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Molecular Identification of Dermo-Mycotic Infection and the Effect of Dietary-Essential Oils on Broiler Chickens in Upper Egypt

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Abstract

The importance of searching for natural alternatives away from chemicals in poultry health and treatment has benefits for humans in many directions, as we control the bad effect of the accumulation of harmful chemicals in their meat, as well as reduce the risk of zoonotic infection and preserve the environment from chemical pollution. Enormous fungi induce a considerable level of annihilation in the poultry industry and human consumers due to their zoonotic implications. This study was designed to explore the effects of keratogenic and toxigenic skin fungal affection and the effects of dietary-essential oils on broiler chickens (n-120). Skin scrapings and feather samples were examined mycologically in association with PCR sequencing for genomes of the culturally detected fungi (in South Korea) based on phylum tree and all Sequences data was deposited in GenBank and each was assigned an accession number. Sera samples of the tested broilers were examined by ELIZA against biogenic amine mainly histamine during the summer season, also a histopathological examination of skin sections before and after taking feed additives (essential oils) as anti-fungal for thirty days, the broiler-fed diet was supplemented with peppermint, thyme, and Carvacrol 70 mg/kg (w/w) in dietary feed. The isolated fungi were: Fifteen fungal species belonging to 9 genera of filamentous fungi which were isolated from skin scrapings and feathers of chickens. *Aspergillus niger* and *A. flavus* are the most prevalent species (20 samples representing 100% of total samples for each. *Rhizopus oryze* 20% and *Fusarium oxysporum* 15% were cultured from total samples respectively. Four fungal species appeared in 10% of the tested samples which are *Aspergillus qudrilineatus*, *Paecilomyces variotii* (*Byssochlamys spectabilis*), *Scopulariopsis brevicaulis* and *Exserohilum rostratum*. Finally, the other seven fungi presented as 5% from tested samples. The average level of serum histamine before treatment was 16.6 ng/ml and after feeding was 12.3 ng/ml (significant decrease, $P < 0.05$) referring to the significant role of the essential oils in broilers ration.

KEYWORDS
Dermomycotic fungi, Essential oils, Broilers

INTRODUCTION

Fungal infections are common in poultry but are less common compared to bacterial and viral infections. However, fungal infections are often devastating agents and therefore require due attention in terms of effective prevention and control measures (Asfaw and Dawit, 2017). Fungal-related diseases can cause great economic damage in the poultry industry through loss of meat, and egg production and high rates of morbidity and mortality either directly or due to the production of mycotoxins which suppresses the immunity of birds, attributed to several microbial infections (Dhama *et al*., 2013). Fungal diseases of poultry have come to the fore all over the world due to the excessive use of antibiotics, which destroy the natural bacterial microflora in the body and give way to infections by opportunistic microorganisms (Dhama *et al*., 2013). In addition, Difficult treatment of fungal infection in poultry and very costly due to lack of proper biosecurity measures, intensive farming, pathogen load in farms where there are no available vaccines and drug resistance is on the rise, making diagnosis uncertain, thus prevention is best dealt with these diseases (Sokolović *et al*., 2015). Importance of prevention as a way to minimize the consequences of zoonosis (Miskiewicz *et al*., 2018). Outwardly healthy birds can be carriers of various fungi/molds that pollute the soil, air, and water that surround their habitat. However, they act as an important source of potential pathogenic microorganisms. The presence of any fungal species on the feathers or / body of any bird is naturally transmitted to others and causes many fungal diseases (Miljković *et al*., 2011). The skin of birds is affected due to contact with contaminated litter, feed, settled dust, etc, these contaminants are a mixture of organic and inorganic particles from litter, feathers, fur (skin material), bacteria, fungi, and mold spores, etc., level of infection according to bird species and the stage of production cycle. Isolation of *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., and *Scopulariopsis* spp., from poultry feathers (BMiljković *et al*., 2011). Detection of potential allergens as, *Alternaria*, *Aspergillus*, Mucor, *Penicillium*, and *Rhizopus* species (Ljaljević *et al*., 2000). Growing of filamentous fungi causing spores production and mycotoxin secretion have bad effects on chicken health (Schnurer *et al*., 1999). Three pathways detected filamentous fungi producing diseases in poultry farms, direct invasions of tissues, secreting toxins, and tissue damage (Friend., 1999). This invasion causes

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specific types of inflammation and allergic infections. (Dhama *et al*., 2013). Mycotoxins secreted from toxigenic fungi such as *Penicillium*, *Fusarium*, *Aspergillus* cause mycotoxicosis, which inhibit the immune response, so broiler chickens exhibition to many bacterial and viral diseases (Asfaw and Dawit, 2017; Oliveira *et al*., 2018). Detection of highly concentrated fungal spores in poultry house presented by a group of allergenic fungi *A. flavus*, *Scopulariopsis*, *Cladosporium cladosporioides*, *Penicillium crysogenum*, *A. fumigatus*, and *Penicillium crysogenum* (Nichita *et al*., 2010). *A. alternata* is one of the potent allergenic fungi and its spores are potent allergens (Salo *et al*., 2006). *Paecilomyces* spp. is one of the heat-resistant fungi that can spoil meat causing economic losses in the food industry (Danielly *et al*., 2018). *Paecilomyces* spp. can resist heat treatment methods during meat processing (Hosoya *et al*., 2014). *Paecilomyces variotii* is a species that has a thermotolerant nature, it can contaminate herbs and spices such as ground red pepper. This may increase its pathogenic potential, leading to human and animal diseases (Houbraken *et al*., 2010; Ham *et al*., 2016; Borba and Brito, 2015). In humans, *P. variotii* was isolated from clinical manifestations that belong to subcutaneous/cutaneous and ocular infections (Vasudevan *et al*., 2013; Borba and Brito, 2015; Evans *et al*., 2015; Trinh *et al*., 2017). More recently, onychomycosis (Pontini. *Et al* 2016). A case was detected of fungal keratitis (keratomycosis) infected by *Exserohilum rostratum* in an immunocompromised patient with ocular trauma (Winai Chaidaroona *et al*., 2019).

No cure for poultry mycotic infection, so prevention is the only effective way to protect poultry farms (Arné *et al*., 2011). Antibiotic resistance is one of the most difficult situations of global significance in veterinary health newly recognized by the WHO. Historically, plants are a great source of drugs whose therapeutic activity is recognized as anti-inflammatory substances, chemotherapeutic compounds, and antimicrobials used all over the world as traditional medicine (Nelson *et al*., 2021). Natural protection against pathogenic fungi through essential oils is a suitable replacement for synthetic chemicals (Michaela *et al*., 2021). Essential oils are used as effective antifungal agents (Nuzhat *et al*., 2013). Oil emulsions in different degrees can penetrate the cell wall and cell membrane of chitin-based fungal hyphae causing inhibition of pathogenic fungal growth (Moghaddam *et al*., 2013). The antimicrobial activity of essential oil is based on its chemical structures which have an aromatic ring and free phenolic hydroxyl group (Tampieri *et al*., 2005). Extraction of Eos, mainly by distillation from aromatic plants has many volatile molecules that act as antioxidants, antibacterial, and antifungal depending on their type and concentration (Bakkali, 2008). The Presence of the delocalized electrons and hydroxyl group system in carvacrol, thymol, and cymene plays an important role in antimicrobial activity (Ultee *et al*., 2002). In many cases, the complex interaction between different classes of EOs containing aldehydes or phenols are more effective in antimicrobial activity for example cinnamaldehyde, carvacrol, citral, thymol, or eugenol complex, make a considerable antimicrobial activity (Dormans *et al*., 2000). A mixture of cinnamaldehyde and thymol has selective properties for inhibiting the growth of fungi, yeasts, and bacteria (Bento *et al*., 2013). The essential oils *Thymus vulgaris* and Mentha piperitha were shown to form a 40 mm diameter inhibition zone on fungal strains. Also (MIC) the Minimum Inhibitory Concentration, *Thymus vulgaris* in of concentration 0.5% had selective activity against fungi also *Malaleuca alternifolia* which have a high antifungal activity (Rūta Mickienė *et al*., 2007). Carvacrol and thymol are active ingredients of the Lamiaceae family; these ingredients have antifungal and antibacterial activity (Memar *et al*. 2017).

In poultry, many studies examined the effects of heat stress on the immune response, especially in summer, which have shown the immunosuppressive effect of heat on broiler chickens and laying hens (Ghazi *et al.,* 2012). Tropical areas with high ambient humidity and temperature enhance the growth of fungi. Similarly, humidity and temperature can enhance fungal contamination in poultry feeds (Okoli *et al*., 2006). Dietary feed additives such as probiotics, antioxidants, minerals, vitamins, essential oils, and prebiotics play an important role in maintaining heat stress. (Lucas *et al* 2013). High temperature, humidity, and crowdedness in poultry houses contribute to fungal contamination, especially with *Aspergillus*, *Penicillium*, and *Fusarium* (Witkowska *et al*., 2010). High concentration of contamination of poultry litter by *Aspergillus* nigri, *Fusarium*, *Cladosporium*, *Rhizopus* spp., *Aspergillus flavi,* yeast, Mucor, and *Penicillium* in summer (Mario *et al*., 2021).

Histamine is released from the mast cells where they are stored to initiate a defensive step against allergy triggers. Increasing its level in the bloodstream causes inflammation in various areas of the body. This condition signals other chemicals from the immune system to protect the body from potential threats. This chain of reactions then leads to allergies (Makati Medical Center 2021). Mast cells and basophils are the main source of histamine contributes significantly to allergic diseases. During allergic skin reactions and anaphylaxis, plasma or tissue histamine reported high levels (White, 1990). Susceptibility to BA, which results in symptoms resembling an allergic reaction, can cause skin rash or inflammation (Guo *et al*., 2015). This study aimed to explore the effects of keratogenic and toxigenic skin fungal affection and the effects of dietary-essential oils on broiler chickens.

MATERIALS AND METHODS

Broiler chickens

This research works on 120 Saso broiler chickens (20 days old) was conducted in the summer season for about 6 weeks from July till August 2021 in Assiut governorate, Egypt. Chicks were vaccinated and fed in commercial ration and water ad libitum.

Sample Collection and Examination

Skin scraping and feathers were taken from the tested birds. Four to five samples were aseptically collected from each bird, skin scraping from the infected area and feathers from the neck area, around the cloaca, outside and inside of the wings, then plucked aseptically and carefully. Samples taken from each bird were mixed and collected in one single sample for each bird. The samples are marked according to the species then packaged into bags with zipper, and transported quickly to the lab to be stored till being analyzed at +4°C.

Mycological analysis of skin scrapings from chicken

Samples of skin scrapings from chickens were cultured in sterile Petri plates containing autoclaved (DRBC) dichloran rose bengal chloramphenicol agar, which contains agar, 15 g; K_2HPO_4 or KH_2PO_4, 1.0 g; peptone (Oxoid), 5 g; glucose, 10 g; $MgSO_4$ 7H20, 0.5 g; distilled water, 1 liter; final pH was 7.2 with K_2HPO_4 and 5.6 with KH_2PO_4 (King *et al*., 1979). Rose Bengal (25ug/ml) and Chloramphenicol (100ug/m) were incorporated into the medium as bacteriostatic agents. Cultures were incubated for 7-10 days at 28ºC, examination and identification of the growing fungi.

Phenotypic Identification of fungi

Isolated fungi were grown in Czapek`s yeast extract medium (CYA) (Pitt and Hocking, 2009). This medium composed of (g/L): CuSO4, 0.005; ZnSO4, 0.01; FeSO4, 0.01; MgSO4.7H2O, 0.5; KCl, 0.5; K2HPO4, 1; Na2NO3, 2; Sucrose, 30; chloramphenicol, 0.25; yeast extract 5 and agar 15, (final pH 7.3) Cultures were incubated at 28° C for 7 days. Identification of the growing fungi according to colony characteristics (texture and reverse pigmentation, growth rate, color) and on microscopic features (conidiogenous cells, shape of conidiophores, and conidial dimensions). The slide was stained with lactophenol cotton blue for better visualization of fungal hyphae and conidia. Axiostar trinocular microscope, made by Zeiss, Germany was used for examination. Main references used in identification (Domsch *et al*., 2007 and Ismail *et al*., 2015).

Molecular identification of fungi based on ITS

Culture of selected fungi on Petri plates containing CYA medium (Pitt and Hocking, 2009) and incubated at 28°C for 5-7 days. The growing cultures under DNA extraction in the Molecular Biology Research Unit, Assuit University using a Patho-gene-spin DNA/RNA extraction kit were provided by Intron Biotechnology Company, Korea. The extracted fungal DNA was sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and rRNA gene sequencing. PCR was performed using ITS1 (forward) and ITS4 (reverse) primers which were incorporated in the reaction mixture Primers have the following composition: ITS1 (5′ - TCC GTA GGT GAA CCT GCG G - 3′), and ITS4 (5′- TCC TCC GCT TAT TGA TAT GC -3′). The purified PCR product (amplicons) was sequenced with the same primers, incorporating ddNTPs in the reaction mixture (White *et al*., 1990). The PCR reaction mixture was prepared by using Solgent EFTaq as follows: 10X EF-Taq buffer 2.5 µl, 10 mMdNTP (T) 0.5 µl, primer (F10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5U) 0.25µl, template1.0 µl, DW to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95°C for 15 min followed by 30 cycles of denaturation at 95°C for 20 s, annealing at 50°C for 40 s and extension at 72°C for 1 min, with a final extension step of 72°C for 5 min. The PCR products were then purified with the SolGent PCR Purification KitUltra (SolGent, Daejeon, South Korea) prior to sequencing. The purified PCR products were reconfirmed (using a size marker) by electrophoreses of the PCR products on 1% agarose gel. The bands were eluted and sequenced. Each sample was sequenced in the forward and backward directions. Contigs were created from the sequence data using CLCBio Main Workbench program. The sequence obtained from each isolate was further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained together with those retrieved from the GenBank database were subjected to Clustal W analysis using MegAlign (DNAStar) software version 5.05 for the phylogenetic analysis. Sequence data was deposited in GenBank, and accession numbers were given for them.

Molecular identification-based beta-tubulin sequences

The fungal isolate was cultured in sterile Petri plates containing autoclaved Czapek`s agar (CZA) medium and incubated for 7 days at 28°C (Pitt and Hocking, 2009). The cultures were sent to the Molecular Biology Research Unit, Assuit University for DNA extraction using a Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. Then fungal DNA samples were sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and rRNA gene sequencing. The Beta-tubulin gene was targeted for PCR using Beta-tubulin primer pairs βtub-F (5′- TGACGGGTGATTGGGATCTC-3′) and βtub-R (5′-CGTCCGCTTCTTCCTTGTTT-3′). The purified PCR product (amplicons) was sequenced with the same primers, incorporating ddNTPs in the reaction mixture (White *et al*., 1990). The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

Serum samples were collected before and after feeding of dietary essential oils. Samples stored at -20°C till determining the level of histamine by Quantitative Sandwich ELISA, Serum histamine level was assayed by a Rat Histamine (HIS) ELISA Kit (cat. No: MBS 013382) purchased from U.S.A. The method has been processed according to the instructions for methodology.
Skin samples taken for histopathological examination were stored in formalin 10%.

Essential oils

Carvacrol (Cymophenol), peppermint (*Mentha piperita* L.), and thyme (*Thymus vulgaris* L.).

EOs were obtained from the National Research Center, Cairo, Egypt. A mixture of three oils was used in a concentration of 99% of each oil for detecting the antifungal effect on studied broiler chickens. These EOs' antimicrobial activity is previously provided by the producer. The essential oils are kept at 2-8°C in sealed brown vials until used. The oils were added to the basal experimental diet during preparation as 70 mg of each oil per kg of diet (Ocak *et al*., 2008; Mehran *et al*., 2016). All diets were fed in mash form. Water and food were provided for ad libitum consumption (Friedman, 2017).

RESULTS

Fungi isolated from chickens

Fifteen fungal species belonging to 9 genera of filamentous fungi were isolated from skin scrapings and feathers of chickens. *Aspergillus flavus* and *A. niger* were the most prevalent species from (120) samples representing 100% of the total samples for each. *Rhizopus oryze* 20% and *Fusarium oxysporum* 15% were cultured from total samples respectively. Four fungal species appeared; in 10% of the tested samples, and these were *Aspergillus qudrilineatus*, *Paecilomyces variotii* (*Byssochlamys spectabilis*), *Scopulariopsis brevicaulis* and *Exserohilum rostratum*. The remaining fungal species occurred only 5% in the tested samples; *Absidia corymbifera*, *Absidia cylindrospora*, *Alternaria alternate*, *Aspergillus tamari*, *Aspergillus versicolor*, *Fusarium semitectum*, *Syncephalastrum racemosum*, and Yeasts.

HIS level

The average level of serum histamine before treatment was 16.6 ng/ml, and after feeding was 12.3 ng/ml (significant decrease, $P < 0.05$), referring to the significant role of essential oils used as a broiler feed additive.

DISCUSSION

Isolation of several genera of fungi in our study posed a

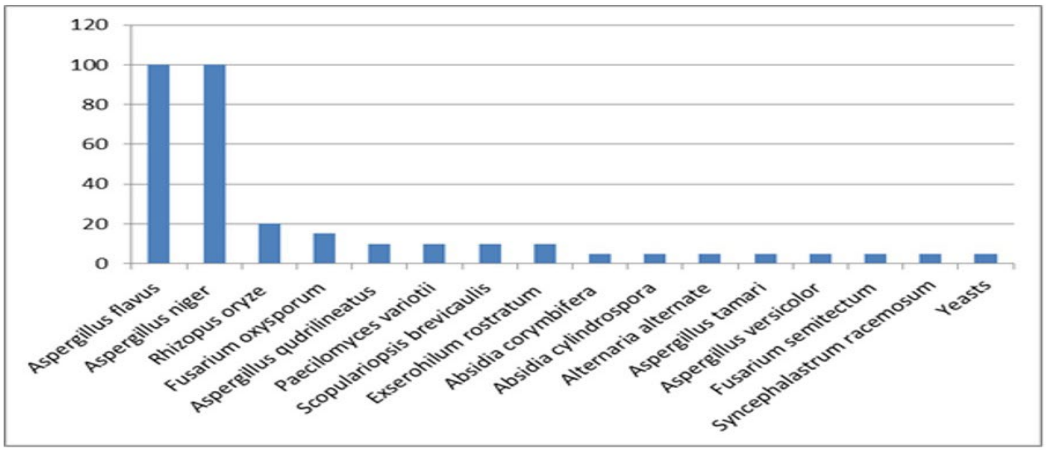

Figure 1. The percentage of the isolated fungi.

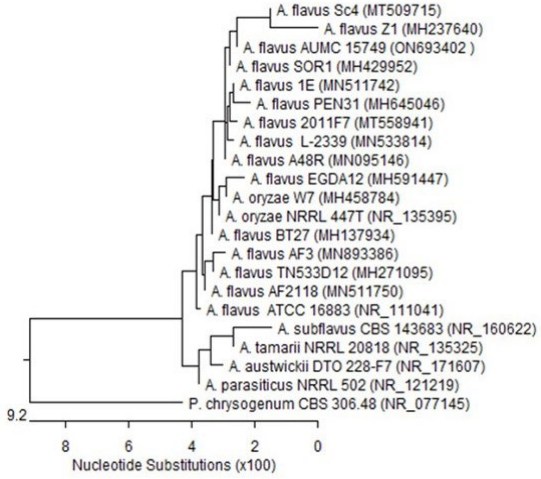

Figure 2. Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (*Aspergillus flavus* AUMC 15749 with GenBnk ccession no. ON693402, arrowed) aligned with closely related strains in the GenBank. This strain showed 100% identity and 99% -100% coverage with several strains including the type material *Aspergillus flavus* ATCC16883 (gb: NR_111041). *Penicillium chrysogenum* represents an outgroup strain.

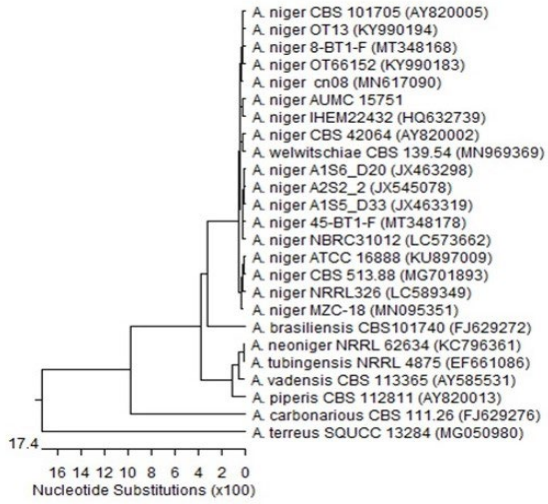

Figure 3. Phylogenetic tree based on beta tubulin gene sequences of the fungal sample isolated in the present study (*Aspergillus niger* AUMC 15751, arrowed) aligned with closely related strains in the GenBank. This strain showed 98.92% - 99.46% identity and 97% -100% coverage with several strains of the same species including the type material *A. niger* ATCC 16888 (gb: KU897009). *Aspergillus terreus* represents an outgroup strain.

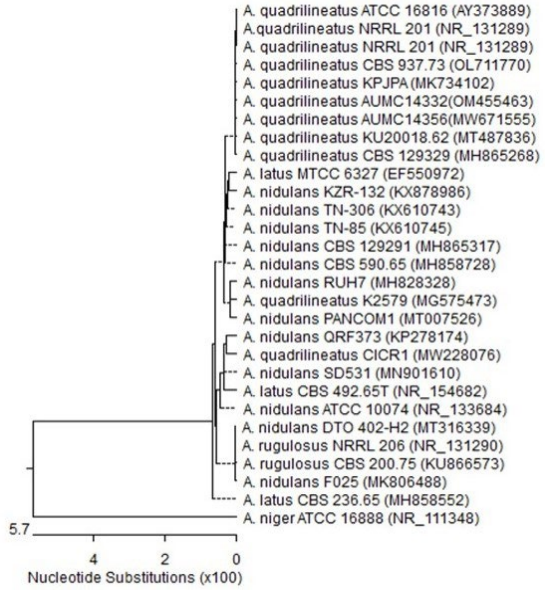

Figure 4. Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (*Aspergillus quadrilineatus* AUMC14332 with GenBank accession No. OM455463, arrowed) aligned with closely related strains accessed from the GenBank. It showed 100% identity and 99% - 100% coverage with several strains of *A. quadrilineatus*. *Aspergillus niger* is included in the tree as an outgroup strain.

Notes: As a member of *Aspergillus* Section Nidulantes, *A. quadrilineatus* shared both phenotypic (mainly asexual stage) and genotypic characteristics with *A. nidulans*, A. latus and A. rugulosus. These species can be discriminated microscopically by the shape of ascospores (sexual stage).

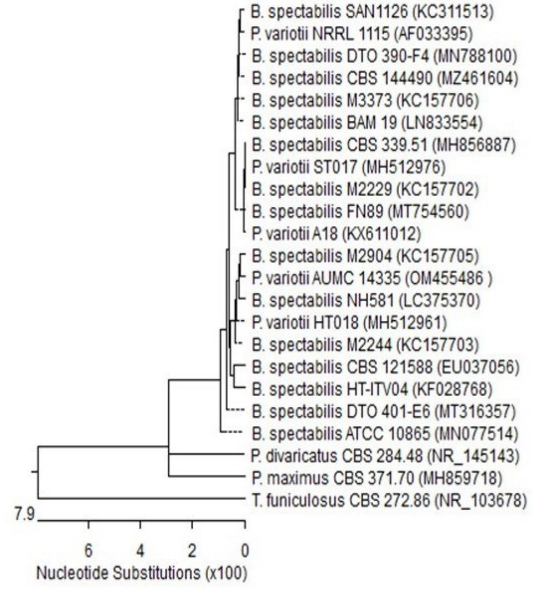

Figure 5. Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (*Paecilomyces variotii* (=*Byssochlamys spectabilis*) AUMC14335 with GenBank accession No. OM455486, arrowed) aligned with closely related strains accessed from the GenBank. It showed 98.65% - 99.50% identity and 97% - 99% coverage with several strains of *P. variotii*. A strain of Talaromyces funiculosus is included in the tree as an outgroup strain.

Notes: *Byssochlamys spectabilis* represents the sexual (ascosporic) state of *Paecilomyces variotii*. It is rarely observed because *P. variotii* is heterothallic requiring mating of positive and negative strains.

health risk to pathogens themselves or their producers of mycotoxins that may cause severe poisoning in animals and humans. *Aspergillus* spp. identified as opportunistic pathogenic fungi in humans, especially those impaired with immune systems (Miljković *et al*., 2011; Miskiewicz *et al*., 2018). The most detected fungi were *A. flavus*, *A. niger*, *Penicillium* spp., and Mucor, but reported no seasonal differences were significant (Mario *et al*., 2012). *A. flavus*, *A. niger*, A. terreus, and *A. nidulans* alone or in combination, also posed systematical or local risks to human or animal health, similar to our study (Aengwanich, 2008; Bozkurt *et al*., 2012). Another species, *Fusarium* spp has wide a range of evidence of infections that are complicated in treatment, which can cause onychomycosis, skin infections, and keratitis. (Deng *et al*., 2012). Similarly, the risk of poisoning by mycotoxins from *A. Flavi, Nigri,* and *Fusarium*, which are known to produce mycotoxins (Deng *et al*., 2012; Bartlett and Smith 2003). In poultry, *Aspergillus fumigatus* is the main cause of 95% of all cases of aspergillosis (Mario *et al*., 2021), In contrast to our study we did not find any species from the *Aspergillus Fumigati* in our skin samples. Investigation of the prevalence of isolated fungi in poultry feed was *Fusarium*, *Aspergillus*, *Rhizopus*, *Penicillium*, Mucor, and *Alternaria*. (Krnjaja *et al*., 2008 and 2010). *Scopulariopsis brevicaulis* has been shown to cause loss of hair covering the skin of two goats, a detected case report in Turkey (Ozturk *et al*., 2009). In Egypt, detection of fungal infection in poultry feed with *Aspergillus*, *Fusarium* spp. Mucor spp., *Scopulariopsis brevicaulis*, *Penicillium* spp., and *Rhizopus* spp. were reported (Moharram *et al*., 1987).

Mint oil in dilutions of 1ml and 0.5ml showed an antifungal effect stronger than gentamicin or synthetic menthol (Moghtader, 2013). Klarić *et al*. (2007) detected a broad-spectrum fungicidal activity of thyme essential oil. Omran *et al*. (2009) reported that among the tested EOs, thyme essential oil presented the most inhibitory effect against fungi and yeast giving a larger inhibitory zone in comparison with amphotericin B. Katooli *et al*. (2012) said that thyme essential oil at concentrations 50, 75 and 100% completely inhibited mycelial growth and thyme essential oil has the strongest antifungal activity compared to eucalyptus oil. Rusenova *et al*. (2009) also identified the activity of thyme essential oil in veterinary importance as the most effective oil against many species of fungi and bacteria. Dutkiewicz *et al*. (1994) detected the presence of molds in broiler houses even after cleaning and disinfection, observing that the concentration of air fungal spores in the broiler's production cycle was close to 3 $\log_{10}$ CFU/m^3, this percent is considered a risk factor for animals' respiratory diseases. Improving the hygienic standards in poultry houses using essential oils was investigated by (Bakutis *et al*., 2011). Mituniewicz *et al*. (2008) confirmed that poultry litter obtained and stored in climatic conditions is considered a main source of fungal infec-

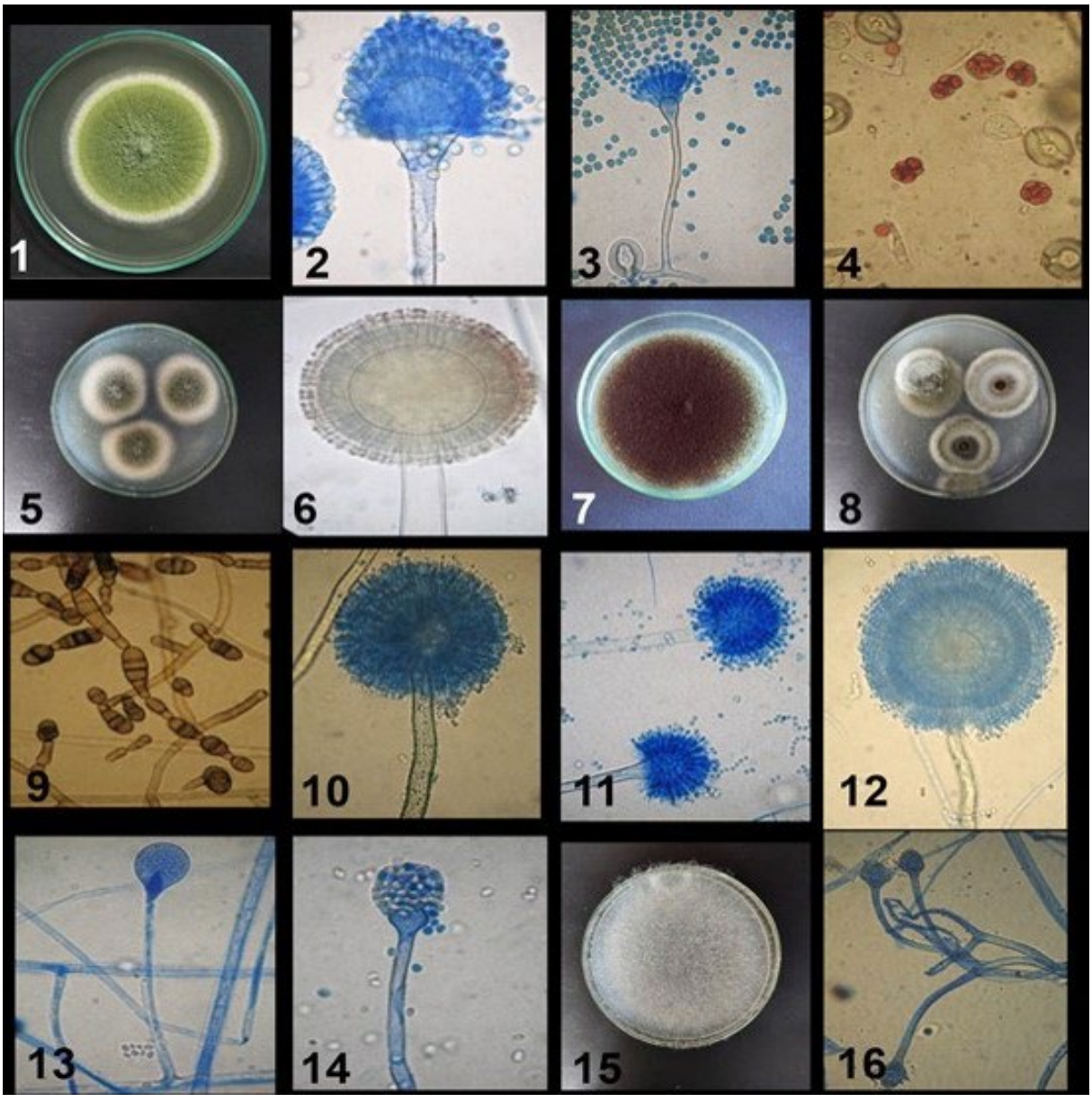

Figure 6. 1: *Aspergillus flavus* colony, 2: *Aspergillus flavus*, 3: *Aspergillus nidulans*, 4: *Aspergillus nidulans* asci, 5: *Aspergillus nidulans* colony, 6: *Aspergillus niger* conidial head, 7: *Aspergillus niger* on CYA, 8: *Alternaria alternate*, 9: *Alternaria* alternata-, 10: *Aspergillus* ochraceus-, 11: *Aspergillus versicolor*, 12: *Aspergillus wentii*, 13: *Absidia corymbifera*-, 14: *Absidia cylindrospora* , 15: *Absidia cylindrospora* colony, 16: *Absidia cylindrospora*.

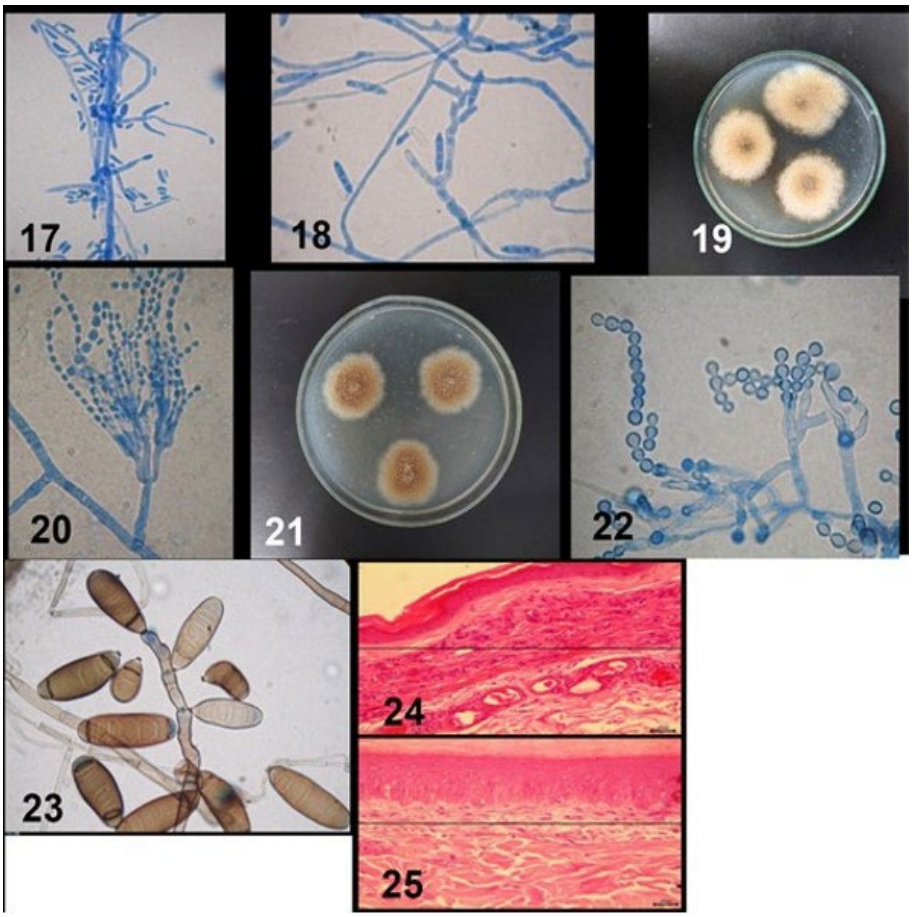

Figure 7. 17: *F. oxysporum*, 18: *Fusarium semitectum*, 19: *Paecilomyces variotii* colony, 20: *Paecilomyces variotii*, 21: *Scopulariopsis brevicaulis* colony, 22: *Scopulariopsis brevicaulis*, 23: *Setosphaeria rostrata*, 24: Avian skin section stain (H&E) before taking food additives reveal sever inflammation and presence of inflammatory cells; eosinophil and mast cell, 25: Avian Skin section stain(H&E) after taking food additives reveal normal cell appearance.

tion in poultry houses so, it must be misted with mint oil and other poultry environments must be treated with essential oils first before placing chicks, this is for proper animal welfare (Kędzia *et al*., 2007) Examined antimicrobial activity of more than fifty essential oils showed that thyme oil has a powerful effect against fungi and peppermint oil and is more effective at high concentrations (Yang and Clausen, 2007) say that essential oil is able to provide long-term protection from mold establishment on cellulose-based building materials under conditions of high humidity and also, detected that thyme oil has a great effect against *Aspergillus* and *Penicillium*. Quantitative analysis of the essential oils to compare their natural antimicrobial compound revealed that there is a predominance of both aromatic alcohols in carvacrol being the most abundant component in this group (Arrebola *et al*., 1994). The antioxidant effect of oregano plants may be related to the presence of carvacrol and thymol in essential oils (Lagouri *et al*., 1993). The *C. citratus* is the first followed by *M. piperita*, in the antifungal effect (Valentina *et al*. 2018). Oregano and citrus have a great antifungal effect against some heat-resistant molds and this effect depends on oil concentration. The highest oil concentration gives the highest inhibitions of mycelial growth of *P. variotii* and *A. fumigatus* (Ghasempour, *et al*., 2016). Ocak *et al*. (2008) reported that the use of EO as a growth stimulant replacement in broiler feed does not always enhance production efficiency and sometimes even worsens it. This may be due to incorrect concentration of oil or too short application time. Differences in reported results between references may be due to weak chickens, biosafety violations, or environmental factors such as litter, lighting, equipment, rodents, etc. This conflict may be due to dietary errors during the experiments such as unbalanced feed or contaminated drinking water.

Many factors, such as high temperature (the summer season), the humidity of the air, and litter contribute to fungal contamination (Wójcik *et al* 2010). *A. alternata* is distributed widely in more than one temperate region but it significantly increases in summer (Wójcik *et al*., 2006). (Bartlett and Smith, 2003) say that the exposure of the broiler to heat stress inhibits the total circulating antibodies in first and second immune responses. Generally, many studies show the immunosuppressing action of heat stress on broiler chicks and laying hens, but (Lucas *et al*., 2013) suggested that there are no effects of heat stress on broiler chicks and laying hens either for growth reduction and egg production or on reduced quality and safety of poultry and eggs. The high-level concentrations of histamine and other BAs may suppress growth rate in broilers (Qaisrani *et al*., 2015). Other investigations to detect the synergistic antimicrobial action of oils and their compounds, ideal doses, and application methods in the field are still required as a preventive measure against mycological infection.

CONCLUSION

Essential oils are of significant values as feed additives in broilers intensive farming for protection and treatment to reduce biogenic amines as natural antinflamatory and antifungal.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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