



Journal homepage:
<http://www.bsu.edu.eg/bsujournals/JVMR.aspx>
 Online ISSN: 2357-0520 Print ISSN: 2357-0512



Original Research Article

Correlation between *Aspergillus fumigatus* isolates recovered from human and broiler chickens

Radwan.I.A; Kamel. M.F; Hamdy.D.A and Mahmoud .Z.A

Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

ABSTRACT

A total of 225 samples were collected from suspected cases and they were 75 human samples (40 ear swabs, 29 vaginal swabs and 6 sputum samples) and 150 from broiler chickens for microbiological examination and fungal isolation, they collected from different areas in El-Fayoum and Beni-Suef governorates, from which 129 fungal isolates were recovered, 22 fungal isolates (29.3%) were recovered from human; of which 15 isolates of ear swabs (*A.fumigatus* 37.5%) while 7 (24.1%) *A.fumigatus* isolates were recovered from women, but there was no *A.fumigatus* recovered from sputum samples, as well as there were 53 *A.fumigatus* isolates (35.3%) recovered from broiler chicken.

The antifungal activities of thymol and carvacrol oils against the recovered fungal isolates were tested using agar dilution method. Thymol and carvacrol oils completely inhibited the growth of different fungal isolates at concentrations of 1% and 0.1%. On the other hand, the concentration of 0.01% was too weak to inhibit the fungal growth, but it completely reduced the colour of the fungal colony converted it into white coloured aerial mycelium.

PCR assay using oligonucleotide primer that amplifying 250bp fragment in *its* Gene of *A.fumigatus* and *A.niger* was performed. Sequence analysis of two isolates of *A.niger* and *A.fumigatus* using its Gene was performed.

ARTICLE INFO

Article history:

Received 3/2019

Accepted 4/2019

Online 4/ 2019

Keywords:

A.fumigatus,
 Human, Broiler
 chickens

*Corresponding author. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. Email: microbiologist111@yahoo.com

Introduction

Fungal infections either community-acquired or nosocomial fungal infections have been increased in recent years due to a growing number of high-risk patients, particularly immunocompromised hosts (Pinto et al., 2006). Unfortunately, few anti-fungal medicines are available for treating fungal infections, not to mention that most of them have serious side effects (Gang-sheng et al., 2012).

Fungal infections were called hidden killer in human beings specially in immune-suppressed ones because the impact of these fungal diseases on human health is not widely appreciated (Gordon et al., 2012).

Fungal infection has been described in respiratory affections in poultry, Aspergillosis occurs in young birds resulting in high morbidity and mortality (Arné et al., 2011). As well as it has been reported in domestic birds including broiler breeders (Martin et al., 2007) and grower chickens (Akan et al., 2002), moreover, *Aspergillus* species remains the most recovered fungal agent in dead-in-shell embryos (Abd El-Aziz, 2015).

Some of *Aspergillus* species, especially *A.niger* produce oxalic acid as a fermentation byproduct. The acid combines with calcium ions at physiological PH to form insoluble calcium oxalate crystals that are mainly deposited at local sites, and this is often seen in lungs or rarely, in renal tubules as it recorded with pulmonary and renal aspergilloma (Pradeep and Uma, 2009).

As systemic anti-mycotic drugs are little and cause immune-suppression, recommendation of new anti-fungal agents are needed to match the currently increasing rate of fungal infection and the development of resistant fungal strains (Seibold et al., 2003).

Aromatic medicinal plants have been used in folkloric medicine as anti-microbial agents since ancient times (Pinto et al., 2006), it is known that most of their properties are due to

presence of their volatile essential oils which are extracted and isolated phytochemically (Carmo et al., 2008).

The essential oils from many plants are known to possess both anti-bacterial and anti-fungal activity (Cowan, 1999).

Fifty medicinal plants belonging to 26 families were studied for their anti-microbial activity, among them; 72% showed positive results, but only 9 plant extracts showed anti-fungal activity against both filamentous and non-filamentous fungi (Srinivasan and Sanggetha, 2001).

There were 20% of 114 species of medicinal plants inhibit growth of *Fusarium oxysporum* (Paul and Agus, 1995). The anti-fungal efficacy of thymol, carvacrol and mixture of both pure essential oils has been proved against *Penicillium digitatum* and *P.italicum* in vivo and vitro (Coperez et al., 2012).

Anti-fungal effects of the essential oils depended on the application method. Larger phenolic compounds such as thymol, thyme, cinnamon and clove had best effect if applied directly to medium (Suhr and Nielsen, 2003). Previous studies reported anti-fungal activity of clove oil and eugenol against yeasts and mycelial fungi (Lopez et al., 2007).

Therefore, the recurrent study aimed to determine the antifungal activity of some essential oils as well as, trials were done for detection of percentage of identity between *A.fumigatus* isolates recovered from human and broiler chickens using DNA sequencing of ITS gene.

Material and Methods

Samples. A total of 225 samples were collected of which 75 human samples (40 ear swabs, 29 vaginal swabs and 6 sputum samples) and 150 from broiler chickens collected from (air sacs, liver, heart and proventriculus) from different areas in El-Fayoum and Beni-Suef governorates

during the period from February 2016 to April 2018.

Fungal isolation. All samples were taken immediately and transferred directly into pre-enrichment broth (Malt Extract broth, Oxoid) and incubated at 37°C for 24-48 h, then cultured on sabouraud dextrose agar medium (oxoid) to be incubated at 37°C for 24-48 h.

Identification of fungal isolates. The recovered fungi were identified morphologically according to **Rippon (1988)**, mycelial fungi were identified by examination of mycelial morphology, the reverse colour as well as examination of lactophenol cotton blue stained smears.

Agar dilution method for detection of antifungal activity of essential oils. According to the method of **Jeff-Agboola et al., (2012)** the antifungal activity of thymol and carvacrol against 10 selected *A.fumigatus* isolates were done. The tested isolates included 5 *A.fumigatus* isolates from human and 5 of *A.fumigatus* isolates from broiler chickens.

The tested fungi were grown on SDA at 37°C for 48 hours. Cells were suspended in saline, and the suspension was adjusted to 1×10^6 CFU.

SDA was prepared and autoclaved at 121°C for 15 minutes and kept at 55°C and then

Results

Results of identification of fungal isolates:

Colonial appearance of *A.fumigatus*: *A.fumigatus* was rapidly growing mold (2 to 6 days) producing a fluffy to granular, white to

the oil was sterilized by filtration through a membrane filter (pore size, 0.45 µm), and were mixed together. The oil-agar medium (10 ml) was then poured into sterile petri dishes and was solidified. About 10^6 CFU of fungal cells suspended in saline was inoculated with a multiple inoculator onto the agar plates. The plates were then incubated in an ordinary incubator at 35°C for 24 hours and then examined daily for 8 days. MICs for fungi were determined.

Polymerase chain reaction. PCR, using Oligonucleotide primers that amplify a 250bp fragment in *its* Gene of *A.fumigatus* by using primer pairs PEX1 and PEX2 (**Muñoz et al., 2003**) was applied on 5 selected *A.fumigatus* isolates (2 from human and 3 from broiler chickens).

Primers: PEX1 and PEX2.

Method of sequence analysis:

DNA Sequencing of ITS gene was conducted in both directions and a consensus sequence of 450 bp was used for nucleotide (nt.) analysis. The original sequences were trimmed to remove vague nt. sequences usually exist in the beginning of the sequencing reaction.

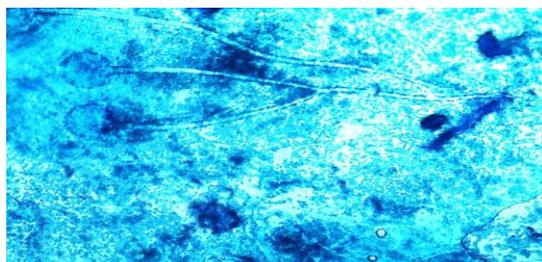
blue green colony. Mature sporulating colonies most often exhibited the blue green powdery appearance (**Fig 1**).



Fig(1) Showed clonial appearance of *A.fumigatus* recovered from human ear swab.

Microscopical examination of *A.fumigatus*:

A.fumigatus was characterized by the presence of septated hyphae and short or long conidiophores having a characteristic cell at their base. The tip of the conidiophore



Fig(2) Showed microscopical morphology of *A.fumigatus* ewcovered from human vaginal swab.

The result of fungal isolation from human and from broiler chickens:

Table (1) showed result of fungal isolates from human and from broiler chickens:

Source of samples	No of <i>A.fumigatus</i> isolates	of %
40 human ear swabs	15	37.5%
29 human vaginal swabs	7	24.1%
150 samples from broiler chickens	53	35.3%

expanded into large, dome-shaped vesicle containing bottle-shaped phialides covering the upper half or two thirds of its surface. Long chains of small (2 to 3 µm in diameter), spherical, rough-walled green conidia formed a columnar mass on the vesicle. (Fig 2).

Out of these, the recovered isolates of ear swabs were (*A.fumigatus* 37.5%) ; but results of incidence of recovered isolates recovered from vaginal swabs were (*A.fumigatus* 24.1%) and the result of incidence of *A.fumigatus* from broiler chickens were 53 *A.fumigatus* (35.3%)

Anti-fungal effect of thymol oil using agar dilution method:

Results illustrated in table (2) and Fig (3) that showed the antifungal activity of thymol oil at concentrations of 0.01%, 0.1% and 1% against 10 selected *A.fumigatus* isolates using agar dilution method. The tested isolates included (5 isolates of *A. fumigatus* from human and 5 isolates from broiler chicken).

The results revealed that thymol oil completely inhibited the fungal growth at concentrations; 0.1% and 1%, on the other hand, thymol oil at concentration of 0.01% were too weak to inhibit the fungal growth, but it completely reduced the black coloured conidia converted it into white coloured arial mycelium (Fig No 1).

Table (2): Antifungal effect of different concentrations of thymol oil against *A.fumigatus*.

Thymol oil conc	<i>A.fumigatus</i> isolates						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
0.01%	-	-	-	+	++	++	+++
0.1%	-	-	-	-	-	+	+
1%	-	-	-	-	-	-	-

No growth: - Moderate growth:+ Luxuriant growth:++



Fig(3) showed antifungal effect of thymol oil against *A.fumigatus*

Anti-fungal effect of carvacrol oil using dilution method:

Results illustrated in **table (3) and Fig (4)** that showed the antifungal activity of carvacrol oil at concentrations of 0.01%, 0.1% and 1% against 10 selected *A.fumigatus* isolates were done using agar dilution method. The tested isolates 5 isolates of *A. fumigatus* from human and 5 isolates from broiler chickens).

The results revealed that carvacrol oil completely inhibited the fungal growth at concentrations; 0.1% and 1%, on the other hand, carvacrol oil at concentration of 0.01% were too weak to inhibit the fungal growth, but it completely reduced the black coloured conidia converted it into white coloured arial mycelium.

Table (3): Antifungal effect of different concentrations of carvacrol oil against *A.fumigatus*.

Carvacrol oil *A.fumigatus* isolates

conc	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
0.01%	-	-	-	+	++	++	+++
0.1%	-	-	-	-	-	+	+
1%	-	-	-	-	-	-	-

No growth: _

Moderate growth: +

Luxuriant growth: ++



Fig(4) showed antifungal effect of carvacrol oil against *A.fumigatus*

Results of PCR of the extracted DNA from different sources of *A.fumigatus* isolates:

The conventional PCR (CPCR) using ITS gene was applied on randomly selected 5 isolates which were morphologically identified of *A.fumigatus* (2 isolates from human and 3 isolates from broiler chickens)

The CPCR also, was applied on 1 isolate of *A.fumigatus* previously treated by carvacrol oil at conc. 0.01% that allowed fungal growth but

with reduction of colour of conidia converted it into white mycelium.

The results in **Fig (5)** revealed that all 5 isolates gave positive results with PCR.

The 1 isolates treated by carvacrol revealed positive result with *A.fumigatus*

Each species-specific primer pair was tested in simplex PCR assays. All *Aspergillus fumigatus* strains were successfully amplified by PEX1–PEX2 primer pair producing amplicons of 250 bp (**Fig5**).

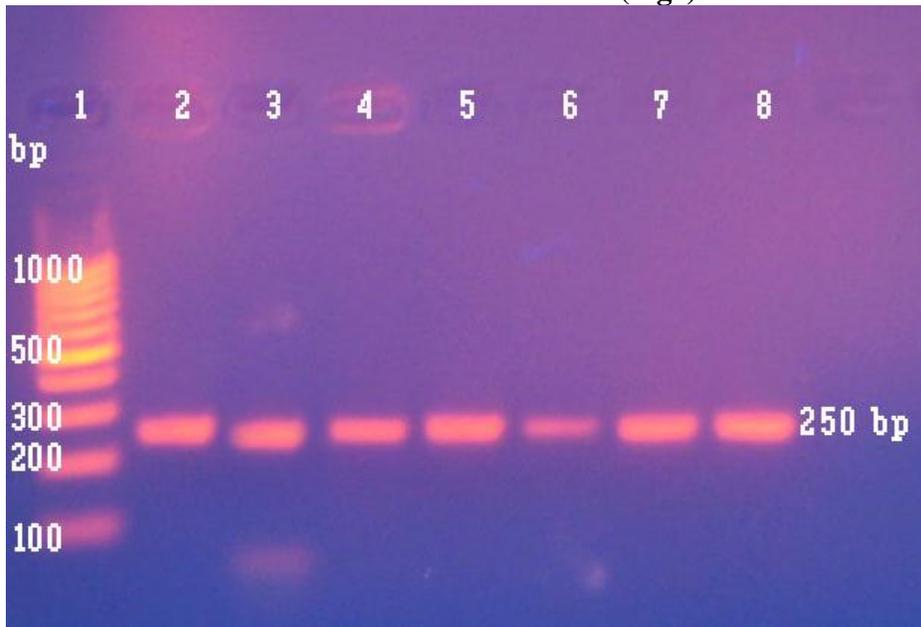


Fig (5): electrophoretic pattern of PCR product of *A.fumigatus*

Lane 1: 100 bp DNA, ladder.

Lane 2: *A.fumigatus* (control +ve)

Lane 3: showed tested sample

Lane 4: showed tested sample

Lane 5: showed tested sample

Lane 6: showed tested sample

Lane 7 : showed tested sample

Lane 8: showed tested sample

Results of sequence analysis:

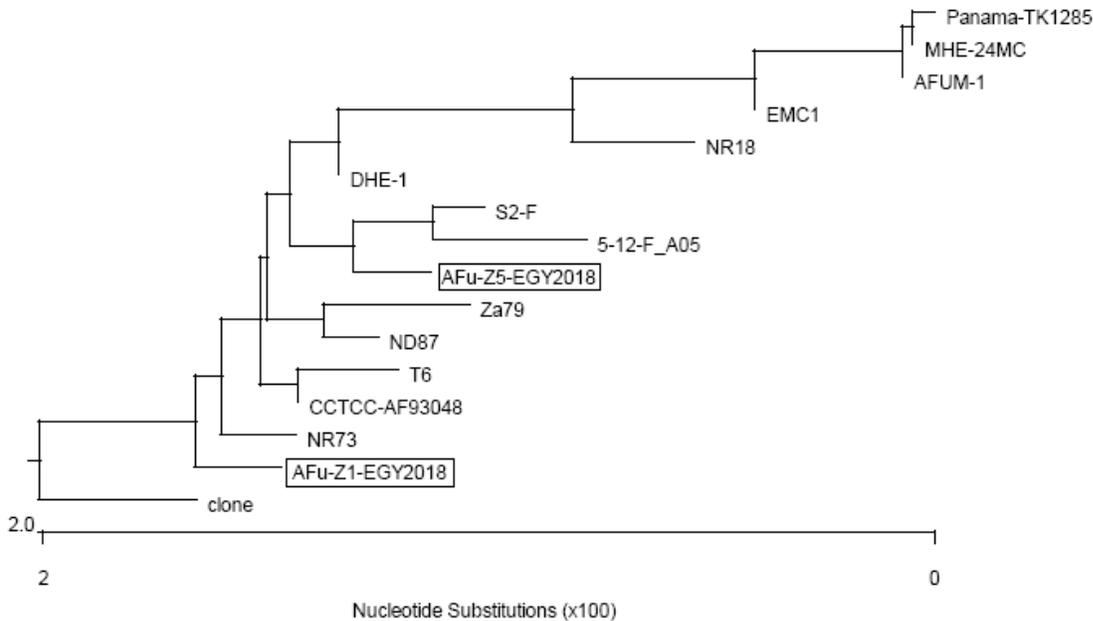
Fig (No6) showed *A.fumigatus* (ITS) identity.

		Percent Identity																	
Divergence		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
	1	■	99.5	99.6	99.1	99.1	99.1	99.1	99.3	99.5	99.8	98.4	98.9	99.1	98.4	98.4	99.6	1	AFu-Z1-EGY2018
	2	0.5	■	99.3	99.3	99.5	99.3	98.6	99.3	99.3	99.8	99.1	99.1	99.5	99.1	99.1	99.3	2	AFu-Z5-EGY2018
	3	0.4	0.7	■	99.6	99.1	99.1	99.1	99.3	99.8	100.0	98.4	99.1	99.1	98.7	98.7	99.6	3	NR73
	4	0.9	0.7	0.4	■	99.6	98.7	98.7	99.3	99.5	100.0	98.4	99.3	99.1	98.7	98.7	99.1	4	S2-F
	5	0.9	0.5	0.9	0.4	■	98.7	98.2	98.9	99.3	99.8	98.4	99.3	98.7	98.7	98.7	98.7	5	5-12-F_A05
	6	0.9	0.7	0.9	1.4	1.4	■	98.7	99.3	99.3	100.0	99.3	99.1	98.7	99.3	99.3	99.1	6	NR18
	7	0.9	1.4	0.9	1.3	1.8	1.4	■	98.9	99.1	99.3	98.0	98.4	98.7	98.0	98.0	99.1	7	clone
	8	0.7	0.7	0.7	0.7	1.1	0.7	1.1	■	99.8	100.0	98.7	98.9	99.3	98.7	98.7	99.3	8	T6
	9	0.5	0.7	0.2	0.5	0.7	0.2	0.9	0.2	■	100.0	99.1	99.1	99.5	99.1	99.1	99.5	9	CCTCC-AF93048
	10	0.2	0.2	0.0	0.0	0.2	0.0	0.7	0.0	0.0	■	99.8	99.8	100.0	99.8	99.8	100.0	10	DHE-1
	11	1.6	0.9	1.6	1.6	1.6	0.7	2.0	1.4	0.9	0.2	■	99.8	98.4	100.0	100.0	98.9	11	Panama-TK1285
	12	1.1	0.9	0.9	0.7	0.7	0.9	1.6	1.1	0.9	0.2	0.2	■	99.1	99.8	99.8	99.3	12	EMC1
	13	0.9	0.5	0.9	0.9	1.4	1.4	1.3	0.7	0.5	0.0	1.6	0.9	■	98.4	98.4	99.6	13	Za79
	14	1.6	0.9	1.4	1.4	1.4	0.7	2.0	1.4	0.9	0.2	0.0	0.2	1.6	■	100.0	98.9	14	MHE-24MC
	15	1.6	0.9	1.4	1.4	1.4	0.7	2.0	1.4	0.9	0.2	0.0	0.2	1.6	0.0	■	98.9	15	AFUM-1
	16	0.4	0.7	0.4	0.9	1.4	0.9	0.9	0.7	0.5	0.0	1.1	0.7	0.4	1.1	1.1	■	16	ND87

Out of these, there was high similarity between the two tested isolates AFu-z1-EGY2018 and AFu-Z5-EGY2018. Also, DHE-1 no(10) showed high similarity with the two tested isolates.

On the other hand, Panama-TK1285 no (11), MHE-24MC no(14) and AFUM-1 no(15) showed lowest identity with the two tested isolates of this study.

Fig (No7): *A.fumigatus* of ITS phylogenetic



tree.

Out of these, the tested isolate AFU-Z5-EGY 2018 showed similarity with DHE-1 that present in the same cluster. And the another tested isolate AFU-Z1-EGY 2018 showed similarity with CCTCC-AF93048.

The two tested isolates showed low similarity with Panama-TK1285, MHE-24MC and AFU-1 present in separated branch.

Discussion

The number of fungal species reported to cause diseases in man was increasing rapidly and very few of these fungi are capable of infecting a normal host (Richardson, 1991).

Unfortunately, few anti-fungal agents are available for treating of fungal infection, not to mention that most of them have extreme side effects (Wang et al, 2012).

The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Lee et al., 2007). Therefore, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. Those are; in the first place, extracts and essential oils of spices and herbs (Smid and Gorris, 1999)

Aromatic medicinal plants have been used in folk medicine as antimicrobial agents since ancient times (Pinto et al., 2006). It is known that most of their properties are due to their volatile essential oils, extracts and isolated phytochemicals (Carmo et al., 2008).

The anti-fungal activity of some E.Os was due to presence of a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components.(Fadil et al., 2008).

In some cases,otomycosis progressed to cause perforation of a tympanic membrane due to the obvious presence of fungal bloom in the external ear canal , the tympanic membrane appeared white and opaque.As time progressed the white area disintegrated forming a perforation.(Hurst, 2001).

The human beings and chickens may share the hazards of contracting aspergillosis and the presence and distribution of *Aspergillus* Sp.in poultry farms suggesting a possible

occupational health problem and the predisposing factors play an important role in such infection (Zaki et al., 2016).

As shown in table (1) , a total of 40 fungal isolates were recovered from total 75 samples of human origin of which 15 isolates of ear swabs were *A.fumigatus* which represented 37.5%) .

In this study, *A.fumigatus* is the main fungal isolates that causing otomycosis and, that disagreed with (Tawlar et al., 1988) who proved that *A.niger* took the main role in otomycosis and otitis externa.

Our results were higher than those of (Jagdish et al., 1996) who collected a total of 110 patients of symptomatic otomycosis; and the commonest isolate was *A.niger* in 46(57.5%) and *A.fumigatus* was only 3 isolates (3.7%).

Our study recorded fungal infection in human vaginal swabs was *A.fumigatus* 24.1% from total 29 collected samples.

According to the small number of tested population in this study, it agreed with the result of (Sallam, 2001) who examined 1344 vaginal swabs from married women; and the results were *Candida albicans* 28%, *Trichomonas vaginalis* 8.7%, *Aspergillus* Spp. 7.4%, *Streptococci* 4.6% and *Chlamydia trachomatis* 4.2%.

In this study table (3) showed that a total 89 fungal isolates were recovered from a total 150 specimens of broiler chickens by incidence rate of (59.3%); the mold isolates were 53 isolates of *A.fumigatus* (59.5%).

Our result revealed that *A.fumigatus* was the most dominant fungus involved in mycosis of poultry, and that in agreement with (Richard et al., 1980).

Also, (Pascal *et al.*, 2011) cited that *Aspergillus fumigatus* was considered as a major respiratory pathogen in birds. Virulence of it represented the ability of a pathogen to invade the host, overcome its sensing a favourable environment, *A.fumigatus* conidia germinated producing enzymes that degraded organic materials into nutrients for further assimilation.

These result was relatively with agreement with result obtained by Salem and Ali (2014) who reported the the incidence of *A.fumigatus* isolation was (13%) than *A .niger* (8%). Similarly, Abd El-Tawap *et al.*,(2015) who proved that *A.fumigatus* was the most isolated spp. by 141(21%).

These results were supported by Musa *et al.*,(2014) who reported that *A.fumigatus* was responsible for a disseminated aspergillosis in a 5-week old broiler. Also, Radwan *et al.*,(2014) found that all mycelial isolates recovered from broiler chickens belonged to *A.niger* and *A.flavus*.

Table (2), Fig(3) and table(3), Fig(4) showed the anti-fungal activity of thymol oil and carvacrol oil against 10 randomly selected fungal isolates of *A.fumigatus* using agar dilution method. The isolates were 5 isolates of *A.fumigatus* from human & 5 from broiler chickens).The result revealed that thymol and carvacrol oils completely inhibited the fungal growth of tested samples at conc 1%, on the other hand; it allowed the fungal growth at conc.of 0.01% but with reduction of the colour of the fungal colony of all tested isolates converted it into white mycelium.

Our results was supported by (Bernardos *et al.*, 2015) who cited the remarkable activity of thymol and carvacrol in inhibition of fungal growth up to 30 days.

Also, (Nachman *et al.*, 1995) studied the anti-fungal activity of E.Os from oregano and thyme were applied for 24h as fumigants against the mycelia and spores of *A.flavus*,

A.niger and *A.ochraceus*. The minimal inhibitory concentration (MIC) of oregano oil needed to inhibit the mycelial growth of the fungi was 2.0µl/L; while spores were eradicated following exposure to 2.0 to 2.5µl/L.

Moreover, (Suwanamornlert *et al.*, 2018) proved that the lag phase of fungal mycelia exposed to thymol and carvacrol increased, while the maximum colony diameter and maximal growth rate decreased. A combination of thymol and carvacrol oils exhibited an antagonistic effect against *G.candidum* but an indifferent effect against *Lasiodiplodia* Sp., *Phomopsis* Sp. And *Pestalotiopsis* Sp.

In this study, Fig(5) showed the result of applying conventional **CPCR** using (ITS) of the extracted DNA from randomly selected 5 isolates which were morphologically identified of *A.fumigatus* from different sources of human and broiler chickens (2 isolates from human and 3 isolates from broiler chickens).

The CPCR also, was applied on 1 isolate of *A.fumigatus*) on (ITS) gene previously treated by carvacrol oil at conc. 0.01% that allowed fungal growth with reduction of the colour of colony.

The results revealed that all 5 isolates gave positive results with PCR.

The 1 isolate treated by carvacrol revealed positive result with *A.fumigatus*.

In general, it could be concluded the importance of PCR amplification of *its* Genes which were proven to be useful and easy tool to diagnose different fungal spp. this conclusions was further supported by Arvanitis *et al.*,(2014) who found that PCR diagnosis of invasive aspergillosis had a specificity of 95% and sensitivity of 64% for invasive infection.

According to Fig(No 6) *A.fumigatus* (ITS) identity,, Panama-TK1285 no (11),

MHE-24MC no(14) and AFUM-1 no(15) showed lowest identity with the two tested isolates of this study because there was high bootstrap.

Also, **photo(No 6): *A.fumigatus* of ITS phylogenetic tree**, the tested isolate AFU-Z5-EGY 2018 showed similarity with DHE-1 that present in the same cluster. And the another tested isolate AFU-Z1-EGY 2018 showed similarity with CCTCC-AF93048.

The two tested isolates showed low similarity with Panama-TK1285, MHE-24MC and AFU-1 present in separated branch.

Martinez and Roman (2007) suggested that phylogenetic analysis can be used to develop relationship between closely related species.

References:

Abd El-Aziz R.M (2015). Characterization and differentiation of fungal and bacterial isolates associated with dead-in-shell embryos in breeder layer; M.V.SC. Thesis in microbiology department, Bani-suef University.

Abd El-Tawab A.A; Ahmed, A.A.M; El-Hofy F.I and Ahmed K.S.M (2015). Molecular characterization of some fungi isolated from broiler chickens farm, Banha Veterinary medical Journal ,29(2): 106-118,2015.

Akan Mehmet; Hazirođlu Rifki; Ilhan Ziya; Sareyyüpođlu Bariř and Tunca Recai (2002). A case of aspergillosis in a broiler breeder flock, Avian diseases 46(2), 497-501,2002.

Arné, P., S. Thierry, D.Wang, M.Deville and G. Le Loch (2011). *Aspergillus fumigatus* in poultry, International Jour.Microbiol., 10.1155/2011/746356.

Arvanitis Marios; Ziakas D.Panayiotis, Zacharioudakis M. Loannis; Zervou N.Fainareti and Angela (2014). Polymerase Chain Reaction in the diagnosis of invasive Aspergillosis: A

Meta-analysis of diagnostic performance; Journal of clinical microbiology, JCM.01365-14,2014.

Bernardos.A; Marina.T; Źáček .P; Pérez-Esteve.Ē; Martinez-Mañez.R; Lhota.M; Kouřimska.L; Pulkrábek.J and Klouček.P (2015). Antifungal effect of essential oil components against *Aspergillus niger* when loaded into silica mesoporous supports; Journal of sci. Food Agriculture; 2015.

Carmo, E. S.; Lima, E. O.; de Souza, E. L. and de Sousa, F. B. (2008). Effect Of *Cinnamomum Zeylanicum* Blume Essential Oil On The Growth And Morphogenesis Of Some Potentially Pathogenic *Aspergillus* Species. Braz. J. Microbiol. 39:91-97.

Coperez-Alfonso, D.Martinez-Romero and P.J.Zapata (2012). The anti-fungal efficacy of thymol and carvacrol, International journal of food microbiology.

Cowan (1999). Plant products as antimicrobial agents; Clinical microbiology reviews 12(4), 564-582, 1999.

Fadil Bakkali, Simone Averbeck, Dietrich Averbeck and Mouhamed Idaomar (2008). Biological effects of essential oils, food & chemical Toxicology 46(2), 446-475,2008.

Gang-sheng, W.; Jie-hua, D.; Yao-hui, M.; Min, S. and Bo, L. (2012). Mechanisms, clinically curative effects, and antifungal activities of cinnamon oil and pogostemon oil complex against three species of *Candida*. J Traditional Chinese Medicine; 32(1): 1-2.

Gordon D Brown, David W Denning, Neil AR Gow, Stuart M Levitz, Mihai g Netea, Theodore C White (2012). Hidden killers : human fungal infections; vol 4,issue 165, pages 165rv13,19/12/2012.

Hurst W.B (2001). Perforation of tympanic membrane due to mycotic otitis, The

- Journal of Laryngology & Otolaryngology, Cambridge-org, 2001.
- Jagdish Chander, Sangeeta Maini, Subha Subrahmanyam; Aru Handa (1996). Otomycosis- a clinic- mycological study and efficacy of mercurochrome in its treatment; *Mycopathologia* 135(1), 9-12,1996.
- Jeff-Agboola, Y. A.; Onifade, A. K.; Akinyele, B. J. and Osho, I. B. (2012). In vitro antifungal activities of essential oil from Nigerian medicinal plants against toxigenic *Aspergillus flavus*. *J. Medicinal Plants Res.*, 6(23):4048-4056.
- Lee, S. O.; Choi, G. J.; Jang, K.S. and Kim, J. C. (2007). Antifungal Activity of Five Plant Essential Oils as Fumigant Against Postharvest and Soil borne Plant Pathogenic Fungi. *Plant Pathol.*, J. 23(2): 97-102.
- Lisa A.Tell(2005). Information about fungi, *Medical mycology* 43(supplement-1),S71-S73.
- López, P.; Sánchez, C.; Batlle, R. and Nerín, C. (2007). Development of flexible antimicrobial films using essential oils as active agents. *J. Agric. Food Chem.*,55(21):8814-8824.
- Martin, M.P., K.P.Bouck, J.Helm, M.J.Dykstra, D.P.Wages and H.J.Barnes (2007). Disseminated *Aspergillus flavus* infection in broiler breeder pullets. *Avian Dis.*, 51: 626-631.
- Matinez-Culebras, P.V and Ramon D. (2007). An ITS-RFLP method to identify black *Aspergillus* isolates responsible for OTA contamination in grapes & wine. *Int J. Food Microbiology*, 113:147-153.
- Muñoz, B.C.; Boldo; X.M; Villa-Tanaca.L and Hernández-Rodriguez.C. (2003). Identification of *Candida* spp. by randomly amplified polymorphic DNA analysis and differentiation between *Candida albicans* and *Candida dubliniensis* by direct PCR methods. *J. Clinical microbiology* 41(1): 414-420.
- Musa, I.W; Aliyu, G. and Ismail, A. (2014). Aspergillosis in broiler chickens: Reports of three cases from a commercial and two broiler breeder farms in Zaria, Nigeria, *Int. J.Curr.Microbiol. App. Sci.*, 3(6): 932-938,2014.
- Nachman Paster, Mazal Menasherov, Uzi Ravid, Benjamin Juven (1995). Thyme as fumigants against fungi attacking stored grain; *Journal of food protection* 58 (1), 81-85, 1995.
- Pascal Arné, Simon Thierry, Dongying Wang, Manjula Deville, Guillaume Le Loc'h, Anaïs Desoutter, Françoise Féménia, Adélaïde Nieguitsila, Weiyi Huang, René Chermette and Jacques Guillot (2011). *Aspergillus fumigatus* in poultry; *International Journal of Microbiology* ; Vol.2011, Article ID 746356, 14 pages.
- Paul W. Grosvenor and Agus Supriono (1995). 20% of 114 medicinal plants inhibit growth of *Fusarium oxysporum*.
- Pinto E., Pina-Vas C., Salgueirol, Concalves MJ., Costa-de-Oliveira S., Cavaleiro C., Palmeira A., Rodrigues A .and Martinez-de-Oliveira (2006). Aromatic medicinal plants are used as anti-microbial agents since ancient times, *Journal of Microbiology*.
- Pradeep Vaideeswar, Uma M Sakhdeo (2009). Pulmonary aspergilloma with renal oxalis: fatal effect at a distance; *Mycoses* 52(3), 272-275,2009.
- Radwan.I.A; Abed A.H; Abeer M.R; Ibrahim M.A and Abdallah A.S (2014). Effect of Thyme, Clove and Cinnamon essential oils on *Candida albicans* and moulds isolated from different sources.
- Richard L, Cutlip R.C, Thurston J.R (1980). Aspergillosis in birds; 1980.
- Richardson. M. D. (1991). Opportunistic and pathogenic fungi; *Journal of Antimicrob.chemotherapy*, 1991.
- Rippon, J. W. (1988). *Medical Mycology-The Pathogenic Fungi and the Pathogenic Actinomycetes* (3rd Ed.).

- Salem M.A. Lobna, and .Ali Abdel Fatah (2014). Epidemiological study of Aspergillosis in chickens and human contacts in chicken farms at Kalyoubia Governorate; Dept. of zoonoses, Dept. of Clinical Pathology, Fac.Vet.Med., Banha University.
- Sallam S.A, Mahfouz A.A and Dabbous N.I; El Barrawy M; El Said MM (2001). Reproductive tract infections among married women in upper Egypt, East Mediterr Health Journal, 2001.
- Seibold M; Schaller M.S; Korting H.C (2003). New anti-fungal agents are recommended to match the currently increasing rate of fungal infection and the development of new resistant fungal strains.
- Smid, E. J. and Gorris, L. G. M. (1999). Natural antimicrobials for food preservation, in Handbook of Food Preservation. Ed. M.S. Rahman, Marcel Dekker, New York pp. 285-308.
- Srinivasan and Sangetha Nathan (2001). Only nine plants from 114 medicinal plants showed anti-fungal activity, Journal of ethanopharmacology, 2001.
- Suhr and Nielsen (2003). The anti-fungal activity of essential oils depended upon the application technique, Journal of Applied Microbiology, 665-674.
- Suwanamornlert.P; Sangchote .S; Chinsirikul.W; Sane.A and Chonhenchob.V (2018). Antifungal activity of plant-derived compounds and their synergism against major postharvest pathogens of longan fruit in vitro, International journal of food microbiology, 271,8-14; 2018.
- Talwar p, Chakrabarti.A, Poonami Jit Kaur, R.K.Pahwa and Ashok Mittal (1988). Fungal infections of ear with special reference to chronic suppurative otitis media, Mycopathologia 104(1), 47-50, 1988.
- Wang Gang-sheng, DENG Jie-hua, MA Yao-hui, SHI Min, LI Bo (2012). The side effect of anti-fungal agents, Traditional Chinese Medicine, March 2012.
- Zaki S.M, Suzan o. Mostafa and Nagwa S.Rabie (2016). Study in infected chicken farm that transport infection into man, National Reaserch Centre, Dokki, Gizza, 2016.