



ISOLATION AND DIAGNOSIS OF FUNGI ASSOCIATED WITH GECKO (*EUBLEPHARIS MACULARIUS*) AND THEIR CONTROL

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Abstract

The current study touched on the gecko animal as it is present in most of our homes and may be a carrier of some pathogenic fungi as it comes into contact with food waste and dirt and transports it to us, which may cause serious diseases.

The study involved collecting twenty different gecko of different ages and randomly from the homes, then the fungi were isolated by the direct isolation method on the SDA medium from the hands, legs, guilt, mouth and abdomen.

The results showed that the fungi have shown differences in number and percentage. The most common fungi were *Aspergillus niger* and it appeared 12 times and formed a percentage of 40% in when the two fungi *Rhizopus stolonifer* and *Penicillium digitatum* came in the second sequence and with 6 isolation times, they formed a ratio of 20% each, while the remaining fungi, *Alternaria alternata* and *Chrysosporium anamorph* came to isolate only twice and formed a ratio of 6.6% each.

Discs diffusion method showed that the use of three antifungal agents in the study, Itraconazole, Ketoconazole, Fluconazole and three concentrations are 0.5, 1, 1.5. The results showed a clear superiority of the itraconazole followed by the Ketoconazole and finally came the antifungal Fuconazole. The MIC Itraconazole was the lowest valuable followed by Ketoconazole and finally Fluconazole.

Keywords: Gecko, *Chrysosporium anamorph*, MIC, *Rhizopus stolonifer*, Itraconazole.

Introduction

The Leopard (gecko) is one of the widespread reptiles and its scientific name is *Eublepharis macularius*. It is present in all over the world except for the polar regions. belongs to this species more than a thousand varieties spread out in all environments in the desert, forests, marshes and savannahs and in the zoo, and it is found in homes of reptiles collectors and breeding in our homes (Phonex,2015).

Despite the unwillingness of many of us to exist due to the cultural legacy from which we are descended, as there are various accounts in its authenticity that it was necessary to kill it at times because it was a human being transcribed to an animal and at times because it kindled the fire on the Prophet Ibrahim. at times because he had deadly teeth and at times because he transferred killer germs and these things can be answered simply if these novels are true, then what is the sin of this animal strain if one mistake from it is one who is held accountable for his mistakes as for the transfer of dangerous germs, this can be confirmed by conducting scientific studies and our study came from this perspective.

The favorite food for the Leopard is insects, small arthropods, leftovers and succulent fruits, and it does not possess lethal teeth, so we are in complete safe from its bites, and it is a shy animal as it escapes quickly when people see him and hides behind pictures and artifacts in the house, and when the enemy grabs him, he cuts his tail to distract the enemy, while he escapes and avoids death at the hands of the enemy, which is cats, dogs, snakes or other reptiles larger than him. These reptiles are distinguished by their spindle body covered with golden, blue or green scales and there are black or walnut motifs permeating them and they are distinctive as there are no two geckos that have identical motifs in proportion of one hundred percent, and the

environment plays the largest role in giving size, color, and shape of gecko, as it needs to hide from enemies as well as for the purpose of latency for prey as it lies for long hours until the appearance of prey and then is swipes it at lightning speed (Ben, 2013).

Gecko can walk on smooth walls and surfaces very gracefully, and the latest studies have shown that gecko legs operate at the nanoscale level, as they have small nano-strings that can stick to all smooth surfaces (which do not look smooth on the nanoscale level) and this matter inspired scientists who manufactured adhesive tapes. It has amazing adhesion force as it can stick to a car and easily pull it horizontally and to remove it, we only need to lift it up vertically (Domenico, 2003).

As we mentioned above, there are people who love and collect all kinds of gecko, breed and proliferate it. There are special private shops to sell gecko and there are types of them that are expensive, especially those rare and those that have beautiful shapes. It is striking that the gecko proliferate with eggs and the female may remain three years pregnant with eggs before she put it. The pictures below show the distinctive shapes of gecko: -





Fig. 1 : The different forms of gecko and the nature of the legs that help it stick

Fungi are widespread living organisms that are characterized as eukaryotic and reproductive by sexual and asexual methods. They are heterotrophic and do not make their own food because they do not contain green chlorophyll and do not practiced photosynthesis. There are more than 1500 genus of fungi known so far, most of them are not pathogenic except for 100 of them that can cause infection of humans and animals, they are called pathogenic fungi, some of which affect the superficial layer of the skin, some of which affect the skin itself or have been exposed to the skin, and some of it affects the internal organs and tissues, which leads to potentially mortal results (Al-Shibly and Abdel Amir, 2017).

Opportunistic fungi are lived on dead tissues and organic materials. As for pathogenic fungi, they live on living tissue when they are surrounded by appropriate conditions of heat, light, pH, and nutrients, and the host immunity plays the biggest role in infection that the fungus be active when there is no high immunity in the host. Among the factors that cause immunosuppression are chronic diseases, starvation, AIDS, asthma, steroids intake, drug addiction, alcohol, smoking, and other factors, as fungi naturally present as normal flora in the body turn into pathogenic and begin to penetrate and colonize of tissues (Al-Shibly and Abdel Amir, 2017).

There are many insects, worms, reptiles, orchards, and birds that live in our homes, whether we like pet birds, fish, or without our desire, such as leprosy, cockroaches, ants, flies, and mosquitoes. They search for shelter and food that you find in our homes, and these organisms may transfer pathogens, including fungi, into and through the house. Many studies have touched on this matter, but they did not address at least at the local level to the fungi transmitted by gecko, so our study came out to highlight on this important aspect because the gecko lives with us in our homes and may walk on or eat from our food and touch our clothes and personal needs (Al-Shibly and Al-Zamily, 2017).

The aim of the study: - To identify pathogenic fungi that may be transmitted to us through the gecko and the possibility of controlling them.

Materials and Methods

Collection of gecko samples

Twenty gecko animals were collected randomly from homes of different sizes and genders during October 2018, each of which was placed in a clean glass container and brought to the laboratory as soon as possible for the purpose of isolation.

Isolation and purification

The culture medium SDA was prepared the antibiotic chloramphenicol (0.025mg) was added to prevent the growth of bacteria and mix the medium well then poured into plastic petri dishes diameter 9 cm and left to cool and harden at room temperature then the dishes pollinated of gecko by anesthetizing it, then passing its hands, legs, tails, and mouth over the culture medium directly, and left some of the dishes without occultation for comparison, after that the dishes were all transferred to an incubator with a degree of 27 C^o and left for a week, after the growths appeared, each new growth was transferred to a clean dish that contain SDA with chloramphenicol to obtaining of pure colonies (Adimi *et al.*, 2013).

Diagnosis

Diagnosis was made based on the cultivated characteristics, such as the shape, color, nature of the colonies growth, height, smell, and the pigments it secreted in the culture medium, as well as on the microscopic characteristics such as the form of hyphae and the reproductive and physical structures of the fungus and compare them with considered taxonomic keys (Watanabe, 2002).

Pharmacological sensitivity test for antifungal agents

Disc diffusion method

The fungal inoculum was prepared as mentioned in (Toplon *et al.*, 2012) by transferring part of the pure fungal colony that cultured on the SDA medium by using a carrier to a sterile glass vial containing the physiological solution and shaking the solution well using a mixer, the number of cells was calculated to obtain the concentration 10⁶ spore / ml using a Hemocytometer count slide (Kahnosh, 2015).

The examination was carried out using Disc diffusion method as the method mentioned by (Al-Faramawi and Mostafa, 2013) by publishing it on the SDA medium prepared for this purpose and after pollinating the medium with a fungus by about 0.1 ml using a carrier so that the entire dish was covered with the fungal inoculum, and the antifungal agents were spread using Sterile tweezer by one disk for each antifungal agent and for three replicates. Then the dishes were incubated in the incubator at a temperature of 28-30 m for a period of three days, after which the inhibition areas were measured in mm using a ruler, which represent the areas surrounding each disc which is free from fungal growth.

Determination of the minimal inhibitory concentration (MIC)

Identify the minimal inhibitory concentration (MIC) and minimal fungicidal concentration for three antifungal agents, Fluconazole, Itraconazole, and Ketoconazole, against isolated fungal species, according to (Caillabet, 2013). The minimal inhibitory concentration (MIC) was determined according to the method of agar dilution described (Mc Ginnis, 1980), as follows:

- 1- Take (2 ml) of the original solution and add it to (7 ml) of SDB medium), a concentration of (1000 µg/ ml) was obtained and we symbolize it with the number (1).
- 2- Take (7 ml) of solution No. (1) and add to (7 ml) of the medium (SDB), we obtain a concentration of (500 µg / ml) and denoted by the number (2) and we continue on this method to a concentration limit of (25.0 µg / ml) also, the pointing continues to No. (13).
- 3- From each dilution, drawing (3 ml) and add to two tubes, each of which contains (27 ml) of medium (SDA), mix the mixture and each (30 ml) of it is drawing to a glass dish and left to cool, then drawing (05.0 ml) of the

fungal suspension was placed on the surface of the dishes and spread uniformly, leaving the dishes without moving in order for the suspension to be absorbed by the medium.

- 4- The inoculated dishes by fungi were incubated at a temperature of (28-30 °C) until the colonies growth appeared and then (MIC) was recorded as the lowest antifungal concentration showing no growth of the fungi.

Results and Discussion

Isolation

The results of the study shown in table (1) showed that there are a number of opportunistic and pathogenic fungi present on the body of gecko. These fungi have shown differences in number and percentage. The most common fungi were *Aspergillus niger*, and it appeared 12 times and formed a percentage of 40% in when the two fungi *Rhizopus stolonifer* and *Penicillium digitatum* came in the second sequence and with 6 isolation times, they formed a ratio of 20% each, while the remaining fungi, *Alternaria alternata* and *Chrysosporium anamorph*, came to isolate only twice and formed a ratio of 6.6% each.

Table 1 : Numbers and percentage of fungi isolated from gecko on SDA medium

Numbers and percentage	Isolation region					Fungi
	Hands	Legs	Abdomen	Tail	Mouth	
(6.6%) 2	0	0	0	2	0	<i>Chrysosporium anamorph</i>
(40%)12	6	2	4	0	0	<i>Aspergillus niger</i>
(20%)6	2	0	2	0	2	<i>Rhizopus stolonifer</i>
(6.6%)2	0	0	2	0	0	<i>Alternaria alternata</i>
(20%)6	0	0	2	2	2	<i>Penicillium digitatum</i>
(6.6%)2	2	0	0	0	0	<i>Fusarium solani</i>
(100%)30	10	2	8	4	4	Total

A.niger is one of the fungi widespread in the world for its ability to adapt, survive and spread as it only needs some moisture for germination and growth on organic materials such as wood, paper, leather, clothing, plant residues, fruits, etc. It is produce toxins and antibiotics that help it to survive and spread. This fungus characterized by produce a huge number of small-sized reproductive units that spread in the air and when they reach to a suitable place, they germinate and give a septate, thin and transparent mycelium, also this fungus distinguishes by its asexual composition called the conidial head, which results from arrangement of spores at the end of the conidiophore, the fungus is also known for its resistance to drought, high salinity and extreme pH. Therefore, this fungus appeared at the front of the fungi isolated from gecko, as its spores are exist everywhere and can stick to the gecko body and its scales (Gamble *et al.*, 1997).

This same speech upper applies to the fungus *P. digitatum* except that it does not have any conidial head, but that its proliferative composition is similar to the brush. This fungus came in second place in terms of isolation and the percentage of appearance by sharing with the fungus *R. stolonifer*, The last fungus from division zygomycota, is known for its rapid growth and high resistance to antifungal agents, difficult conditions, non-septate mycelium and its spherical sporangiospore (Toplon *et al.*, 2012).

A. alternata, *F. solani*, and *Ch. anamorph* appeared twice and each accounted for 6.6%. *A. alternata* and *F.*

solani fungi are restorative, their presence may be due to feeding gecko on food contaminated with these fungi.

As for the fungus, *Ch. anamorph* is considered the most important fungus isolated from gecko, because it is a keratinophilic fungus that preferred keratin, which causes a lot of problems for reptiles, as it attacks gecko, snakes, chameleon, and others. It remains a source of danger for people who deal with it in direct contact, especially when their immunity is weak (Kahnosh,2015) .

The fungi were concentrated in the areas of the hands and abdomen, and the reason for this is that the hands are the first organ that comes into contact with surfaces and pollutants and the remains of food and other stuffs, the abdomen is always in contact with outer environment, its the biggest area so it was the most number of innate isolates (Caillabet, 2013) .

The antifungal effects on the isolated fungi

Discs diffusion method

Table No. 2 shows the effect of antifungal against fungi isolated from the gecko using the disc diffusion method on the solid media (SDA), in terms of the inhibiting zones around the discs. The antagonists included itraconazole, ketoconazole and flucanazole. Itraconazole at a concentration of 1.5 mg / ml was the most effective in inhibition of fungi, followed by ketoconazole. Most of our fungi resisted Flucanazole (Vantrubova *et al.*,2010).

Itraconazole is an antifungal medication used to treat a number of fungal infections. This includes aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. It may be given by mouth or intravenously (Vantrubova *et al.*, 2010).

Itraconazole was patented in 1978 and approved for medical use in the United States in 1992. It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. The

wholesale cost in the developing world is about 0.29 USD per day of treatment as of 2015. In the United States, as of 2017, the wholesale cost of this dose is 6.36 USD (Maurya *et al.*, 2019).

The mode of action of itraconazole is Inhibits fungal cytochrome P450 3A dependent enzyme, decreases ergosterol synthesis and inhibits cell membrane formation (Maurya *et al.*, 2019)

Table 2 : Effect of antifungal against fungi isolated fungi from gecko(discs diffusion method)

			Antifungals(mg/ml)						Fungi
Itraconazole			Ketoconazole			Fluconazole			
1.5	1	0.5	1.5	1	0.5	1.5	1	0.5	
8.6	7.3	6.6	5.3	3	3.1	0.4	0	0	<i>Chrysosporium anamorph</i>
7.9	5.8	3.9	3.3	0	1.7	3.1	2.2	1.8	<i>Aspergillus niger</i>
2.1	2	0	2.5	1.7	0	0	0	0	<i>Rhizopus stolonifer</i>
9.3	8	0	4.8	4.7	0	0.6	0	0	<i>Alternaria alternata</i>
7.1	4.7	2.2	3.3	2.7	1	1.6	0	0	<i>Penicillium digitatum</i>

MIC method

With regard to the lowest inhibitory concentration, Table 3 shows that the itraconazole antifungal gave the best results, as the fungi under study were inhibited with a concentration that is the lowest in comparison with the other antigens and as *Chrysosporium anamorph* comes 1.5mg/ml, *Aspergillus niger* 1.8 mg/ml *Rhizopus stolonifer* 2.2 mg/ml, *Alternaria alternata* 1.3mg/ml and *Penicillium digitatum* 1.7 respectively (Fernandez-Torres *et al.*, 2001).

This test depends on obtaining the best results of inhibition with a low cost, as the use of high concentrations of antibiotics has very many negative effects. It is costly and tired and causes the emergence of resistance isolates. With the difficult circumstances, this made health institutions resort to adopting the least concentrations that inhibit germs (Nweze *et al.*, 2007).

Table 3 : The minimum inhibitory concentration $\mu\text{g} / \text{ml}$ for fungi isolated from gecko

Itraconazole	Ketoconazole	Fluconazole	Fungi
1.5	3.2	8.4	<i>Chrysosporium anamorph</i>
1.8	4.6	6.3	<i>Aspergillus niger</i>
2.2	3.1	7.1	<i>Rhizopus stolonifer</i>
1.3	2.1	3.8	<i>Alternaria alternata</i>
1.7	3.3	4.6	<i>Penicillium digitatum</i>
2.6	3.1	6.2	<i>Fusarium solani</i>

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