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Isolation and identification of fungi from two hospitals in Baghdad city and effect of disinfectants on some fungi

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Abstract:

Swabs from two hospitals at Baghdad city were collected from the period 28-October-2013 to 31-March-2014, to found out the prevalence and distribution of fungi. These isolates were collected from hospitals' environment and most of the items used for hospital work in order to detect the nosocomial pathogens persistent. All fungal isolates were identified depending on the morphological and microscopic examinations as well as biochemical tests. Out of 220 environmental samples, 146 fungal isolates was obtained, *Aspergillus flavus* was more frequency and occurrence 23.29% and 15.45% respectively, followed *Aspergillus niger* was 15.57% and 10.45% then *Penecillium* spp. was 13.27% and 8.64%. In Al -Yarmouk Teaching Hospital more places contamination was burns 75%, followed dialysis 72.72% then RCU units 69.23%, while more places contamination in Imam Ali General Hospital was wards 70%, followed burns and Kitchen 58.33% then operation hall 57.14%. Determining the minimum inhibitory concentration for five types of disinfectants which are used in hospitals, the MIC values of disinfectants against fungal isolate were Chlorhexidin 7.5%, Chloroxlenol 1.25%, Formaldehyde 1.25%, Povidine iodine 10% and Sodium hypochlorite 7.5%. Chloroxlenol had significantly the most effective compound on fungi, followed formaldehyde then Sodium hipochlorite. This study found that hospitals contain different fungal species and genus, antiseptics and disinfectants have an effect on the fungus if it used correctly and permanent change in the use of antiseptics and disinfectants to avoid resistance of fungi.

Keywords: Nosocomial infections, Disinfectants, Environmental swabs.

عزل وتشخيص الفطريات من مستشفيات في مدينة بغداد وتأثير المطهرات على بعض الفطريات

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الخلاصة:

تم جمع عزلات فطرية بطريقة اخذ المسحات من مستشفيات في مدينة بغداد للفترة من 28-10-2013 الى 31-3-2014 للتعرف على مدى تلوثها وانتشار وتوزيع الفطريات فيها. تم اخذ العينات البيئية من اماكن مختلفة في المستشفى شملت معظم الوحدات والاجهزة الطبية، للكشف عن تواجد الفطريات التي تسبب عدوى المستشفيات. تم تشخيص الفطريات اعتمادا على المظهر الخارجي والفحص المجهرى والاختبارات البايوكيميائية. تم الحصول على 146 عزلة فطرية مختلفة من 220 مسحة المجموع الكلي للعينات البيئية الماخوذة، كان *Aspergillus flavus* الاكثر ظهورا وترددا بنسبة 23.29% و 15.45% على التوالي،

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تليها *Aspergillus niger* بنسبة 15.57% و 10.45% ثم *Penecillium spp.* بنسبة 13.27% و 8.64%. في مستشفى اليرموك التعليمي كان اكثر الاماكن تلوثا هي وحدة الحروق 75% تليها وحدة غسل الكلى 72.72% ثم وحدة الانتعاش 69.23%، اما في مستشفى الامام علي العام فان الاكثر تلوثا كانت ردهات رفود المرضى 70%، تليها وحدة الحروق والمطبخ 58.33% ثم صالة العمليات 57.14%. كانت قيم التركيز المثبط الادنى 7.5% Chlorhexidin و 1.25% Chloroxyleneol و Formaldehyde و 1.25% و 10% Povidine iodine و 7.5% Sodium hypochlorite. وجدت هذه الدراسة ان المستشفيات تحتوي على انواع واجناس فطرية مختلفة، وان المطهرات لها تاثيرا على الفطريات فيما لو استخدمت بشكل صحيح، ويجب التغيير المستمر في نوعية المطهرات المستخدمة لتجنب مقاومة الفطريات لهذه المطهرات.

Introduction

Hospitals consider favorite places for the emergence of a wide variety of pathogenic and opportunistic fungi. Different fungi causing infections, some is occurring commonly infections while others is a rare [1]. Airborne fungal conidia are inhaled by everyone, because their concentration in the air is high, approx. 1–100 conidia per m³ [2]. This may be the reason why nosocomial acquired infections and community acquired infections quite often develop in immunocompromised as well as in immunocompetent people [3]. Like any other microbial pathogen, fungal infection also involves some basic steps such as (1) entry and adherence to the host tissue, (2) invasion of the host tissue, (3) multiplication, colonization and dissemination in the tissues, and (4) evasion of the host immune system and damage to the tissues [4]. The frequency of severe fungal diseases has increased in the last few decades. Opportunistic pathogens from the genera *Candida* and *Aspergillus* can invade human organism and may lead to mucosal and skin infections or to deep-seated mycoses of almost all inner organs, especially in immunocompromised patients[5]. In the last decades the problem of severe nosocomial fungal diseases has become more serious. Among all the fungi only few species are pathogenic to humans. The most frequently diagnosed fungal infections are caused by pathogens from the genera *Candida*, and *Aspergillus*. These fungi are ubiquitous and can be acquired from host surroundings or are components of normal endogenous flora [6]. Many human fungal pathogens are dimorphic (capable of reversible transitions between yeast and hyphal forms) [7]. Usually disinfectants are "cidal" in that they kill the susceptible potential pathogenic agents. The selection of a disinfectant should be based on the function the disinfectant is expected to perform. Ideally, a disinfectant should be broad spectrum, nonirritating, nontoxic and noncorrosive. Selection include effectiveness against the potential pathogenic agent, safety to people, impact on equipment and the environment [8]. Aims of study: Control fungal contamination hospitals and prevention of nosocomial infections.

Materials and Methods:

Collection of sample: Swabs were taken from hospitals by using sterile transport media swabs.

Culturing laboratory: Swabs cultivated directly on the media culture appropriate (Sabourad dextrose agar, Potato dextrose agar, Czapek agar), incubated dishes under temperature at 29 °C for 7-35 days, and then examine the growth after that period and done sub culture of dishes that showed a positive result [9].

Purification, isolation and identification of fungi: Colony morphology, colony reverse and microscopic characteristics were taken in consideration in the identification [10].

Determination of disinfectants activity: Different concentrations were prepared (1.25, 2.5, 5, 7.5 and 10 %) for purpose of determining minimum inhibitory concentration, cultured on Sabourad dextrose agar containing different concentrations of disinfectants, incubated dishes under temperature at 29 °C for 7 days, after incubated period, measured diameters of fungal colonies growth and comparing it with control treatment.

Percentage of inhibition: Calculated the percentage of inhibition of measuring the diameter of the colony transactions relative to the control treatment according to the following equation [11].

$$\text{Percentage of inhibition} = \frac{\text{Diameter of colony control} - \text{diameter of colony treatment}}{\text{Diameter of colony control}} \times 100$$

Frequency Percentage: Calculated the percentage frequency of species isolated by applying the following equation [11].

$$\text{Percentage frequency} = \frac{\text{The number of isolates of the same species}}{\text{The total number of isolates of all kinds}} \times 100$$

Occurrence Percentage: Calculated the percentage for the Occurrence of each species isolated according to the following equation [11].

$$\text{Percentage of emergence} = \frac{\text{The number of samples that appeared to show one type}}{\text{The total number of samples}} \times 100$$

Results and Discussion:

Aspergillus flavus more frequency and occurrence fungi 23.29 and 15.45 respectively, according to [12], Table-1 shows number of swabs, percentages of contamination.

Table 1-The number of swabs and percentages of contamination.

Hospitals	Al -Yarmouk Teaching Hospital	Imam Ali General Hospital	Total
Swabs	125	95	220
Positive	78	49	127
%	62.4	51.58	57.72
Negative	47	46	93
%	36.7	48.42	42.28

The most common fungi generally collected are *Cladosporium*, *Aspergillus* and *Penecillium*. *Alternaria*, *Rhizopus*, *Mucor*, yeast, and *Fusarium* species are often being found as well, Table-2 shows fungal species, frequency and occurrence percentages.

Table 2-The species and genus fungal isolates and the frequency and occurrence percentages.

Hospitals Isolates	Al -Yarmouk Teaching Hospital	Imam Ali General Hospital	Total	Frequency percentage	Occurrence percentage
Fungal isolation no.	88	58	146		
<i>Aspergillus flavus</i>	25	9	34	23.29	15.45
<i>Aspergillus niger</i>	11	12	23	15.57	10.45
<i>Penecillium</i> spp.	11	8	19	13.01	8.64
<i>Aspergillus fumigates</i>	11	4	15	10.27	6.89
<i>Rhizopus</i> spp.	6	6	12	8.22	5.45
<i>Fusarium</i> spp.	5	5	10	6.85	4.54
<i>Mucor</i> spp.	5	3	8	5.48	3.64
<i>Yeasts</i>	4	3	7	4.79	3.18
<i>Aspergillus nidulans</i>	2	3	5	3.42	2.27
<i>Alternaria</i> spp.	3	2	5	3.42	2.27
<i>Cladosporium</i> spp.	3	2	5	3.42	2.27
<i>Aspergillus terrus</i>	2	2	4	2.74	1.89
<i>Scopulariosis</i> spp.	1		1	0.68	0.45
<i>Curvularia</i> spp.	1		1	0.68	0.45

Fungal isolates was 164 from different sources have been isolated, higher was 20 isolates from Hospital wards, while lowest was 9 isolates from CCU, Table-3 shows sources of swabs, fungal isolates.

Table 3-Sources of swabs and fungal isolates in all units.

Location	Swabs	Isolates	Fungi
Burn units	24	14	4 <i>A. flavus</i> 3 <i>Penecillium</i> spp. 2 Yeasts <i>Rhizopus</i> spp. <i>A. fumigates</i> <i>Fusarium</i> spp. <i>Mucor</i> spp. <i>Cladosporium</i> spp.
CCU units	24	9	3 <i>A. flavus</i> 3 <i>A. fumigates</i> <i>Penecillium</i> spp. <i>Rhizopus</i> spp. <i>Fusarium</i> spp.
Dialysis units	22	14	4 <i>A. flavus</i> 3 <i>A. niger</i> 2 <i>Penecillium</i> spp. 2 <i>Rhizopus</i> spp. <i>A. fumigates</i> <i>Alternaria</i> spp. <i>Cladosporium</i> spp.
Emergency units	24	15	4 <i>A. niger</i> 3 Yeasts 2 <i>A. flavus</i> 2 <i>Fusarium</i> spp. 2 <i>Mucor</i> spp. <i>Penecillium</i> spp. <i>A. fumigates</i> <i>Rhizopus</i> spp. <i>Cladosporium</i> spp.
Endoscopy units	18	13	5 <i>A. flavus</i> 3 <i>A. niger</i> 2 <i>A. nidulans</i> <i>A. fumigates</i> <i>Rhizopus</i> spp. <i>Alternaria</i> spp.
Hospital kitchen	12	12	4 <i>A. niger</i> 2 <i>A. flavus</i> 2 <i>Penecillium</i> spp. 2 <i>Fusarium</i> spp. <i>A. fumigates</i> <i>Mucor</i> spp.
Neonatal units	22	12	4 <i>Penecillium</i> spp. 2 <i>A. niger</i> 2 <i>Rhizopus</i> spp. 2 <i>Fusarium</i> spp. <i>A. fumigates</i> <i>A. nidulans</i>

Obstetrics theaters	13	11	3 <i>Penecillium</i> spp. 3 <i>A. flavus</i> 2 <i>A. nidulans</i> <i>A. fumigates</i> <i>Rhizopus</i> spp. <i>Mucor</i> spp.
Operating Theaters	28	12	5 <i>A. flavus</i> 2 <i>A. niger</i> 2 <i>Rhizopus</i> spp. <i>Penecillium</i> spp. <i>Fusarium</i> spp. Yeasts <i>Cladosporium</i> spp.
RCU units	13	13	3 <i>A. niger</i> 3 <i>A. fumigates</i> 2 <i>A. flavus</i> 2 <i>Penecillium</i> spp. <i>Rhizopus</i> spp. <i>Mucor</i> spp. <i>Cladosporium</i> spp.
Hospital wards	20	20	4 <i>A. flavus</i> 4 <i>A. terrus</i> 3 <i>Alternaria</i> spp. 2 <i>A. niger</i> 2 <i>A. fumigates</i> 2 <i>Fusarium</i> spp. 2 <i>Mucor</i> spp. Yeasts <i>Scopulariosis</i> spp. <i>Curvularia</i> spp.

Burn units: The findings of this unit different with [13], revealed that *Candida albicans* the most frequent isolate. This environment is difficult to decontaminate because of continuous reinoculation of organisms from the patients.

CCU units: The findings of this unit agree with [14], is observed that *Penicillium spp.* and *Aspergillus spp* the most frequent isolates. The indoor moulds are usually originated from outdoor sources and their presence in large numbers or altered dominance indicates a source of colonization inside the hospitals [15].

Dialysis units: The findings of this unit different with [16], that *Candida albicans* the most frequent isolates. The use of modern more permeable dialyzer membranes has increased the risk of contamination.

Emergency units: The findings of this unit different with [17], that *Candida spp.* the most frequent isolate. Possible sources of airborne nosocomial infection in emergence unit include ventilation or air-conditioning systems, decaying organic material, dust, ornamental plants, food and water [18]. Figure-1 was showed 5 species of fungi were isolated from emergency unit in Al -Yarmouk Teaching Hospital.



Figure 1-Fungal colonies isolated from Al -Yarmouk Teaching Hospital.

Endoscopy units: Since the late 1970s there have been sporadic reports of nosocomial infections linked to endoscopic procedures. Additionally, fungi can be transmitted via endoscopic procedures [19].

Hospital kitchen: The findings of this unit different with [20], revealed that *Candida spp.* the most frequent isolate. The role of surfaces in food preparation areas in the transmission of hospital-acquired infections has been long recognized. Figure-2 was showed 4 species of fungi were isolated from kitchen in Imam Ali General Hospital.

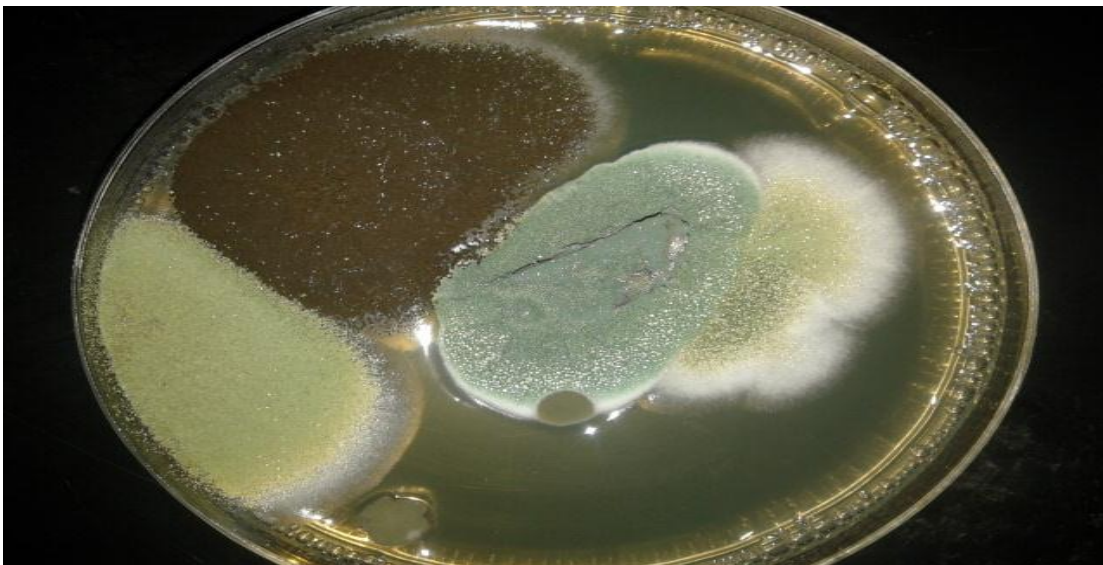


Figure 2-Fungal colonies isolated from Imam Ali General Hospital.

Neonatal units: Infants who remain hospitalized for extended periods of time and who undergo numerous invasive procedures is most susceptible to nosocomial infections [21].

Obstetrics theaters: Complex medical and surgical problems, disruption of natural barriers and multiple invasive procedures are some of the factors contributing to the alarming increase of fungal infections [22].

Operating Theaters: The findings of this unit different with [23], yeast the most frequent isolated. Fungi was isolated from surfaces in operating theatres, hospital environmental control procedures can be an effective support in reducing nosocomial infections [24].

RCU units: RCU patients are at great risk of acquiring nosocomial infections, therefore RCU is the workplace where number of direct contacts between the hands of workers and the patients [25].

Hospital wards: The findings of this unit agree with [26], revealed that *Aspergillus niger* *Aspergillus flavus* the most frequent isolate. A high correlation is reported between the prevalence of infection and the duration of hospitalisation.

Effect of some chemical disinfectants on fungi

The findings agree with [27], indicating that Dettol 10% concentration was higher efficient than less concentrations. The findings different with [28], which have shown that Hepeten maintains inhibition effective at 2.5% concentration, and different with [29], referred to Dettol lowest effective than formaldehyde and Hepeten. This different of findings may be due to widely used sterilizers and other disinfectants in hospitals, and more used one antiseptic without others leads to prevalence of microorganisms resistance.

Sodium hypochlorite (Alwazir): The MIC is 10% for *A. niger*, *Penecillium* spp. and *Mucor* spp., while 7.5% is the MIC for *A. flavus*, 2.5% is the MIC for *Rizopus* spp. and 5% is the MIC for *Fusarium* spp. and *A. fumigatus*. The results were summarized in Figure-3.

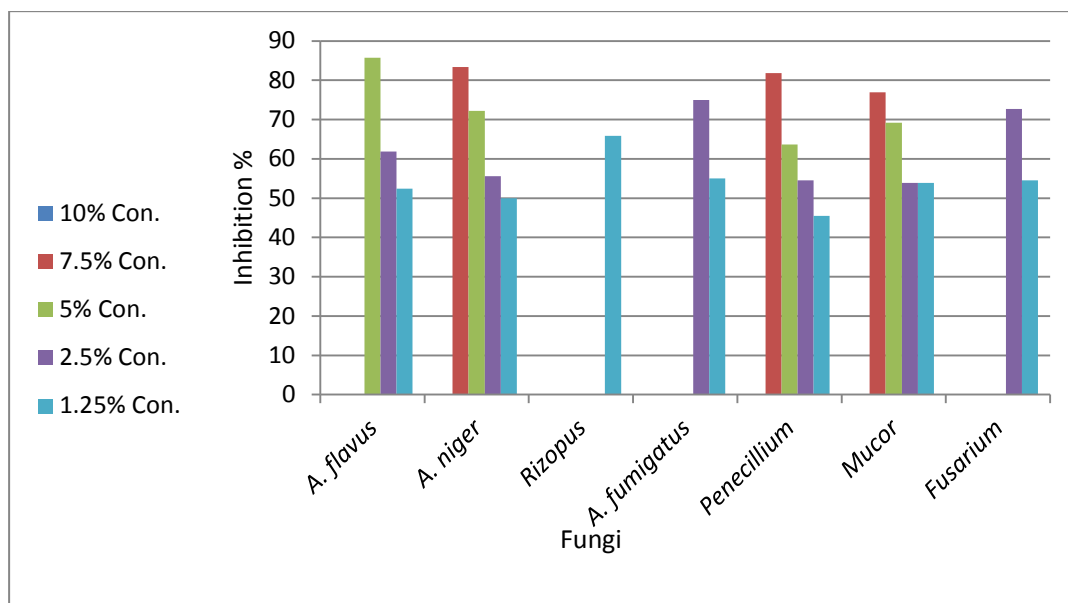


Figure 3-Effect of Sodium hypochlorite (Alwazir) on fungi growth.

Strong concentrations of hydrochloric acid 6 M are routinely employed in amino acid analysis to completely destroy proteins breaking them into their individual amino acid constituents [30].

Formaldehyde (Formalin): The MIC is 2.5% for all fungi. The results were summarized in Figure-4.

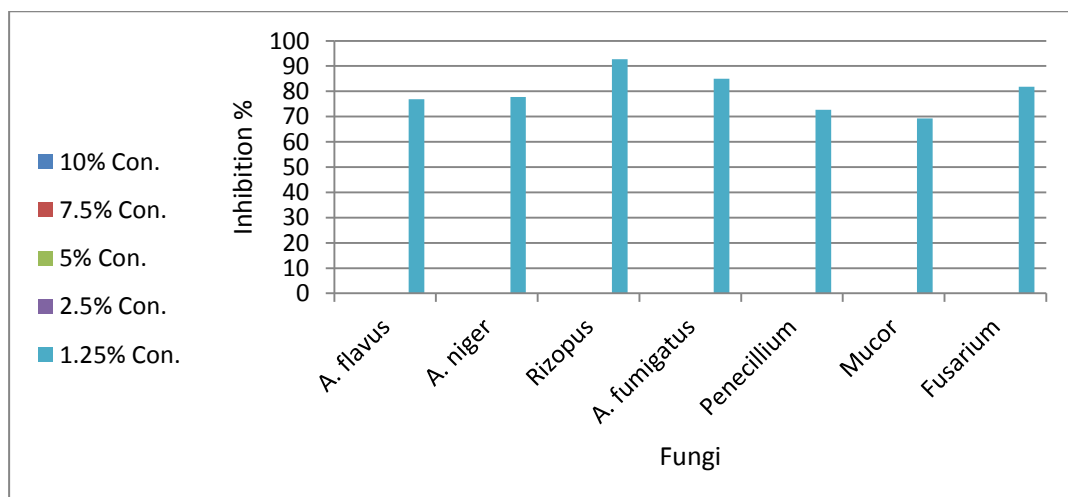


Figure 4- Effect of Formaldehyde (Formalin) on fungi growth.

Formaldehyde at approximately 5% in a solution with water is used as a disinfectant and fumigant in hospitals as it is effective in killing most fungi.

Chlorhexidin (Hepeten): The MIC is 10% for *A. Flavus*, *A. fumigates*, *Penecillium* spp. and *Rizopus* spp., while 7.5% is the MIC fo *A. niger*, *Fusarium* spp. and *Mucor* spp. The results were summarized in Figure-5.

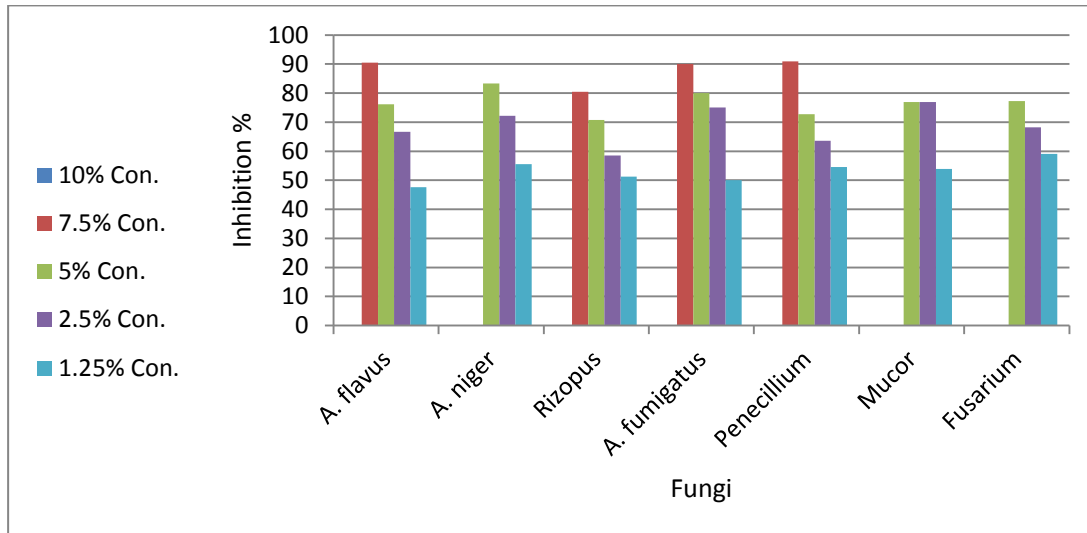


Figure 5-Effect of Chlorhexidin (Hepeten) on fungi growth.

Chlorhexidine has been reported to be effective against fungi *in vitro*, and *in vivo* [31].

Povidine iodine (Povidone): The MIC is 10% for *A. fumigatus*, *Penecillium* spp. inhibited at 10% and did not inhibited at 7.5, 5, 2.5 and 1.25%, *A. flavus*, *Rizopus* spp. and *Mucor* spp. inhibited at 10, 7.5 and 5% and did not inhibited at 2.5 and 1.25%, *A. niger* inhibited at 10, 7.5, 5 and 2.5% and did not inhibited at 1.25%, while *Fusarium* spp. inhibited at all concentration. The results were summarized in Figure-6.

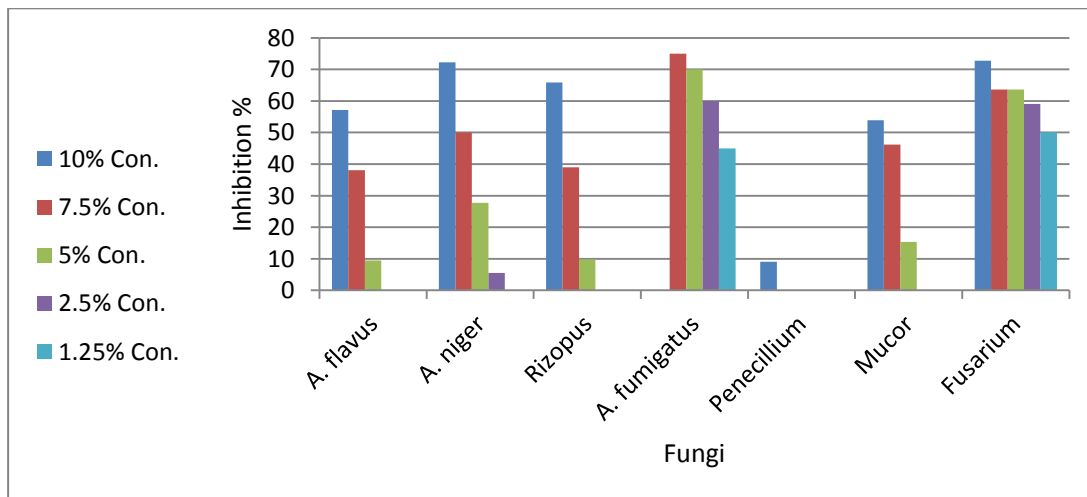


Figure 6-Effect of Povidine iodine (Povidone) on fungi growth.

Povidone iodine is a topical antiseptic that effects sporicidal, fungicidal, by the gradual liberalization of iodine [32].

Chloroxylenol (Setol): The MIC is 2.5% for *A. flavus*, *Mucor* spp. and *Fusarium* spp., while *A. niger*, *Rizopus* spp., *A. fumigates* and *Penecillium* spp. did not growth at all concentration. The results were summarized in Figure-7.

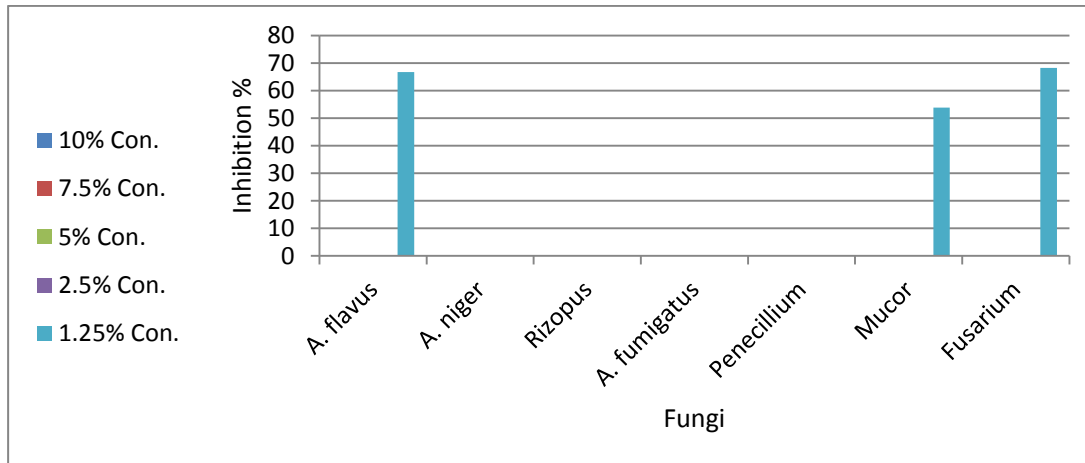


Figure 7-Effect of Chloroxylenol (Setol) on fungi growth.

Chloroxylenol, is a phenolic derivative, the key halophenol used in many antiseptic or disinfectant formulations [33].

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