used in a synergistic combination compared to the dose required when used alone, while still maintaining the same level of effect (Chou, 2008; Intakhan et al., 2024). The DRI of fluconazole and esomeprazole was calculated using the following formula:

$$DRI = \frac{IC50 of drug A alone}{IC50 of drug A in combination}$$

A DRI > 1 indicates a favorable dose reduction (the combination allows you to use less of the drug to get the same results, which reduces toxicity). DRI = 1 indicates no dose reduction is achieved (additive effect). DRI < 1 indicates a negative dose-reduction (the combination is less effective than the drug alone).

Statistical analysis

Data were presented as means of three replicates ±standard error. The differences were compared statistically using an independent-samples t-test and one-way analysis of variance in GraphPad Prism version 10.5. Differences between groups were considered significant at $p \leq 0.05$.

Ethical approval: Not necessary for this manuscript

RESULTS

Species identification

Among the 19 collected samples, 12 (63.2%) were culture-positive for fungi following culture on SDA. Among the culture-positive samples, 8 isolates (66.7%) were identified as A. fumigatus, and 4 (33.3%) were identified as C. albicans. Fungal identification was initially based on macroscopic colony morphology and microscopic features, and was confirmed by PCR amplification of the ITS region.

Median inhibitory concentrations

The IC50 of fluconazole alone against A. fumigatus isolates ranged between 36.15 and 347.33 µg/mL, and the values against C. albicans isolates were between 5.45 and 9.37 µg/mL (Table 1). The combination of esomeprazole and fluconazole reduced fluconazole's IC50 in all tested isolates of both fungal species, indicating enhanced antifungal activity. A consistent decrease in IC50 values occurred when esomeprazole was added to fluconazole. For example, in isolate 1 of A. fumigatus, the average fluconazole IC50 was 36.15 µg/mL, which decreased significantly to 14.05 µg/mL (mean difference = 22.09 µg/mL; $p = 0.028$) when esomeprazole was added. The highest reduction was observed in A. fumigatus isolate 8, where the IC50 decreased from 347.33 µg/mL (fluconazole alone) to 13.80 µg/mL (fluconazole + esomeprazole).

A similar pattern was observed for C. albicans isolates. For example, the IC50 of fluconazole against C. albicans isolate 1 was 9.04 µg/mL, which decreased significantly to 4.15 µg/mL (mean difference = 4.89 µg/mL; $p = 0.011$) when esomeprazole was added (Table 1). Also, fluconazole's IC50 decreased from 9.37 µg/mL when used alone to 5.39 µg/mL when esomeprazole was introduced to the fungal culture.

The effect of esomeprazole on fluconazole's antifungal activity on all the A. fumigatus and C. albicans isolates is summarized in Figure 1. Adding esomeprazole significantly improved fluconazole's antifungal activity against both fungal species, as reflected by reduced IC50 values.

In the case of A. fumigatus, the mean IC50 of fluconazole alone was 120.96 µg/mL, whereas its combination with esomeprazole significantly reduced the IC50 to 17.02 µg/mL ($p < 0.05$), indicating esomeprazole's synergistic effect with fluconazole against all A. fumigatus isolates. A similar trend was observed for C. albicans: the IC50 of fluconazole alone was 7.45 µg/mL, and the addition of esomeprazole significantly reduced it to 3.90 µg/mL ($p < 0.05$). This outcome further confirmed the potentiating effect of esomeprazole on fluconazole's antifungal activity against C. albicans. Collectively, these results indicate that the fluconazole-esomeprazole combination significantly enhances fluconazole's antifungal activity against both A. fumigatus and C. albicans, with a synergistic interaction under the in vitro conditions in this study.

Minimum fungicidal concentrations

The MFC of fluconazole against A. fumigatus ranged between 341.33 µg/mL and 1365.33 µg/mL, and the values for C. albicans isolates ranged between 64:00 and 128:00 µg/mL (Table 2). The addition of esomeprazole at 30 µg/mL reduced the MFC values to 85.33–213.33 µg/mL. The combination of fluconazole + esomeprazole significantly reduced the MFC values against A. fumigatus isolates 1, 2, 5, 6, 7, and 8 ($p < 0.05$). This reduction indicates a potential synergistic interaction between the two compounds. Combining fluconazole and esomeprazole significantly reduced the MFC against isolate A. fumigatus 1 from 341.33 µg/mL to 85.33 µg/mL, a decrease of 256.00 µg/mL ($p = 0.044$). A similar pattern was observed in isolate A.

fumigatus 2, with the MFC declining from 512.00 µg/mL to 160.67 µg/mL (a decrease of 341.33 µg/mL; $p = 0.001$). Significant declines in MFC values were also detected in isolates 5, 6, 7, and 8. The greatest decrease in the MFC value was observed in isolate 8, declining from 1365.33 µg/mL to 137.33 µg/mL (mean difference = 1228.00 µg/mL; $p = 0.023$). However, even though the MFC values against isolates 3 and 4 decreased when esomeprazole was added to the culture medium, the decrease was not statistically significant ($p > 0.05$).

In contrast to A. fumigatus isolates, the fluconazole + esomeprazole combination had less pronounced effects against C. albicans. Although the MFC values decreased across all 4 C. albicans isolates, none of these differences reached statistical significance ($p > 0.05$), as shown in Table 2. For example, the MFC against isolate 1 of C. albicans decreased from 128.00 µg/mL to 85.33 µg/mL, a mean difference of 42.67 µg/mL, but the decline did not reach statistical significance. A similar pattern of non-significant difference was observed for isolates 2, 3, and 4.

When the overall fungicidal effect of esomeprazole and fluconazole was assessed against all the A. fumigatus and C. albicans isolates, the combination had a marked effect against A. fumigatus (Figure 1). The overall MFC of fluconazole alone against A. fumigatus averaged 736.00 µg/mL, but when esomeprazole was added, the MFC was 166.51 µg/mL, a significant reduction of 569.49 µg/mL ($p < 0.01$). This outcome shows a strong positive interaction between fluconazole and esomeprazole. However, although fluconazole's MFC against C. albicans decreased from 112.00 µg/mL when used alone to 58.67 µg/mL when esomeprazole was added, the difference was not statistically significant.

These findings indicate that esomeprazole significantly enhances fluconazole's fungicidal activity against most A. fumigatus isolates tested, whereas its stimulatory effect on fluconazole's fungicidal activity against C. albicans is limited and statistically non-significant under these in vitro conditions.

Dose reduction index

The DRI values of the tested drugs against C. albicans and A. fumigatus are shown in Figure 2. All A. fumigatus isolates exhibited DRI values above 1, indicating that the fluconazole-esomeprazole combination produced a synergistic interaction. However, the magnitude of synergism varied substantially between isolates, as the DRI ranged between 2.39 in A. fumigatus isolate 1 and 26.02 in A. fumigatus isolate 8.

Similar to A. fumigatus, all C. albicans isolates had DRI values above 1, confirming synergism across all tested isolates. However, the DRI values were consistently lower than those observed for most A. fumigatus isolates, ranging between 1.58 (for C. albicans isolate 3) to 2.26 (for C. albicans isolate 4). Compared with A. fumigatus, the DRI values for C. albicans were less variable and clustered within a narrower range, indicating a more consistent but generally weaker synergistic interaction.

DISCUSSION

The present study assessed the potential synergistic effect of esomeprazole with fluconazole against different isolates of A. fumigatus and C. albicans isolated from sputum samples of patients initially diagnosed with tuberculosis. The results showed that combining esomeprazole with fluconazole significantly enhanced fluconazole's antifungal activity against most fungal isolates used in this study—the marked reduction in the IC50 values evidenced this synergistic effect. Also, the substantial decrease in MFC against most A. fumigatus isolates further confirmed the initial hypothesis about esomeprazole's synergistic interaction with fluconazole. The IC50 values in this study showed that a higher concentration of fluconazole was required to suppress the growth of A. fumigatus isolates than that of C. albicans. This outcome was consistent with previous studies showing that Aspergillus species are less susceptible to azoles, including fluconazole (Guinea et al., 2008; Gao et al., 2024). Generally, C. albicans is more susceptible to azole antifungals than Aspergillus species, because its Cyp51 enzyme possesses a higher intrinsic activity to azoles (Bassetti et al., 2020; Hosseini et al., 2024).

Fluconazole is generally regarded as a fungistatic antifungal against Candida albicans, not a strongly fungicidal drug (Fioriti et al., 2022). Therefore, a combination that increases intracellular fluconazole accumulation may substantially improve growth inhibition without necessarily causing fungal death.

Resistance to azole compounds among Candida and Aspergillus species is emerging, possibly due to their broad clinical use (Vitiello et al., 2023). This resistance occurs mostly via two main mechanisms. The first is the overexpression of ABC and the major facilitator superfamily (MFS) transporters, which results in increased drug efflux and decreased intracellular concentrations of the azole compound (Jaiswal and Kumar, 2024). Another cause of this resistance is the TR34/L98H mutation. The TR34 is a 34-base-pair tandem repeat in the promoter region of the gene cyp51A, leading the fungus to overproduce the target enzyme (lanosterol 14α-demethylase). Also, the L98H is a point mutation that substitutes leucine for histidine at codon 98, altering the enzyme's structure so that azoles can no longer bind (De Francesco, 2023).

The DRI analysis demonstrated that combining fluconazole with esomeprazole enhanced antifungal activity against both C. albicans and A. fumigatus, as all tested isolates yielded DRI values above 1. This finding confirms that the combination allowed a lower effective dose of fluconazole when combined with esomeprazole to achieve the same antifungal effect compared with fluconazole alone, indicating a favorable pharmacodynamic interaction.

Several possible mechanisms may explain the synergistic interaction in this study. PPIs such as esomeprazole disrupt intracellular pH regulation in microbial cells and can inhibit fungal efflux pumps, leading to the accumulation of antifungal drugs within fungal cells (Gao et al., 2024). This disruption of fungal cell proton pumps impairs membrane permeability, facilitating the intracellular accumulation of antifungal agents, such as fluconazole, and thereby enhancing antifungal efficacy (Li et al., 2025).

Another explanation for the observed synergistic interaction between fluconazole and esomeprazole in this study is the alteration of environmental pH by esomeprazole. Extracellular pH conditions can influence fungal growth and drug susceptibility by altering the ionization state of fungal cells and their cellular permeability. Hence, esomeprazole may facilitate fluconazole's penetration into the fungal cytoplasm and improve its antifungal effectiveness (Fernandes et al., 2023).

Although the fungal isolates used in this study were derived from human clinical samples, the findings remain relevant to veterinary medicine within a One Health framework. A. fumigatus and C. albicans are ubiquitous opportunistic pathogens capable of infecting both humans and animals, with frequent overlap in environmental exposure sources such as soil, organic matter, and contaminated feed (Gisi, 2022). Moreover, antifungal resistance mechanisms, including efflux pump overexpression and target enzyme alterations, are highly conserved across isolates from different hosts (Lee et al., 2023). Therefore, human clinical isolates can serve as appropriate models for studying antifungal susceptibility and drug interaction patterns that are translatable to veterinary fungal infections. Nevertheless, future studies incorporating animal-derived isolates would further strengthen the veterinary applicability of these findings.

The clinical implications of these findings are noteworthy. Opportunistic fungal infections, especially in immunocompromised individuals and patients with chronic pulmonary diseases like tuberculosis, represent a critical therapeutic challenge. The emergence of antifungal-resistant pathogenic fungi further complicates treatment outcomes. Drug repurposing, including the incorporation of non-antifungal therapies that potentiate the effectiveness of existing antifungal drugs, has become of increasing interest recently. The present findings showed that esomeprazole can become a potential adjuvant that improves the antifungal efficacy of fluconazole. However, despite the positive results, the study was conducted in vitro, and the synergistic interactions may not fully reflect the pharmacodynamic synergism in vivo. Another limitation is that the number of fungal isolates used in the study was relatively limited, which may affect the generalizability of the findings. Also, the molecular mechanisms underlying the synergistic interaction between fluconazole and esomeprazole remain unclear and require further experimental investigation.

An important limitation of the present study is that Aspergillus fumigatus is widely recognized as intrinsically resistant to fluconazole because of the low affinity of its target enzyme and other intrinsic resistance mechanisms. Consequently, fluconazole is not recommended for the clinical management of aspergillosis. Therefore, the findings of this study should not be interpreted as evidence supporting the clinical use of fluconazole against A. fumigatus. Rather, fluconazole was used as a model azole compound to investigate whether esomeprazole could modify fungal susceptibility and overcome resistance-associated mechanisms.

The marked reduction in fluconazole IC50 and MFC values observed after the addition of esomeprazole suggests that proton pump inhibitors may influence mechanisms involved in azole resistance, such as drug efflux, membrane transport, or intracellular pH regulation. Nevertheless, the residual fluconazole concentrations required to inhibit fungal growth remained substantially higher than those typically observed for clinically active anti-Aspergillus azoles. Therefore, further studies should evaluate whether similar potentiating effects occur with clinically relevant anti-Aspergillus agents such as itraconazole, voriconazole, posaconazole, or isavuconazole.

Future research should focus on the molecular mechanisms underlying the pharmacological interactions between PPIs and antifungals in vivo, as well as on the mechanisms responsible for the observed synergistic interaction. Also, a broader range of fungal isolates needs to be tested to provide further insight into the clinical applicability of the combination.

CONCLUSION

This study demonstrated that esomeprazole markedly potentiates the antifungal activity of fluconazole against A. fumigatus and C. albicans in vitro. The combination significantly reduced the IC50 of fluconazole against both fungal species, indicating enhanced antifungal activity. The combination also significantly reduced the MFC of fluconazole against most A. fumigatus isolates, suggesting a robust synergistic fungicidal potential. Esomeprazole enhanced the in vitro activity of fluconazole, highlighting the potential of incorporating it as an adjuvant to potentiate fluconazole activity. However, further studies with clinically relevant anti-Aspergillus azoles are warranted.

Acknowledgments

The University of Sulaimani provided the necessary laboratories to conduct this study.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This research received no funds.

Authors contributions

H.O.D. conceptualized the study, analyzed data, and reviewed and finalized the manuscript.

B.A.M. contributed to the conceptualization and design of the study, conducted the research, and drafted the manuscript. K.K.F., M.S.A., D.B.M., E.R.M., and H.A.S. conducted the research and analyzed data.

Data availability

All data supporting the findings of this study are available within the manuscript, and no additional data sources are required.

REFERENCES

1. Al-Shahrani, M.H.; Mahfoud, M.; Anvarbatcha, R.; Athar, M.T. and Al Asmari, A. 2017. Evaluation of antifungal activity and cytotoxicity of Thymus vulgaris essential oil. Pharmacogn. Commun 7, 34-40.
2. Bassetti, M.; Vena, A.; Bouza, E.; Peghin, M.; Muñoz, P.; Righi, E.; Pea, F.; Lackner, M. and Lass-Flörl, C. 2020. Antifungal susceptibility testing in Candida, Aspergillus and Cryptococcus infections: are the MICs useful for clinicians? Clinical Microbiology and Infection 26, 1024-1033.

3. Brown, G.D.; Ballou, E.R.; Bates, S.; Bignell, E.M.; Borman, A.M.; Brand, A.C.; Brown, A.J.; Coelho, C.; Cook, P.C. and Farrer, R.A. 2024. The pathobiology of human fungal infections. Nature Reviews Microbiology 22, 687-704.
4. Carmo, A.; Rocha, M.; Pereirinha, P.; Tomé, R. and Costa, E. 2023. Antifungals: from pharmacokinetics to clinical practice. Antibiotics 12, 884.
5. Chou, T.-C. 2008. Preclinical versus clinical drug combination studies. Leukemia & lymphoma 49, 2059-2080.
6. Daneshnia, F.; de Almeida Júnior, J.N.; Ilkit, M.; Lombardi, L.; Perry, A.M.; Gao, M.; Nobile, C.J.; Egger, M.; Perlin, D.S. and Zhai, B. 2023. Worldwide emergence of fluconazole-resistant Candida parapsilosis: current framework and future research roadmap. The Lancet Microbe 4, e470-e480.
7. De Francesco, M.A. 2023. Drug-resistant Aspergillus spp.: a literature review of its resistance mechanisms and its prevalence in Europe. Pathogens 12, 1305.
8. de Jong, J.E.; Heuvelink, A.E.; Dieste Pérez, L. and Holstege, M.M. 2025. Aspergillus spp., aspergillosis and azole usage in animal species in Europe: Results from a multisectoral survey and review of recent literature. Medical Mycology 63, myaf009.
9. Dieste-Pérez, L.; Holstege, M.M.; de Jong, J.E. and Heuvelink, A.E. 2025. Azole resistance in Aspergillus isolates from animals or their direct environment (2013–2023): a systematic review. Frontiers in Veterinary Science 12, 1507997.
10. Eknure, C.S.; Agarwal, A.P.; Bachhav, Y. and Kumar, M.S. 2025. Unravelling azole resistance in fungal pathogens: molecular mechanisms, diagnostic challenges, and therapeutic strategies. World Journal of Microbiology and Biotechnology 41, 476.
11. Eschenauer, G.A. (2024). Antifungal therapies for Aspergillus spp.: present and future. Thieme Medical Publishers, Inc. 061-068.
12. Fatima, I.; Saba, M.; Ullah, F.; Akram, W.; Batool, M.; Ullah, M.; Noor, A. and Maula, F. 2025. Culturing, Molecular Characterization and Antibacterial and Antifungal Properties of Fungal Endophytes Isolated from Solanum surattense. Biology Bulletin Reviews 1-15. Fernandes, T.R.; Mariscal, M.; Serrano, A.; Segorbe, D.; Fernández-Acero, T.; Martín, H.;
13. Turrà, D. and Di Pietro, A. 2023. Cytosolic pH controls fungal MAPK signaling and pathogenicity. MBio 14, e00285-00223.
14. Fioriti, S.; Brescini, L.; Pallotta, F.; Canovari, B.; Morroni, G. and Barchiesi, F. 2022. Antifungal combinations against Candida species: from bench to bedside. Journal of Fungi 8, 1077.
15. Gao, L.; Xia, X.; Gong, X.; Zhang, H. and Sun, Y. 2024. In vitro interactions of proton pump inhibitors and azoles against pathogenic fungi. Frontiers in Cellular and Infection Microbiology 14, 1296151.
16. Gisi, U. 2022. Crossover between the control of fungal pathogens in medicine and the wider environment, and the threat of antifungal resistance. Plant Pathology 71, 131-149.
17. Glajzner, P.; Bernat, A. and Jasińska-Stroschein, M. 2024. Improving the treatment of bacterial infections caused by multidrug-resistant bacteria through drug repositioning. Frontiers in Pharmacology 15, 1397602.
18. Guinea, J.; Peláez, T.; Recio, S.; Torres-Narbona, M. and Bouza, E. 2008. In vitro antifungal activities of isavuconazole (BAL4815), voriconazole, and fluconazole against 1,007 isolates of zygomycete, Candida, Aspergillus, Fusarium, and Scedosporium species. Antimicrobial agents and chemotherapy 52, 1396-1400.
19. Halliday, C.; Alguacil-Cuéllar, L.; Chen, S.C.-A. and Alastruey-Izquierdo, A. 2025. Optimizing antifungal therapies for Candida infections: evidence, resistance, and emerging approaches. Clinical Microbiology and Infection
20. Hardy, T.; Erdei, N.; Sipos, D.; Verebélyi, V. and Eszterbauer, E. 2023. Experimental exposure of rainbow trout eggs to water mould, Saprolegnia parasitica (Oomycota): intraspecific differences in pathogenicity. Acta Veterinaria Hungarica 71, 101-111.
21. Haroun, M.; Tratrat, C.; Mathew, R.T.; Munir, M.; Sattar, M.N.; Shawky, M.; Kochkar, H.; Aldakhilallah, O.N.; Ghafoor, A. and Turk, K.G.B. 2026. Avian Candidiasis: A Comprehensive Review of Pathogenesis, Diagnosis, and Control. Veterinary Sciences 13, 171.
22. Hosseini, P.; Keniya, M.V.; Sagatova, A.A.; Toepfer, S.; Müller, C.; Tyndall, J.D.; Klinger, A.; Fleischer, E. and Monk, B.C. 2024. The molecular basis of the intrinsic and acquired resistance to azole antifungals in Aspergillus fumigatus. Journal of fungi 10, 820.

23. Intakhan, N.; Saeung, A.; Rodrigues Oliveira, S.M.; Pereira, M.d.L. and Chanmol, W. 2024. Synergistic effects of artesunate in combination with amphotericin B and miltefosine against Leishmania infantum: Potential for dose reduction and enhanced therapeutic strategies. Antibiotics 13, 806.
24. Jaiswal, N. and Kumar, A. 2024. Modulators of Candida albicans membrane drug transporters: a lucrative portfolio for the development of effective antifungals. Molecular Biotechnology 66, 960-974.
25. Kashyap, V.H.; Mishra, A.; Bordoloi, S.; Varma, A. and Joshi, N.C. 2023. Exploring the intersection of Aspergillus fumigatus biofilms, infections, immune response and antifungal resistance. Mycoses 66, 737-754.
26. Langfeldt, A.; Gold, J.A. and Chiller, T. 2022. Emerging fungal infections: from the fields to the clinic, resistant Aspergillus fumigatus and dermatophyte species: a one health perspective on an urgent public health problem. Current Clinical Microbiology Reports 9, 46-51.
27. Lee, Y.; Robbins, N. and Cowen, L.E. 2023. Molecular mechanisms governing antifungal drug resistance. npj Antimicrobials and Resistance 1, 5.
28. Li, Y.; Wang, H.; Wang, H.; Wang, T.; Wu, D. and Wei, W. 2025. Molecular mechanisms of pathogenic fungal virulence regulation by cell membrane phospholipids. Journal of Fungi 11, 256.
29. Liu, F.; Zeng, M.; Zhou, X.; Huang, F. and Song, Z. 2024. Aspergillus fumigatus escape mechanisms from its harsh survival environments. Applied Microbiology and Biotechnology 108, 53.
30. Lu, M.; Yan, H.; Yu, C.; Yuan, L. and Sun, S. 2020. Proton pump inhibitors act synergistically with fluconazole against resistant Candida albicans. Scientific reports 10, 498.
31. Nji, Q.N.; Babalola, O.O. and Mwanza, M. 2023. Soil Aspergillus species, pathogenicity and control perspectives. Journal of Fungi 9, 766.
32. Pohlman, L.M. and Chengappa, M. 2022. Yeasts: Cryptococcus, Malassezia, and Candida.Veterinary microbiology 405-417.
33. Rivelli Zea, S.M. and Toyotome, T. 2022. Azole-resistant Aspergillus fumigatus as an emerging worldwide pathogen. Microbiology and Immunology 66, 135-144.
34. Saini, V.; Safwan, S.M.; Mehta, D.; Das, E.E. and Bajaj, A. 2025. Recent advances in the development of antifungal agents: beyond azoles, polyenes, and echinocandins. ACS Infectious Diseases 11, 1271-1295.
35. Sen, P.; Vijay, M.; Singh, S.; Hameed, S. and Vijayaraghavan, P. 2022. Understanding the environmental drivers of clinical azole resistance in Aspergillus species. Drug Target Insights 16, 25.
36. Shin, J. and Bae, S. 2023. In vitro effects of omeprazole in combination with antifungal compounds against Malassezia pachydermatis. Veterinary Medicine and Science 9, 2594-2599.
37. Simões, D.; De Andrade, E. and Sabino, R. 2023. Fungi in a one health perspective. Encyclopedia 3, 900-918.
38. Stilz, C.R.; Kunkel, M.R.; Keel, M.K.; Fenton, H.; Weyna, A.A.; Niedringhaus, K.D.; Andreasen, V.A.; McKinney, A.S.; Maboni, G. and Nemeth, N.M. 2025. Aspergillosis in 41 wild bird species in the eastern United States: a 22-year retrospective review. Journal of Veterinary Diagnostic Investigation 37, 305-316.
39. van de Veerdonk, F.L.; Carvalho, A.; Wauters, J.; Chamilos, G. and Verweij, P.E. 2025. Aspergillus fumigatus biology, immunopathogenicity and drug resistance. Nature Reviews Microbiology 23, 652-666.
40. Van Rhijn, N. and White, P.L. 2025. Antifungal treatment strategies and their impact on resistance development in clinical settings. Journal of Antimicrobial Chemotherapy 80, 3208-3226.
41. Vitiello, A.; Ferrara, F.; Boccellino, M.; Ponzo, A.; Cimmino, C.; Comberiati, E.; Zovi, A.; Clemente, S. and Sabbatucci, M. 2023. Antifungal drug resistance: an emergent health threat. Biomedicines 11, 1063.
42. Wołowiec, Ł.; Osiak-Gwiazdowska, J.; Jaśniak, A.; Janiak, M.; Wydeheft, L.; Łukasiak, M.; Pellowska, M. and Grześk, G. 2025. Pharmacodynamics, pharmacokinetics, interactions with other drugs, toxicity and clinical effectiveness of proton pump inhibitors. Frontiers in Pharmacology 16, 1507812.
43. Yang, G.; Shi, W.; He, W.; Wu, J.; Huang, S.; Mo, L.; Zhang, J.; Wang, H. and Zhou, X. 2024. The mitochondrial protein Bcs1A regulates antifungal drug tolerance by affecting efflux pump expression

in the filamentous pathogenic fungus Aspergillus fumigatus. Microbiology Spectrum 12, e01172-01124.
44. Zakaria, L. 2024. An overview of Aspergillus species associated with plant diseases. Pathogens 13, 813.
45. Zhang, H.; Zhou, Y.; Yang, H.; Tao, X.; Chen, Y.; Dong, F. and Sun, Y. 2025. In vitro interactions of berbamine hydrochloride and azoles against Aspergillus fumigatus. Microbiology Spectrum 13, e03184-03124.

Table 1. Median inhibitory concentration (IC50) of fluconazole against A. fumigatus and C. albicans, with and without esomeprazole

	IC50 (µg/mL)		**Mean difference (µg/mL)**	**95% CI of the difference (µg/mL)**	
Isolate	**Flu**	**Flu + Eso**			**P value**
A. fumigatus 1	36.15 ± 4.11	14.05 ± 0.99	22.09	5.57–38.62	0.028
A. fumigatus 2	161.67 ± 10.37	21.99 ± 2.31	139.68	97.68–181.67	0.004
A. fumigatus 3	137.57 ± 16.99	22.16 ± 4.71	115.4	48.40–182.41	0.016
A. fumigatus 4	37.47 ± 3.04	12.65 ± 0.90	24.82	12.93–36.70	0.01
A. fumigatus 5	90.74 ± 12.49	20.79 ± 1.75	69.95	17.59–122.31	0.028
A. fumigatus 6	92.77 ± 5.98	13.68 ± 1.07	79.1	54.40–103.79	0.005
A. fumigatus 7	63.96 ± 3.49	17.03 ± 1.09	46.93	33.39–60.47	0.003
A. fumigatus 8	347.33 ± 9.91	13.80 ± 1.78	333.54	292.62–374.45	0.001
C. albicans 1	9.04 ± 0.64	4.15 ± 0.20	4.89	2.43–7.35	0.011
C. albicans 2	9.37 ± 0.78	5.39 ± 0.19	3.98	0.86–7.11	0.03
C. albicans 3	5.45 ± 0.35	3.44 ± 0.18	2.01	0.76–3.26	0.015
C. albicans 4	5.94 ± 0.21	2.63 ± 0.02	3.31	2.40–4.22	0.004

Values are presented as means of three replicates ±SEM. A p-value < 0.05 indicates a significant difference in IC50 between the two groups. The highlighted p-values are higher than 0.05, indicating a non-significant difference. The statistical analysis was conducted using an independent samples t-test. Flu: fluconazole; Flu + Eso: fluconazole + esomeprazole.

Table 2. Minimum fungicidal concentration (MFC) of fluconazole against A. fumigatus and C. albicans, with and without esomeprazole

	MFC (µg/mL)		**Mean difference (µg/mL)**	**95% CI of the difference (µg/mL)**	
Isolate	**Flu**	**Flu + Eso**			**P value**
A. fumigatus 1	341.33 ±	85.33 ±	256	11.79–500.21	0.044

	85.33	21.33			
A. fumigatus 2	512.00 ± 0.00	170.67 ± 42.67	341.33	222.87–459.79	0.001
A. fumigatus 3	682.67 ± 170.67	213.33 ± 42.67	469.33	-19.10–957.76	0.056
A. fumigatus 4	426.67 ± 85.33	170.67 ± 42.67	256	-8.89–520.89	0.055
A. fumigatus 5	512.00 ± 0.00	170.67 ± 42.67	341.33	222.87–459.79	0.001
A. fumigatus 6	853.33 ± 170.67	213.33 ± 42.67	640	-42.01–1322.01	0.022
A. fumigatus 7	1024.00 ± 0.00	170.67 ± 42.67	853.33	734.87–971.79	0
A. fumigatus 8	1365.33 ± 341.33	137.33 ± 9.33	1228	279.95–2176.05	0.023
C. albicans 1	128.00 ± 0.00	85.33 ± 21.33	42.67	-16.56–101.90	0.116
C. albicans 2	170.67 ± 42.67	64.00 ± 0.00	106.67	-11.79–225.13	0.067
C. albicans 3	85.33 ± 21.33	42.67 ± 10.67	42.67	-23.56–108.89	0.148
C. albicans 4	64.00 ± 0.00	42.67 ± 10.67	21.33	-8.28–50.95	0.116

Values are presented as means of three replicates ±SEM. A p-value < 0.05 indicates a significant difference in MFC values between the two groups. The highlighted p-values are higher than 0.05, indicating a non-significant difference. The statistical analysis was conducted using an independent samples t-test. Flu: fluconazole; Flu + Eso: fluconazole + esomeprazole.

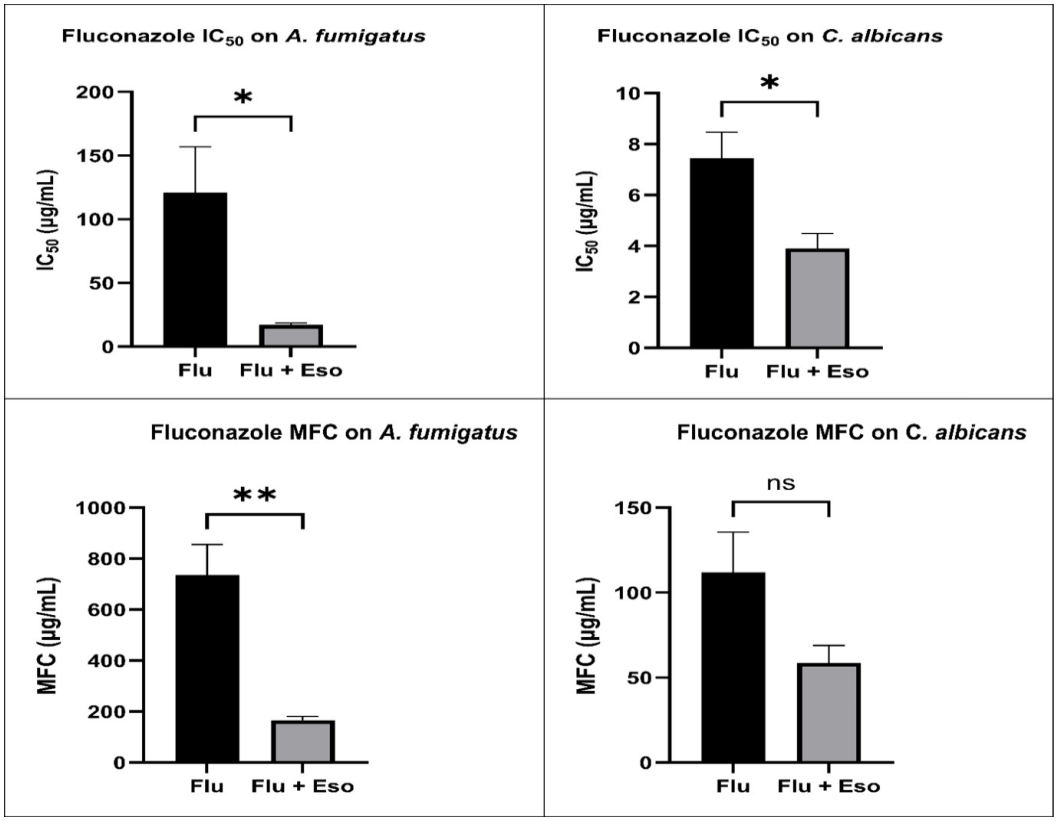

Figure 1. IC50 and MFC values of fluconazole against A. fumigatus and C. albicans with and without esomeprazole. The data represent the cumulative mean (columns) and SEM (error bars) for all A. fumigatus

(eight) and C. albicans (four) isolates. The presence of one asterisk denotes a significant difference between the two groups at $p < 0.05$, while two asterisks denote a significant difference at $p < 0.01$. ns = not significant. The statistical analysis was conducted using an independent samples t-test.

Flu: fluconazole; Flu + Eso: fluconazole + esomeprazole.

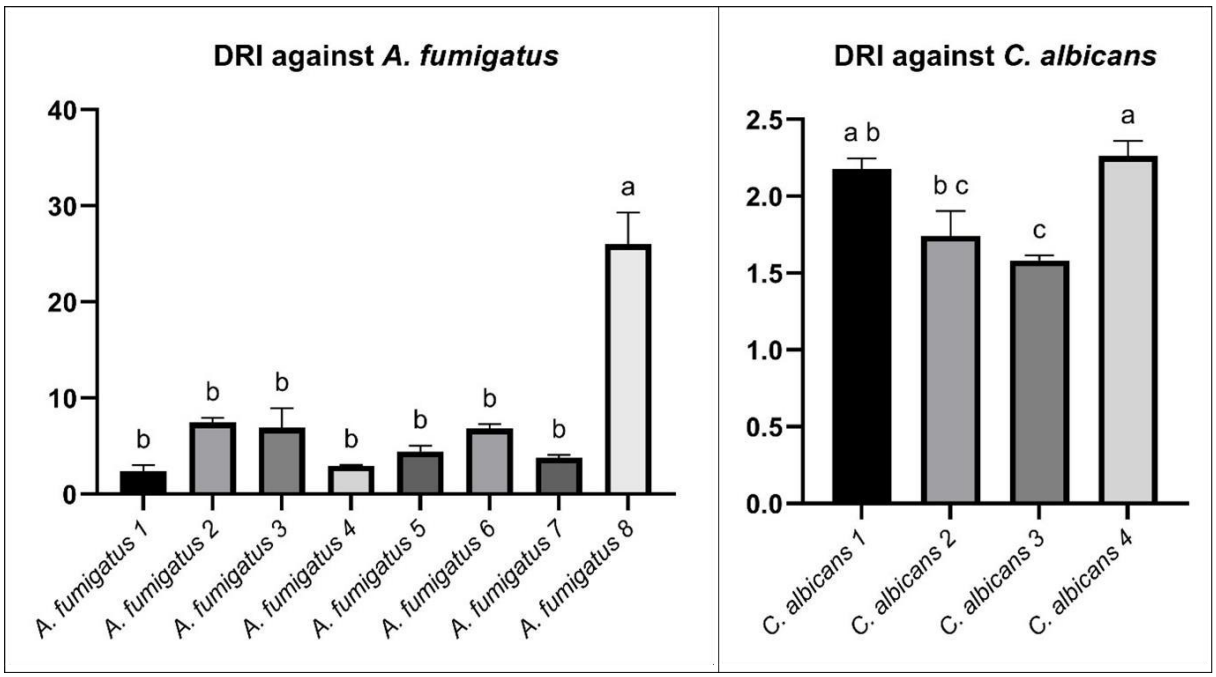

Figure 2. Dose reduction index achieved by the combination treatment of fluconazole and esomeprazole. The left panel shows DRI values for eight A. fumigatus isolates, while the right panel shows the DRI values for four C. albicans isolates.