



## Co-Metabolism and Immobilized Degradation of Some Anesthesia Drugs by *A.Fumigatus*

By Amany H. Aboellil & Fawziah M. Albarakaty

*Umm Al Qura University Makkah AL Moukarramah*

**Abstract** - In this study, three fungi isolated from some hospitals at Makkah Almoukarramah showed promising degrading capabilities towards some anesthesia drugs (propofol, clonazepam, lidocaine and bucaine) commonly used. Based on polyphasic identification, morphological, biochemical and 18SrRNA molecular identification these isolates are nominated as *Aspergillus niger*, *A. fumigatus* and *Rhizopus oryzae*. In general, propofol and clonazepam, were more liable to the biodegradation process when compared to the other two drugs. *A. fumigatus* showed the highest degrading capability towards drugs. The highest fungal wet biomass of *A.fumigatus* was obtained on cultures containing propofol and clonazepam at a final concentration of **2.5** and **1.25mgml<sup>-1</sup>**, respectively and separately. Shaking cultures showed an enhanced degradation when compared to that of static cultures. Moreover, the optimal conditions for drug biodegradation by *A.fumigatus* were pH4 at **28.5C°** and addition of vitamin C to the growth medium. Calcium alginate- immobilized fungal cells of *A. fumigatus* grown on propofol and clonazepam containing media showed improved higher degradation of the two drugs, compared to those of free fungal cells growing on the same media. On the other hand, a chromatogram of Infrared (IR) for the end products derived from the biodegradation of drugs confirmed that these two drugs are efficiently degraded to certain end products by *A. fumigatus* that could be categorized into some identified groups.

**Keywords** : *A. fumigatus*, *Propofol*, *Clonazepam*, *immobilization*, *DNA*, *IR*.

**GJSFR-C Classification**: NLMC Code: *WO 16, WO 20*



*Strictly as per the compliance and regulations of :*



# Co-Metabolism and Immobilized Degradation of Some Anesthesia Drugs by *A. Fumigatus*

Amany H. Aboellil<sup>α</sup> & Fawziah M. Albarakaty<sup>ο</sup>

**Abstract** - In this study, three fungi isolated from some hospitals at Makkah Almoukarramah showed promising degrading capabilities towards some anesthesia drugs (propofol, clonazepam, lidocaine and bucaine) commonly used. Based on polyphasic identification, morphological, biochemical and 18SrRNA molecular identification these isolates are nominated as *Aspergillus niger*, *A. fumigatus* and *Rhizopus oryzae*. In general, propofol and clonazepam, were more liable to the biodegradation process when compared to the other two drugs. *A. fumigatus* showed the highest degrading capability towards drugs. The highest fungal wet biomass of *A. fumigatus* was obtained on cultures containing propofol and clonazepam at a final concentration of 2.5 and 1.25mgml<sup>-1</sup>, respectively and separately. Shaking cultures showed an enhanced degradation when compared to that of static cultures. Moreover, the optimal conditions for drug biodegradation by *A. fumigatus* were pH4 at 28.5C° and addition of vitamin C to the growth medium. Calcium alginate-immobilized fungal cells of *A. fumigatus* grown on propofol and clonazepam containing media showed improved higher degradation of the two drugs, compared to those of free fungal cells growing on the same media. On the other hand, a chromatogram of Infrared (IR) for the end products derived from the biodegradation of drugs confirmed that these two drugs are efficiently degraded to certain end products by *A. fumigatus* that could be categorized into some identified groups.

**Keywords** : *A. fumigatus*, Propofol, Clonazepam, immobilization, DNA, IR.

## I. INTRODUCTION

The most important anesthesia drugs having clinical and therapeutic implications are dipriivan (propofol), clonazepam, lidocaine and bucaine as tablets or injection. Fukada *et al.*, (2008) and Loftus, *et al.*, (2008) reported that microbial infections could occur in drug addicted persons with immune complications.

Some microbes such as *Candida albicans*, *Staphylococcus aureus*, *S. epidermidis*, *Acinetobacter sp.*, *A. fumigatus*, *A. oryzae*, *A. niger*, *A. clavatus* and *A. ustus* could grow well in these contaminated drugs. The ability of the aforementioned microbes to sustain life in these drugs was reported by Robertson and Drummer (1995). On the other hand, it was reported that some of microbes such as *Bacillus megaterium*, *B. subtilis*, *B.*

*cereus*, *Streptococcus faecalis*, *Salmonella typhimurms*, *Escherichia coli* and *Pseudomonas aeruginosa* have the ability to develop some physicochemical modifications for the structure of some drugs (Ohsuka *et al.*, 1994).

Several papers have described the antimicrobial activity of local anesthetics on different organisms. Lidocaine is most active against pathogenic bacteria *in vitro* (Erlach, 1961 and Evron, 1980), an effect also confirmed in an *in vivo* model of surgical wound infections (Frelin *et al.*, 1982). Lidocaine & bupivacaine also showed fungicidal activity against *Candida spp.* by damaging the cytoplasm membrane (Galper and Catterwall, 1979). Additionally, both drugs are potent inhibitors of germ tube formation by *C. albicans* as a result of Ca<sup>+2</sup> channel blockade. Local anesthetics affect the membrane of *Candida*, impairing its permeability to ions e.g Ca<sup>+2</sup>, at low concentration while causing lethal damage at high concentrations (Hill, 1991).

The objective of this study is to isolate and identify some fungi that are accomplished with four narcotic drugs namely, propofol, clonazepam, lidocaine and bucaine, routinely used as anesthetic agents in a wide range of hospitals, in Makkah Moukarrama. The aim is extended to determine the biodegradability of these drugs by tested fungi, in an attempt to present a biotechnological approach in the treatment of drug-addicted person, to help get rid of any residual drugs circulating in their bodies. Moreover, it is to avoid contamination during anesthesia processor in hospital and to bioremediate of these drugs in disposable syringes.

## II. MATERIALS AND METHODS

- Drugs used:** anesthesia drugs were propofol, clonazepam, lidocaine, bucaine were purchased with in a pure form (99%) from Sigma-Aldrich, USA company.
- Isolation of some fungi associated with the process of anesthesia:**
  - Sources of micro-organisms** : Some fungal isolates were isolated from hospitals on Czapek's medium at 28C° for 5-7 days. from old contaminated syringes or ampoule containing the residue of drugs.
  - Preliminary tests to confirm the ability of fungi to grow on some drugs** : by using the method of wells, and measuring the inhibition area, if any.

**Author α :** Biology Department (Microbiology), Faculty of Applied Science (Girl Branch), Umm Al Qura University Makkah AL Moukarramah.

**Author ο :** Botany Department Faculty of Science, Cairo University.

iii. **Identifying fungal isolates:** fungal isolates obtained were traditionally identified after their purification and conservation in a suitable media, at the Public Health Laboratory in the capital of the Holy Capital. Some of them were selected to further study, confirming the identification by a molecular characterization of the gene 18S rRNA, at Alexandria Research Center (UNARC) Department of Biotechnology, Alexandria university.

3. **Effect of some different concentrations of some drugs on the growth of the selected fungi by estimating the wet weight :** Suitable Czapeks broth media fungi with different concentrations, of species of each drug separately (1.25 - 2.50 - 3.75 - 5.00 - 8.3 - 10 mgml<sup>-1</sup>). The media inoculated with fungal isolate separately and on all the previous concentrations. Flasks are incubated at a temperature of 28°C for a period of 5-7 days on shaking incubator at 180 rpm. After the end of the incubation period, the fungal growth could be estimated as biomass.

4. **Preparation of biomass for use in the following further studied experiments :** Fungal cultures were filtered after the incubation period then centrifuged at 2000 rpm, for 10 minutes, and then the precipitate biomass are washed twice by distilled water, try to get rid of water out by using filter papers followed by taking the wet weight (fresh weight g/12ml media). The filtrate is preserved in the refrigerator at a temperature of 4°C until analyzed by infrared spectrum IR.

5. **Optimization the environmental conditions to improve the process of growth and biodegradation :** Tested fungi had been developed in different environmental conditions in order to improve growth at different concentrations of the drugs between (1.25 - 2.50 - 3.75 - 5.00 - 8.3 - 10 mgml<sup>-1</sup>) were studied. Fungi were incubated at different degrees of temperature ranging from (5, 15, 28, 37 and 40°C) as well as different the hydrogen ion concentrations pH 4-7-8-10, the aeration by increasing the number cycles of shaking incubator (100-180-220 rpm). Also the impact of adding different concentrations from ascorbic acid (vitamin C) in the environment 12-36-72-108 mg /12 ml.

6. **The preparation of Biocatalysts :** Cells have been restricted (immobilized) in gels in agar and calcium alginate by dissolving 2g agar and 0.75 g sodium alginate in 60 ml of distilled water at room temperature, sterilized at a temperature of 110°C for 10 minutes and leave to cool at a temperature of 50°C, then 40 ml fungal suspension is added. 20 ml of this mixture is transferred under aseptic conditions to sterile syringe and injected in the form of drops in a cold sterile solution of calcium chloride of 2% concentration with continuous stirring using a magnetic stirrer for two hours. The gel beads were put in 200 ml of potassium phosphate buffer solution, 1.46% base potassium phosphate, 0.226% hydrogen potassium phosphate, for two hours to remove calcium alginate from beads gel. Then the gel beads

is washed with sterile distilled water or sterile saline solution and then filtrate and transferred to Czapeks broth.

7. **Preparation of the selected fungal isolate for examination with scanning electron microscope :** In order to examine the surface view and the interior porous of the restricted cells where it is frozen by cooling and then broken down into two parts and then dried, followed by loading on carbon or gold holder (gold coated prior) to cover the sample and preparing for microscopic examination and photography by the scanning electron microscope at King Saud University in Riyadh in the Central Laboratory of the Company [(JEOL) JSM-6060 LV] (Hayakawa *et al.*, 1991).

8. **Infrared spectrum (IR) analysis of the product of biodegradation of drugs in filtrate of *A.fumigatus* :** Filtrate is prepared resulting from the vital degradation process at the end of incubation, centrifuged at 2000 rpm / min for 15 minutes, to try to precipitate any impurities from the fungal growth or contents of media. To ensure the clearance of the filtrate, it is filtered by Minisart single use filter non-pyrogenic 0.20µm, then getting rid of water by lyophilizing apparatus (to avoid the affect the disturbance during spectrum infrared (IR) measurement through the emergence the peaks of water molecules, where the analysis was performed using Perkin elemspectrum 100 Japan Model FT-IR spectrometer (King Saud University in Riyadh in the central laboratory of the company).

### III. RESULTS

1. **Isolation and Identification of microorganisms associated with anesthesia at hospitals of Makka Almokarrama :** The fungi were isolated, purified and conserved as method mentioned before. It was prepared by subculturing the isolate on Czapek's media, incubated for 5 days at 28°C. Preliminary tests, using well method to estimate the effect of different drugs on the isolates was done by measuring inhibition zones, if occurred, after incubation for 24-72 hrs at 28°C. The surface area of inhibition zones were adopted according to its ability to resist the drug. In this study, three fungal isolates were highly satisfactory to grow against anesthetic drug, were selected for identifying morphologically and biochemically. These isolates were *A.fumigatus*, *A.niger* and *R.oryzae*.
2. **The effect of different concentrations of some aneesthesia drugs on the growth of the tested fungi on shaking culture :** From Table (1) and figure (2) the tested fungi reported different growth rate (represented as fresh weight g/12ml media) under different concentrations for each of propofol, clonazepam, lidocaine and bucaine. The highest growth rates were reported under clonazepam followed by propofol then bucaine and lidocaine

The growth rates of *A.fumigatus* and *A.niger* were the best in all concentration of clonazepam, if compared with the other drugs. *A.fumigatus* gives the highest growth rates of 27.17 and 32.99% at 2.5 mgml<sup>-1</sup> concentrations of propofol and 1.25 mgml<sup>-1</sup> clonazepam respectively, if compared with the growth of other fungi at the same concentrations of propofol and clonazepam.

3. **Classification of fungal isolates according to its abilities to grow against different concentrations of drug** : Table (2) summarized the *in vitro* sensitivities of the three fungal isolates to the four anesthetic drugs as determined by inhibition zone and growth on different drug-doses . Isolates were classified as drug-phile, resistant, tolerant, sensitive and lethal. *A.fumigatus* and *A.niger* were classed as propofol – resistants & clonazepam – phile, while *R.oryzea* was classed as propofol – sensitive, clonazepam – resistant. Bucaine and lidocaine have lethal effect for *R.oryzea*. Bucaine and lidocaine have negatively effect on fungal growth. *A.niger* and *A.fumigatus* showed reduced but persistent growth under all drug doses of bucaine , therefore they were classed as doses tolerant . *A.fumigatus* was selected to further study.
4. **Confirmation the identification of *A.fumigatus* by molecular method** : Fungal isolate was polyphasic identified through microscopical examination, biochemical and molecular identification via sequencing of 18SrRNA gene fig (1). DNA sequences of *A.fumigatus* were subjected to blast programme at National Center for Biotechnology Information (NCBI). Initial alignment with blast programme results in selection of some 18SrRNA fungal sequences, already deposited in the Genbank, that showing high similarity with the query sequences derived from the present study. These selected 18SrRNA fungal sequences along with our query sequences were subjected to another alignment (multiple alignment) in Bioedit software to precisely determine which 18SrRNA fungal sequence is highly similar to our query sequence. Moreover, phylogenetic tree was drawn to determine the genetic affiliation of these fungal isolates. This confirm that the isolate is perfect *A.fumigatus*.
5. **Growth rates of *A.fumigatus* as free and immobilized cell cultures at different drug-doses of propofol and clonazepam** : From fig (2) , the highest growth rates of *A.fumigatus* ( 2.710 and 2.834 g/12 ml ) at 2.5 mgml<sup>-1</sup> and 1.25 mgml<sup>-1</sup> for propofol and clonazepam , respectively were demonstrated as free cell culture . On immobilized cell culture the growth rate reported increased values 92.45 and 74.84% at the same concentrations, if compared with free cell culture. The immobilized cell culture support the growth rate of *A.fumigatus* in the highest concentrations of propofol and clonazepam

by consider values 2.125 and 2.653 g/12ml at 10.0 mgml<sup>-1</sup> for each drug.

6. **Optimizing cultural conditions involved in growth and biodegradation of anesthesia drug by *A.fumigatus*** : The factors as pHs , temperatures , drug concentrations , antioxidants and aeration rates were optimized to improve the growth of *A.fumigatus* . Table (3) showed that optimum cultural conditions were pH 4 and temperature 28 C° . The growth of *A.fumigatus* was improved by addition of 108 and 12 mgml<sup>-1</sup> of ascorbic acid as antioxidant on the media containing 2.5 and 1.25 mgml<sup>-1</sup> of propofol and clonazepam respectively, on orbit shaker for 220 rpm . These previous cultural conditions have been applied on immobilized cell culture for inducing (improving ) of biodegradation of anesthetic drugs .
7. **Electron microscopic scanning** : Fig (4a) illustrated the biocatalyst ( immobilized cells as a bead ) before incubation showing its smooth surface , but fig ( b, c ) showed the heavy growth of *A.fumigatus* hyphae on the biocatalyst .
8. **IR analysis of the products of the biodegradation by *A.fumigatus* for each of propofol and clonazepam** : Fig (5a) illustrated the IR analysis of the principle drug compound of clonazepam . From fig (5b) the IR peak of absorption was shown at 3292cm<sup>-1</sup> illustrated the NH group which already occurred in drug (clonazepam) also another peak was at 1417cm<sup>-1</sup> confirmed the above group (NH). The group C-Cl was demonstrated by the peak of absorption at 768cm<sup>-1</sup>. The IR analyses of the filtrate of *A.fumigatus* grown on clonazepam as free cell was shown in fig (5b). A new absorption peak was observed at 1725cm<sup>-1</sup>. This is evidence the presence of carbonyl group (C=O) which did not recorded in principle drug. C-Cl group was lacked. The two peaks of 2940, 2890 cm<sup>-1</sup> were reduced, due to decrease the intensity of aromatic CH. On the other hand, IR of the filtrate of immobilized *A.fumigatus* grown on medium containing clonazepam was shown in fig (5c). The increase in absorption peak at 3363 cm<sup>-1</sup> illustrate increasing in intensities of NH and C=O groups in compared with the principle compound a peak at 1406 cm<sup>-1</sup> confirmed rising of intensities of these groups .At first time a new peak at 1350cm<sup>-1</sup> involved in the filtrate of immobilized *A.fumigatus* grown on clonazepam, this peak characterized the group of C-O-C . Two peaks at 1727 and 2940 – 2890 cm<sup>-1</sup> increased due to high intensities for acidic carbonyl (C=O) and aromatic CH corresponding to the principle compound and the first filtrate.

From fig (5d) the IR analysis absorption illustrated the principle propofol . A peak at 3353cm<sup>-1</sup> was evidence for presence of OH group with H-bonds . A peak for 2850-2920cm<sup>-1</sup> is due to aromatic OH. On free cell culture, a peak of at 1743 cm<sup>-1</sup> due to acidic C=O

was indicated fig (5e) , but on immobilized culture fig (5f), a high intensity of carbonyl group C=O was demonstrated.

#### IV. DISCUSSION

A survey of fungi associated with anesthesia were isolated on specific media, under appropriate conditions, purified and conserved as the above mentioned method. Three isolates are identified as *Aspergillus niger*, *A. fumigatus*, *Rhizopus oryzae*. *A. fumigatus* is the most prevalent airborne fungal pathogen in developed countries and in immunocompromised patients causes a usually fatal invasive aspergillosis (Latge, 2001). The definition of *A. fumigatus* was confirmed by molecular characterization of the gene 18S rRNA. This method is the best definition, through multiplication of gene by PCR. Sequences of genetic for 18S rRNA was found out and compared with their counterparts in the field of Bioedit software Showing 85% similarity. Phylogenetic tree determined the affiliation gene for this isolate.

In this study, the isolated fungi were classified, on its ability to grow in different concentrations of each drug used, as drug-phile, resistant, tolerant, sensitive or lethal, depending on the doses used. lidocaine or bucaine have inhibiting effects on *A.niger* and *A.fumigatus* and influential fatal to *R. oryzae*. Lidocaine and bucaine are known to inhibit germ tube formation of *C.albicans*, hypothesizing that the effect is due to blockading ionic channels, particularly calcium channel. Therefore, lidocaine can affect morphology and probably also the pathogenesis of *C. albicans*. (Rodrigues *et al*, 2005).

Pina-Vaz *et al.*, (2000) stated that at lower concentrations, these drugs have a fungistatic activity, due to metabolic impairment, while at higher concentrations they are fungicidal, due to direct damage to the cytoplasmic membrane. Ohsuka *et al.*, (1994) suggested that depolarization of the cytoplasmic membrane, preceded by the permeabilization of the outer membrane for gram-negative bacteria, is associated with antibacterial activity of lidocaine. The drugs have a negative impact on the membranes of organism as a result of an imbalance in the permeability and the demise of polar cell membrane and the leakage of ions of potassium K +. Also, it led to the inhibition of activity of some enzymes such as succinic dehydrogenase .

However, numerous studies over the past several decades have elucidated the supplemental role of local anesthetics as antimicrobial agents. In addition to their anesthetic properties, medications such as bupivacaine (bucaine) and lidocaine have been shown to exhibit bacteriostatic, bactericidal, fungistatic, and fungicidal properties against a wide spectrum of microorganisms (Johnson *et al.*, 2008).

In our study, *A .fumigatus* and *A .niger* were propofol- resistant . *R.oryzea* showed sensitivity to propofol. Güzelant *et al.*, (2008) have found that some types of organisms such as *Candida albicans*, *Staphylococcus aureus*, *Acinetobacter calcoaceticus*, *Escherichia coli*, *Enterobacter cloacae*, *Moraxella osloensis*, *Cunninghamella elegans* and *Pseudomonas aeruginosa*, *Streptomyses* were grown well on propofol , they are resistant to the drug. Propofol infusion promoted budding of *Candida* and the germination of *Aspergillus*, latter forming a lipid layer around the hyphae . Propofol infusion , due to its lipidic vehicle, increased the fungal germination and promoted resistance to antifungals. This effect seems to be related to the reduced access and / or permeabilization to fungal cells by antifungals. (Costa-de-oliveira *et al.*, 2008).

*A. fumigatus* and *A. niger* have an affinity to clonazepam but *R.oryzea* showed a resistance to drug. It may be due to their ability to degrade the drug and use it as essential material for their growth and their activities therefore, they are drug-phile. Robertson and Drummer (1995) have studied on the degradation of some drug types of clonazepam in the blood by bacterial species of intestine, *Streptococcus faecalis* and *Clostridium perfringens*. He noted the complete degradation of this drug in the blood .The incubation under anaerobic condition led to a reduction of clonazepam and its transformation into 7-aminoclonazepam and the incubation under aerobic conditions gave 3-hydroxyclozepam, as a key component of the transformation processes. He also found that the process of degradation occurs the same way in animals by the microflora.

Environmental conditions have been improved for enhancing the growth of *A. fumigatus* to best degradation of selected drugs at high concentrations. It has been found that the best concentration of hydrogen ion (pH) is 4, and the best temperature is 28°C. Adding antioxidant ascorbic acid (vitamin C) to media accelerated the degradation of drugs, when using 2.5mgml<sup>-1</sup> concentration of propofol and 1.25mgml<sup>-1</sup> clonazepam . Using shaking culture (220 rpm) helped to improve the degradation of drugs. Güzelant *et al.*, (2008) stated that the temperature is of the important factors to control the activity of microbial, and that the proper temperature promotes the growth of microbes on the drug and lead to an increase in rates of destruction of drugs.

Robertson and Drummer (1995) stated that the rates of metabolism, at 37 C, ranged from 0.1 ng/mL/min of nitrobenzodiazepines for *Streptococcus faecalis* to 8.8 ng/mL/min. The pH had variable effects on the rate of metabolic bioconversion of nitrobenzodiazepines, while increasing temperatures were found to generally increase the rate of nitrobenzodiazepine bioconversion.

Baumgart *et al.*, (2007) noted that the most important chemicals affecting also are antioxidants, which found that intravenous drug abuse led to an increase of antioxidants in blood plasma and this led to increased resistance to large molecules to oxidation. The some fungal species and bacteria can produce some antioxidants, such as carotene and some antioxidant enzymes, such as those produced by bacteria *E.coli*.

Some new technologies have been used to improve the degradation of drugs by microorganism through immobilizing the fungal cells, this immobilized cell is called Biocatalyst (Usha *et al.*, 2010). Calcium alginate used in this study. Modak *et al.*, (2001) has been able to use of the immobilizing process in the detention cells *Pseudomonas putida* for the degradation of phenol. Mattiasson (1983) has proved to that stability enzymatic system of immobilized cells was better than in the free cells, and offers the opportunity to step enzyme multiple accompanied by co-enzyme for the process of degradation in general. On this basis, Wiesel *et al.*, 1993 worked on restricting the entire microbial living cells. This is due to its features, many is that the cells bound to be more resistant to pollution, can be separated metabolites recorded easily, can re-use of biocatalyst recorded more than once, Also, it give the large biomass and protect cells from high concentrations of toxic substances. In our study immobilized cells of *A. fumigatus* has shown resistance, even was able to grow in higher concentrations, it has already grown at a concentration of 10 mgml<sup>-1</sup> of the medical drug.

The immobilized *A.fumigatus* mycelia (biocatalyst) may be allowed the concentrations of nutrients around the fungus away from the toxicity, of high concentrations of narcotic substances. This accelerates the growth process and leads to flourishing growth surface on gel beads which may speed up the process of degradation of drugs.

According to the IR analyses of propofol and clonazepam, the changes of the original compounds after treatment with exposure to fungal growth were studied. In general, functional groups presented showed fluctuation by decreasing or increasing of its the related peaks. Also, some original functional groups disappeared and some new ones appeared, e.g acidic carbonyl (C=O) group was appeared the first time and (C-Cl) group was laked, corresponding to the principle compound of clonazepam.

These changes led to confirm the bioconversion of both drugs by fungal growth. IR has proved the capability of *A.fumigatus* to change the nature of drugs. These indicate that *A.fumigatus* may be biodegraded the drugs to another compounds to avoid its toxicity or to use its derivatives as nutrient.

Also, IR analyses showed that the immobilized cells of *A.fumigatus* recorded the highest degree of degradation for the two drugs than that of the free cells . of *A.fumigatus*.

## V. CONCLUSION

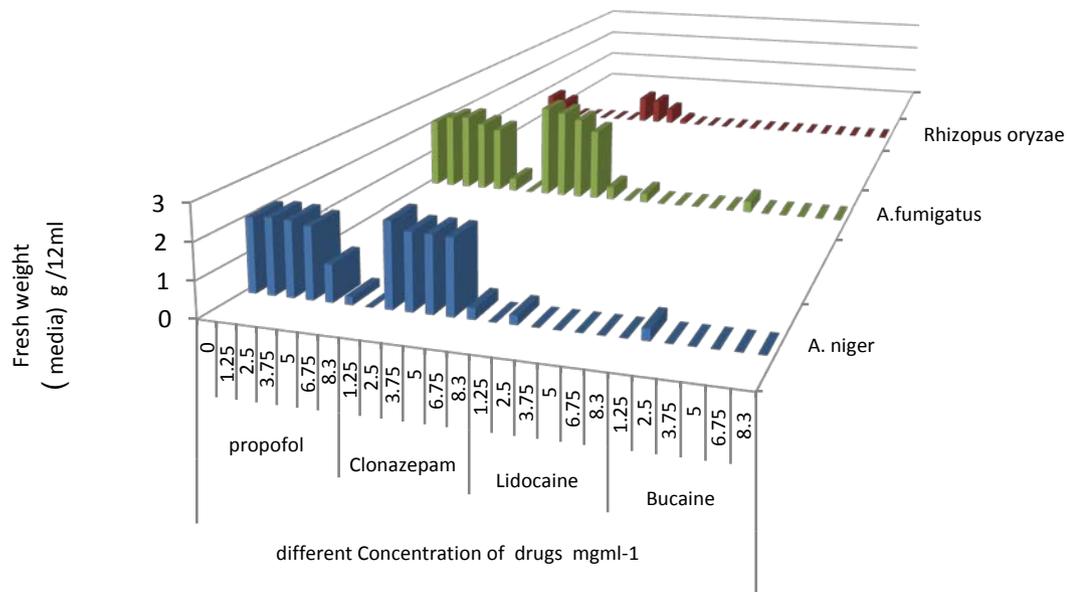
Some drugs may support the growth of some microbes, such as enhancing of propofol for the growth of *A.fumigatus*. The fungus may be able to use the drug clonazepam where it has the ability to convert drug into suitable compound easy to use. Lidocaine and biocaine have to do mutual action, they had the ability to inhibit microbes, where they were working as antimicrobial. Immobilization of the fungus, as biocatalyst under certain circumstances may lead to increased growth of *A.fumigatus* and its ability to degrade some drugs. The vision for the future is to take these advantages of the microorganisms, in a positive step, to treat the addict or the patient, focusing on an attempt to avoid the contamination with microbes during the process of anesthesia, in medical field, to avoid microbial infection. Further studied are necessary to define the clinical significance, prevalence and mechanisms of resistance of these isolates of *A.fumigatus* to drugs .

## REFERENCES RÉFÉRENCES REFERENCIAS

1. Costa-de-oliveira,S. ; Araujo,R. ; Silva-dias,A. ; Pinavaz,C. and Goncalves,A . (2008). Propofol lipidic infusion promotes resistance to antifungals by reducing drug input into the fungal cell. BMC Microbiology, 8:9:10.1186/1471-2180-8-9.
2. Erlich, H. (1961). Bacteriologic studies and effects of anesthetic solutions on bronchial secretions during bronchoscopy. Am Rev Respir Dis 84:414–42.
3. Evron, R. (1980). In vitro phagocytosis of Candida albicans by peritoneal mouse macrophages. Infect Immun 28:963–971.
4. Frelin, C.; Vigne, P. and Ladzunski, M. (1982) Biochemical evidence for pharmacological similarities between a adrenoreceptors and voltagedependent Na<sup>1</sup> and Ca<sup>11</sup> channels. Biochem Biophys Res Commun 106:967–973.
5. Fukada, T.; wakiri, H. and Ozaki, M. (2008). Anaesthetists role in computer keyboard contamination in an operating room. Hosp. Infect., 70(2): 148-153.
6. Galper, J.B. and Catterwall, W.A. (1979). Inhibition of sodium channels by D600. Mol Pharmacol 15:174–178.
7. Güzelant, A.; Apiliogullari, S.; Kara, I.; Turhan, V.;Apiliogullari, B.; Yilmaz, H.; Balasar, M. and Duman, A. (2008). The preventive effect of diphenhydramine on bacterial growth in propofol. European Journal of Anaesthesiology, 25 : 737-740.
8. Haykawa, M. ; Kajiura, T. and Nonomura, H.(1991). New methods for the highly selective isolation of strepto sporangium and Dacty Losporangium from soil.Ferment. Bioeng, 72:327-333.
9. Hill, B. (1991). TheWilcoxon signed rank test. In: Bradford Hill (ed) Principles of medical statistics, 12th ed., London: Edward Arnold, pp., 126–127.

10. Johnson, S.; Saint, M.; John, B.E., and Dine, A.P. (2008). Local anesthetics as antimicrobial agents. *Surg. Infect. (Larchmt).*, 9 (2):205-213.
11. Latge, J.P. (2001). The pathobiology of *Aspergillus fumigatus*, *Trends Microbiol*, 9(8): 382–9.
12. Loftus, R. W.; Koff, M. D.; Burchman, C. M.D.; Schwartzman, J.D. and Thorum, Valerie, M.T. (2008). Transmission of Pathogenic Bacterial Organisms in the Anesthesia Work Area. *Anesthesiology*, 109(3): 399 - 407.
13. Mattiasson, B. (1983). Immobilized cells and organelles. Mattiasson, B. (ed.), Crc- Press, Boca Raton, Florida, P. 152.
14. Modak, J.M. ; Bandopadhyay, K. ; Das, D. and Maiti, B. R. (2001). Mathematical Model for evaluation of mass transfer limitation in phenol biodegradation by immobilized *Pseudomonas putida*. *J. Biotechnol.* , 87 : 211 – 223.
15. Ohsuka, S. ; Ohta, M.; Masuda, K.; Arakawa, Y.; Kaneda, T. and Kato, N. (1994). Lidocaine hydrochloride and acetylsalicylate kill bacteria by disrupting the bacterial membrane potential in different ways. *Microbiol. Immunol.*; 38 (6) :429 – 34.
16. Pina-vaz, C.; Rodrigues, A.; Sansonetty, F.; Martinez-De-Oliveira, j.; Fonseca, A. and Mardh, p. (2000). Antifungal activity of local anesthetics against *Candida* species, *Infect Dis Obstet Gynecol* 8, pp. 124–13.
17. Robertson, M.D. and Drummer, O.H. (1995). Postmortem Drug Metabolism by Bacteria. *Journal of Forensic Sciences (JOFS)*, 40: 5.
18. Rodrigues, A.; Pina-Vaz, G.; Mardh, C.; Martinez-de-Oliveira, P. and Freitas-da-Fonseca, J. A. (2000). Inhibition of germ tube formation by *Candida albicans* by local anesthetics: an effect related to ionic channel blockade, *Curr Microbiol* 40 pp. 145–148.
19. Usha, M.S.; Sanjay, M.K.; Gaddad, S.M. and Shivannavar, C.T. (2010). degradation of H-acid by free and immobilized cells of *alcaligenes latus*. *Brazilian journal of microbiology* 41:931-945.
20. Wiesel, I.; Wubker, S.M. and Rehm, H.J. (1993). Degradation of polycyclic aromatic hydrocarbons by an immobilized mixed bacterial culture. *Appl. Microbiol. Biotechnol.*, 39 : 110 – 116.

Fig 1 : Effect of different concentrations of some anesthesia drugs on the growth of the tested fungi (fresh weight g/12ml) on shaking culture .



**Table 1 :** The growth rate (%) of the tested fungi grown on different concentrations of some anesthesia drugs on shaking culture.

The tested fungi Different concentrations of drugs mgml <sup>-1</sup>		Growth rate %		
		<i>A. niger</i>	<i>A. fumigatus</i>	<i>R. oryzae</i>
propofol	control	100	100	100
	1.25	+ 2.12	+7.84	- 24.65
	2.50	+ 0.86	+ 10.18	≠
	3.75	- 3.66	- 1.64	≠
	5.00	- 50.12	- 6.52	≠
	6.75	- 89.25	- 81.84	≠
	8.30	≠	≠	≠
Clonazepam	1.25	+ 13.49	+ 32.99	+ 25.35
	2.50	+ 2.27	+ 27.69	+12.47
	3.75	+ 1.74	+ 18.82	- 28.83
	5.00	+ 0.63	+ 2.72	- 82.53
	6.75	- 84.82	- 81.42	≠
	8.30	≠	≠	≠
Lidocaine	1.25	- 88.05	- 87.10	≠
	2.50	≠	≠	≠
Bucaine	3.75	- 84.82	- 85.45	≠
	5.00	≠	≠	≠

(+) positive growth

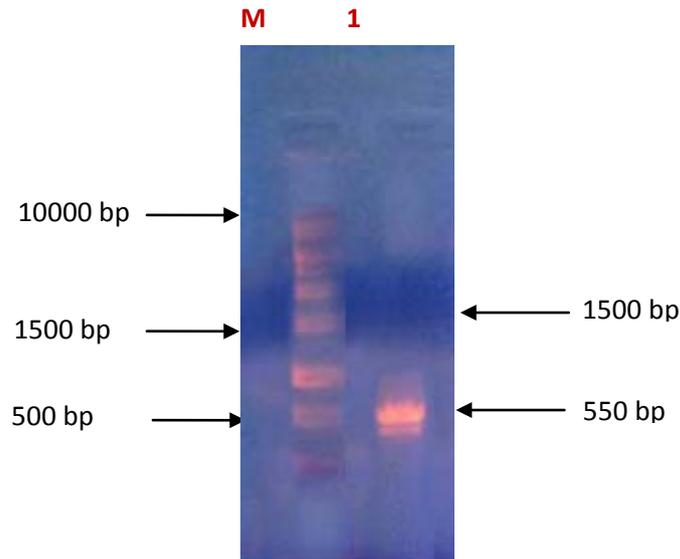
(-) negative growth

(≠) no growth

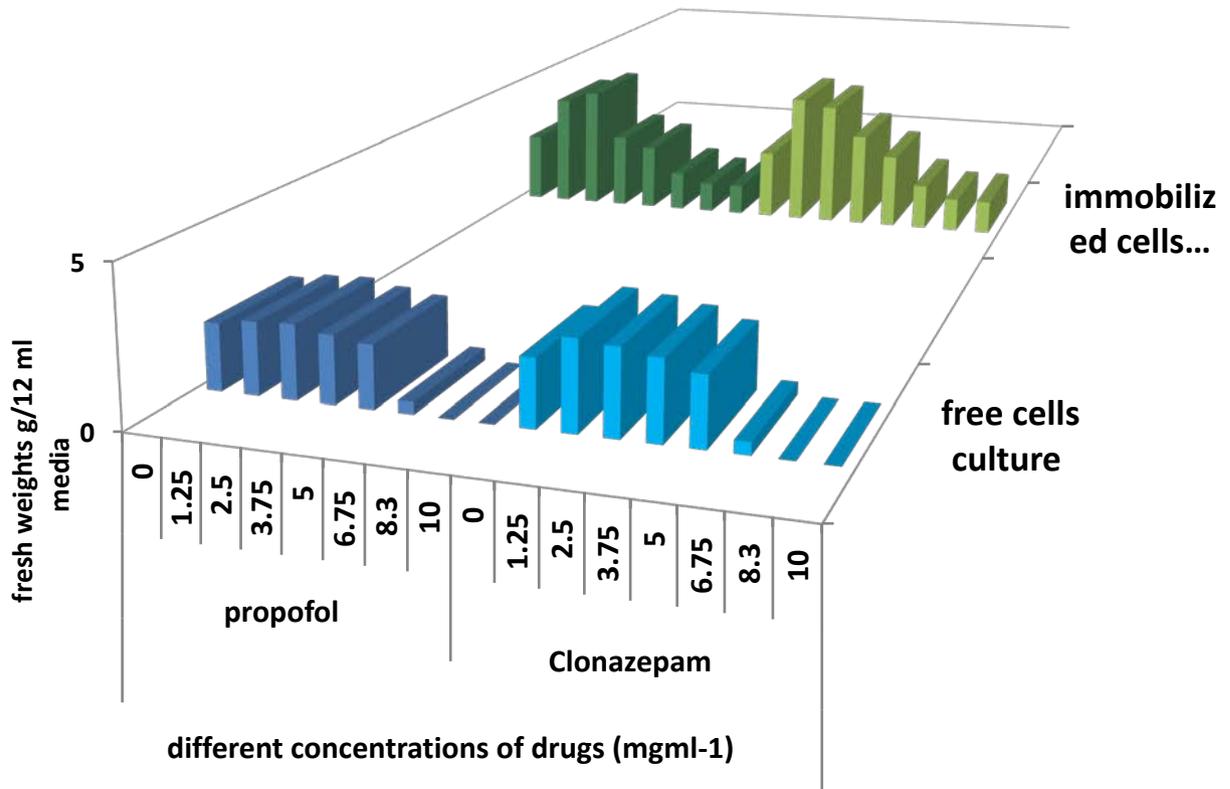
**Table 2 :** Classification of isolates according to its ability to grow against some anesthetic drugs .

Fungi	Types of drugs			
	Propofol	Clonazepam	Lidocaine	Bucaine
<i>A. niger</i>	drug – resistant	drug – phile	drug – sensitive	drug – tolerant
<i>A. fumigatus</i>	drug – resistant	drug – phile	drug – resistant	drug – tolerant
<i>R. oryzae</i>	drug – sensitive	drug – resistant	drug – lethal	drug – lethal

*Fig 2 :* showing PCR product of 18SrRNA gene amplified via PCR from one fungal isolates. M: DNA base pair marker. Lane1: PCR product of the fungal isolate nominated biochemically as *A. fumigatus* .



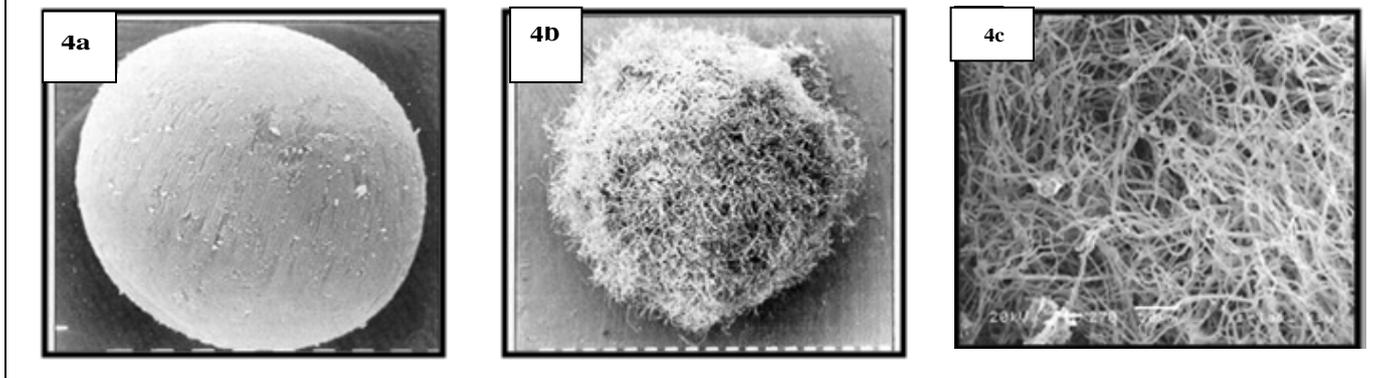
*Fig 3 :* Comparison between the growth of *A.fumigatus* as free cells and immobilized cells on different concentrations of propofol and clonazepam on shaking culture .



**Table 3 :** The optimization of environmental conditions for the growth of *A.fumigatus* grown on media containing some anesthesia drugs .

Drugs mgml-1	Concentration of optimal growth	Final concentration in free cell culture	Final concentration in immobilized cell culture	Incubation temperature (C°)	pH	Number of cycles of shaken culture rpm/min	Vitamin C mgml-1
Propofol	2.50	6.75	10.00	28	4	220	108
Clonazepam	1.25	6.75	10.00	28	4	220	12

**Fig (4) :** Scanning electron micrographs of (A) the surface of calcium alginate bead containing subsurface mycelium of *Aspergillus fumigatus*. (B) the surface of an inoculated calcium alginate bead which was overgrown with mycelia of *A. fumigatus* mycelia during incubation. (C) amplified of (B).



**Fig 5 :** IR Spectra (a): IR spectrum of clonazepam (b): IR spectrum of degrading products of clonazepam on free cell culture (c): IR spectrum degrading of products of clonazepam on immobilized cell culture (d): IR spectrum of propofol (e): IR spectrum of degrading products of propofol on free cell culture (f): IR spectrum of degrading products of propofol on immobilized cell culture.

