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Evaluation of the efficacy of lemon leaf extract and clove essential oil as long- term sources for treating fungus and parasites linked to pet birds

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Recent research has demonstrated that plant essential oils (Eos) can defend plants against both biotic and abiotic challenges. This study attempts to identify and isolate the pathogenic fungus that are present in two different species of pet birds. Additionally, research the viability of employing inexpensive, eco-friendly, natural items as a sanitizer to stop the spread of these fungus. Birds discovered in bird-selling homes provided samples, which were used to research the effectiveness of lemon leaf and clove essential oil (CEO) extracts against isolated fungus. The results revealed variance in the percentages and quantities of isolated fungus. The most prevalent fungus in birds, *Penicillium glabrum*, has a percentage of isolates of 39.5%, whereas *Alternaria alternate* has a percentage of 4% in *Melopsittacus undulates* and *Serinus canaria*, and *Aspergillus niger* has a percentage of isolates of 1.5%. CEO and Lemon leaf extract were examined for their antifungal properties against isolated fungi at doses from 5 mg/ml to 25 mg/ml. Additionally, CEO was more effective in inhibiting the development of fungus than lemon leaf extract, with a modest impact (41.2%) on fungi at 20 mg/ml of CEO compared to 70.6% at 20 mg/ml for lemon leaf extract. One of the most frequent parasite infestations in domestic and wild birds is mite infestation, which causes scratching and pain and is occasionally accompanied by a subsequent bacterial illness. The description of *Isospora serinuse* n. sp. is also based on a single domestic canary Different percentages of *Serinus canaria* forma were noted.

Keywords: Lemon leaf extract; Clove oil; Parasite; Antifungal efficacy; Pet birds

INTRODUCTION

The most prevalent fungi-related ailments in birds of all species include *Aspergillosis, Dactylariosis, Histoplasmosis, Candidiasis, Cryptococcosis, Rhodotorulosis, Torulopsis, Mucormycoses,* and *Cryptococcosis* (Arné et al. 2021). According to Mirhosseini and Khosravi (2023), almost 50% of birds are reservoirs and carriers of fungi that are harmful to both people and birds. According to Malekifard et al. (2023), over 60 diseases can be spread to people through inhalation of bird droppings, and some of these diseases are airborne. Additionally, birds' feathers, ectoparasites, internal organs, respiratory systems, and digestive tracts can spread fungi. Carrier birds get sick because of their high body temperatures, which prevent fungus from growing. It is unusual for carrier birds to fall ill because their high body temperatures prevent the formation of some fungus, which can be deadly to humans and other animals with weakened immune systems (Washington DC, 2019). Therefore, it is important to pay attention to fungal infections and follow the unsuccessful treatment regimens recommended by Washington DC (2019).

The danger arises when people come into contact with the feathers of these birds when they are introduced into households. Domestic bird feathers are known to be carriers of a variety of microorganisms, including a few forms of pathogenic fungus that can infect people and animals anywhere in the world (Miskiewicz et al. 2018).

According to studies, healthy birds can transport a variety of fungi (molds) that contaminate the air, soil, and water around them. Birds are regarded as an appropriate route for the transmission of these fungi due to the infections they carry in their bodies and feathers since they significantly contribute to the emergence of a wide variety of fungal diseases (Bakr et al. 2018).

Although chemical treatments are the most effective, building up the host immune system can reduce the infection's severity. Essential oils are among the natural treatments that show the most promise for fungal control (Kalemba and Kunicka, 2003; Abhishek and Ravi, 2021). Numerous types of EOs made from various herbs or plants displayed antifungal properties (Abhishek and Ravi, 2021; Bilel et al. 2023a; Bilel et al. 2023b). Essential oils may be utilized to regulate, impede, or reduce microbial development (Abhishek and Ravi, 2021; Alotaibi et al. 2022). The Food and Drug Administration classifies EOs as "Generally Recognized as Safe" (GRAS) substances (FDA), Customers accept them more readily than "manufactured" products since it is non- toxic and damaging, and because their origins are natural (Abhishek and Ravi, 2021).

The properties of terpenoids/terpenes, which because of their lipophilic nature and low molecular weight can damage the cell membrane and cause cell death or inhibit the germination and sporulation of pathogenic or non-pathogenic fungi, may cause the antifungal or antimicrobial activity of EOs. Terpenoids/terpenes exhibit less effective antibacterial activity when employed as isolated compounds compared to entire essential oils, according to several in vitro investigations (Abhishek and Ravi, 2021).

Most volatile oils have an anti- microorganism effect, so adding them to skin preparations (ointment - cream - gel) eliminates the use of preservatives. The most important volatile oils in this field are clove oil, mustard oil, and corona oil. Likewise, the vapors of these volatile oils can kill microorganisms in the air, so they can be added to the air conditions used to sterilize the atmosphere of rooms and closed places (Licon et al. 2020). Pet and wild bird parasite infestations cause scratching and pain, and on occasion they are linked to subsequent bacterial infections; mite infestation is one of the most frequent (Zappia et al. 2023). A single domestic canary (*Serinus canaria forma domestica*) (subspecies *S. c. domestica*) from Western Australia was found to have *Isospora serinuse n. sp.* (*Apicomplexa:Eimeriidae*) (Yang et al. 2015). To investigate environmental pollution, the current study aims to analyze fungal infections in birds, classify them, and look at the antifungal efficacy of natural plant extracts on the isolated fungus.

MATERIALS AND METHODS

Sites and Sampling

Isolation of fungi from pet birds

Five samples were collected from shops selling Pet birds (*Serinus canaria* and *Melopsittacus undulates*) in the Sakaka Al- Jouf region. The collection process included taking parts of feathers, faces, and feed, and then placing them in sterile plastic bags. Fungi were isolated on rose-bengal potato dextrose agar (rose-bengal-PDA) by putting 4 pieces of each sample on the rose-bengal PDA medium. Plates were incubated at 27ºC for 7 days and then purified on (PDA) without rose-bengal. The number of purified fungi was identified and counted.

Identification of fungi

Using colonial and microscopic structures, taxonomic keys were used to identify fungal genera and species (Ainsworth et al., 2008; Saleem et al. 2010), whereas molecular criteria based on sequence analysis of the ITS1-5.8S rRNA-ITS2 region were used to identify the most dominant species (Animal health research institute, Dokki, Giza, Egypt).

Plants material and extraction

Clove essential oil (E.O) extraction

Clove Essential oil was obtained by hydro-distillation type Clevenger of 200 g of clove in distilled water (100 ml) for 8 h. Collected oil was separated from water and dried over anhydrous sodium sulfate Na2SO4. Clove E.O. was diluted in ethanol to create five samples at concentrations of 5, 10, 15, 20, and 25 mg/ml. All samples were stored at 4°C for further experiments. The yield of the extracted oil was 5% (w/w). Essential oil extraction was done in triplicate (Bilel et al. 2020).

Lemon leaves extract

From Sakaka- Aljouf in Saudi Arabia, fresh lemon leaves were procured in September 2020. Thirty grams of leaves were put in the Soxhlet extractor's extraction chamber, and 250 ml of ethanol was then put in the distillation flask. After 4 hours of refluxing, the ethanol was removed using a rotary evaporator operating at low pressure. By diluting lemon leaf extract in ethanol, five samples (5, 10, 15, 20, and 25 mg/ml) were created. Three copies of the extraction were made. The oil that was extracted had a 23% (w/w) yield. For future usage, the pure extract was kept in the dark at 4°C.

Determination of chemical composition by GC-MS

Shimadzu's GC-MS-QP2010 ultra with a capillary column (SLB-5MS: 30 mX0.25 mmX0.25 m) and FID detector was used to analyze essential oils using gas chromatography linked to mass spectrometry. The column temperature was increased from 45°C to 160°C at a rate of 5°C/min, then altered to 10°C/min to achieve 300°C, with the injector temperature being set at 200°C. An emission current setting of 10 A and an electron multiplier voltage setting of 1500 V were made for the mass spectrometer. With a total run duration of 40 minutes, the mass analysis was carried out in scan mode between 40 and 650 amu. We made duplicates of each determination.

Evaluation of the antifungal activity of Clove E.O and lemon leaf extract

After 15 ml of PDA media had been added to each Petri dish (9 cm in diameter), they were solidified, leaving a 2-cm-diameter hole in the center. The tested extract, at each of its varied concentrations, was added to each well in a milliliter. Four discs of one of the tested fungi's fungal growths were placed in each Petri dish before it was incubated at 28 °C for seven days. PDA medium was employed as a negative control, while miconazole nitrate 20 mg/ml was employed as a positive control. The effects were statistically examined after each therapy was applied three times. Inhibition zones surrounding each well were formed by determining the diameter from the well's center.

To evaluate the inhibition zone and inhibition (%), one ml of various amounts of clove oil and leaves extract (5, 10, 15, and 20 mg/ml) were added to each well.

The diameter of the inhibitory zones was calculated using Eq. 1 to determine the percentage of antifungal activity.

Inhibition (%)= (DC-DT)/DC * 100

DC is the measurement of the fungal mycelium's diameter in the control petri dish.

DT is the measurement of the diameter of the growing fungal mycelium in the petri dish that has been treated with clove oil or lemon leaf extract.

Identification of parasites

Parasites genera and species were identified using a microscopic examination and counting ectoparasites and comparing them between two species of birds (Zappia et al. 2023).

Aflatoxins are carcinogenic, mutagenic, teratogenic, and immunosuppressive to most animal species: the International Agency for Research on Cancer (IARC) has classified all four aflatoxins as group 1 carcinogens, with aflatoxin B1 being one of the most potent hepato-carcinogens known (Zappia et al. 2023).

Statistical Analysis

All collected data were put through the usual analysis of variance process. Using Mstat-C software, the values of LSD were calculated at the 5% level and used to compare the means in accordance with Snedecor and Cochran (1982).

RESULTS

Table 1 demonstrates the isolation of 6 species of 5 genera from 5 samples taken from a Sakaka, Saudi Arabia shop that sold pet birds.

Table 1: Number and percentages of fungal isolates from pet birds, according to the source of their isolation

Types of birds	Sources of isolates	Fungal species	Number of appearance of isolates*	Percentage of appearance of isolates %*	Means
Melopsittacus undulates	feathers	*Alternaria alternata*	7.66	4.67	57.5%
		A.niger	18.00	10.98	
		Cladosporium cladosporioides	18.33	11.18	
		P. digitatum	30.66	18.70	
		Rhizopus sp	19.66	11.99	
	feed	*P. digitatum*	17.00	10.37	20.9%
		Rhizopus sp	17.33	10.57	
	feces	*A.niger*	19.33	11.79	21.5%
		Rhizopus sp	16.00	9.76	
Serinus canaria	feather	*Alternaria alternata*	16.00	9.30	65.5%
		A.niger	20.00	11.63	
		A. versicolor	15.66	9.11	
		Cladosporium cladosporioides	18.33	10.66	
		P. digitatum	24.33	14.15	
		Rhizopus sp	18.33	10.66	
	feed	*Rhizopus sp*	17.33	10.08	10.1%
	feces	*A.niger*	17.33	10.08	24.4%
		Rhizopus sp	24.66	14.34	

*** = Average of three replications.**

Identification and isolation of fungi from pet birds

The study was started by isolating and identification of important fungi from *Melopsittacus undulates* and *Serinus canaria* which are one of dangerous diseases to humans.

ctgcggaggg atcattacaa gtgacccccg gctccggccg gggatgttca taaccctttgttgtccgact ctgttgcctc
cggggcgacc ctgccttttc acgggcgggg gccccgggtggacacatcaa aactcttgcg taactttgca gtctgagtaa
atttaattaa taaattaaaactttcaacaa cggatctctt ggttctggca tcgatgaaga acgcagcgaa atgcgataagtaatgtgaat
tgcagaattc agtgaatcat cgaatctttg aacgcacatt gcgccccctggtattccggg gggcatgcct gttcgagcgt catttcacca
ctcaagcctc gcttggtattgggcgacgcg gtccgccgcg cgcctcaaat cgaccggctg ggtcttctgt
cccctcagcgttgtggaaac tattcgctaa agggtgccac gggaggccac gccgaaaaac aaacccatttctaaggttga
cctcggatca ggtagggata c

Figure 1: ITS1-5.8S rRNA-ITS2 region of *Penicillium glabrum* sequence analysis

ctgcggaggg atcattacaa gtgacccccg gctccggccg gggatgttca taaccctttgttgtccgact ctgttgcctc
cggggcgacc ctgccttttc acgggcgggg gccccgggtggacacatcaa aactcttgcg taactttgca gtctgagtaa
atttaattaa taaattaaaactttcaacaa cggatctctt ggttctggca tcgatgaaga acgcagcgaa atgcgataagtaatgtgaat
tgcagaattc agtgaatcat cgaatctttg aacgcacatt gcgccccctggtattccggg gggcatgcct gttcgagcgt catttcacca
ctcaagcctc gcttggtattgggcgacgcg gtccgccgcg cgcctcaaat cgaccggctg ggtcttctgt
cccctcagcgttgtggaaac tattcgctaa agggtgccac gggaggccac gccgaaaaac aaacccatttctaaggttga
cctcggatca ggtagggata c

Figure 2: ITS1-5.8S rRNA-ITS2 region of Cladisporum cladosporioide sequence analysis

The most prevalent species' sequence showed 100% identity with Penicillium glabrum (Genbank accession number: MW672597.1) and was closely related to it (Fig 1), and *Cladosporium cladiosporioides* (GenBank accession number, MW672598.1) with 100% similarity (Fig 2).

Rhizopus solani, Aspergillus niger, Penicillium digitatum, Alternaria Alternata, and Cladosporium cladiosporioides were isolated from Melopsittacus undulates, and Rhizopus solani, Aspergillus niger, Aspergillus versicolor, Penicillium glabrum, and Alternaria Alternata were isolated from Serinus canaria. (Fig 3, 4, 5, 6, 7, and 8). The present study reported that Rhizopus sp was the most frequent fungus and represented 33.5% of the total count, followed by *A. niger* which was recovered from 4 samples matching 22.1% of the total count of fungal isolates. *Penicillium digitatum* represented 21.5% of the total count, while *Cladosporium cladiosporioides* gave 15.9% of the total count but with low occurrence in *M. undulates. Alternaria alternata* was found in 7% of the total fungi, Whereas *Aspergillus* species represented by *A. versicolor* had the lowest value obtained sharing 4.5 %.

Regarding the source of isolation, the feathers were the most contaminated with fungi, as the percentages of 57.5% and 65.5% in *M. undulates* and *S. canaria,* respectively, followed by feces which recorded 21.5 % in *M. undulates* and 24.4% in *S. canaria,* while the feed was 20.9 and 10.1% in *M. undulates* and *S. canaria,* respectively as clarified in Table1.

The fungal growth of *Rhizopus solani* on a PDA at 27 °C in the dark is shown in Fig 3 (1) using a normal compound microscope. Sporospores appear bearing spores containing spores (2-5). While figure 6 shows Zygospores with suspension. and pic. 7 showing spores by scanning electron microscopy.

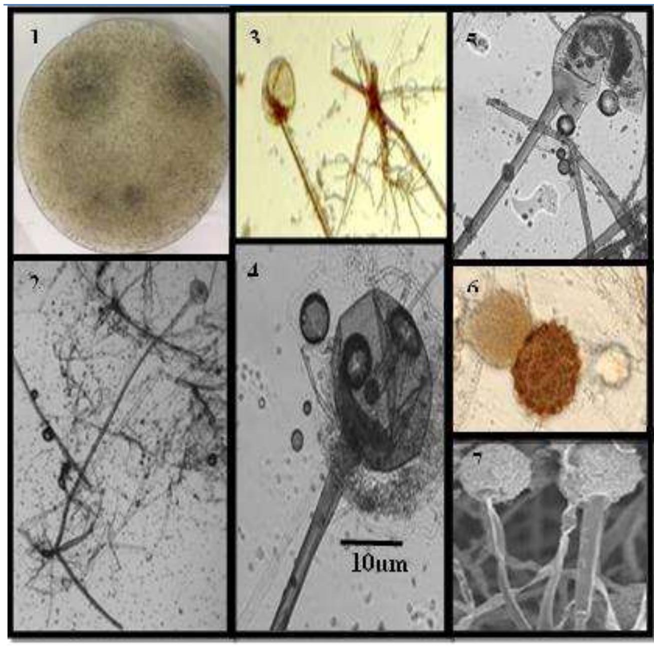

Figure 3: (1) Mycelial growth of *Rhizopus solani* on PDA at 27ºC in the dark. (2 – 5) were taken using normal compound microscope. Sporangospores carrying sporangia containing spores. Bar 10 µm in all pictures. (6) Zygospores with suspensors. 7 Scanning electron microscopy of sporangiospores.

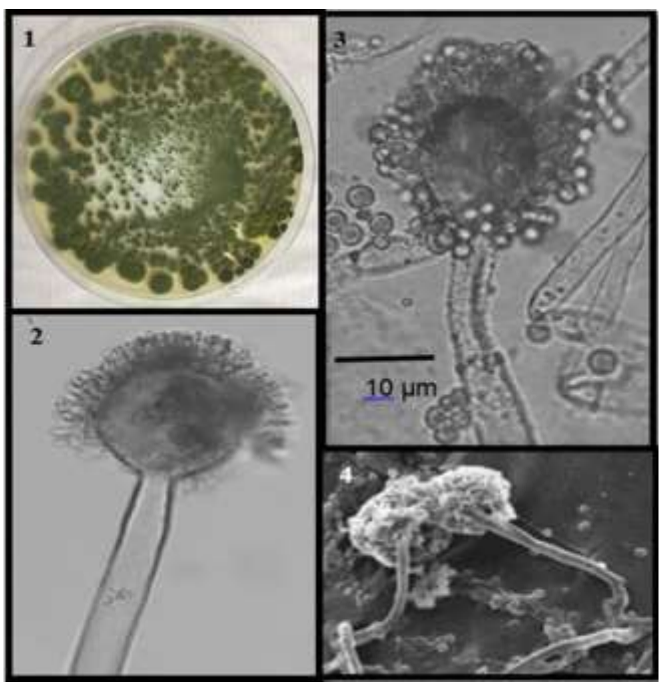

Figure 4: (1) Mycelial growth of *Aspergillus versicolor* PDA at 27ºC in the dark. (2 – 3) were taken using normal compound microscope. Aseptate conidiophores and sub globose heads with tepicaly biserriate sterigmata. Bar 10 µm in all pictures. (4) Scanning electron microscopy of *A. versicolor.*

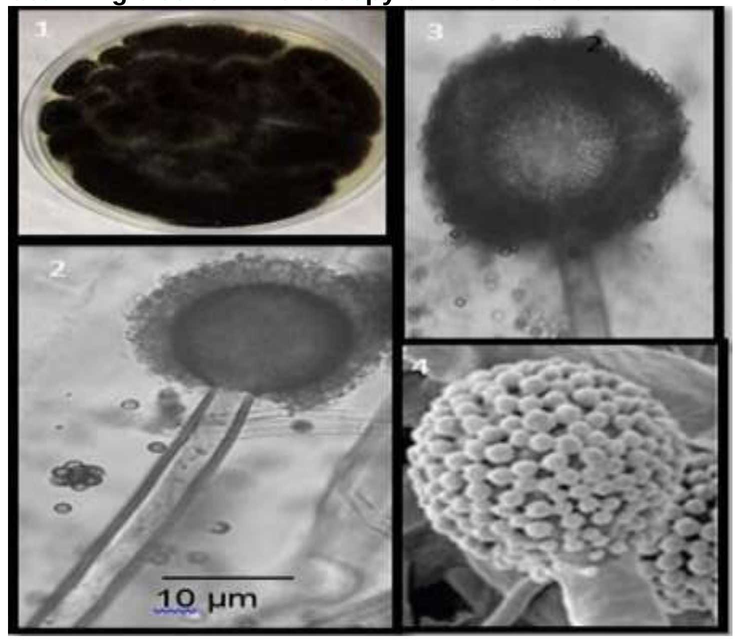

Figure 5: (1) Mycelial growth of *Aspergillus niger* on PDA at 27ºC in the dark. (2 – 3) were taken using normal compound microscope. Aseptate conidiophores and mostly radiate heads with mono and biserriate sterigmata mounted on globose shaped vesicles. Bar 10 µm in all pictures. (4) Scanning electron microscopy of *A. niger.*

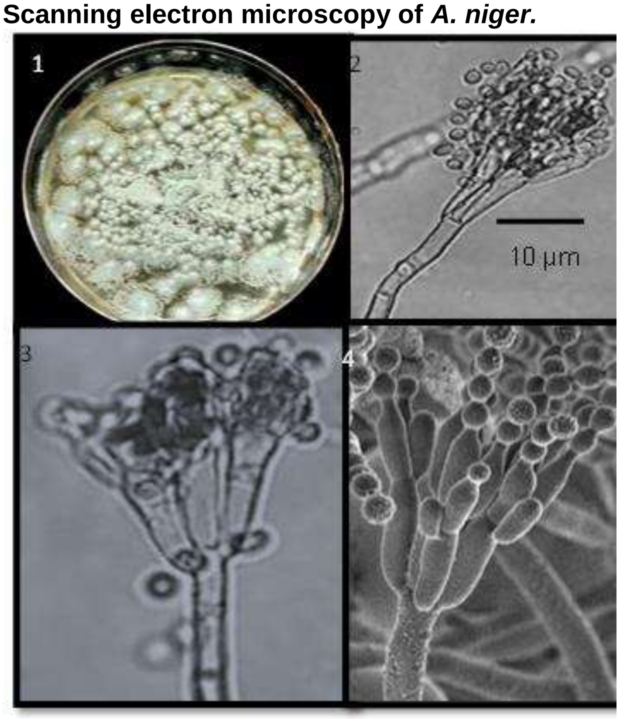

Figure 6: (1) Mycelial growth of *Penicillium digitatium* on PDA at 27ºC in the dark. (2 – 3) were taken using normal compound microscope. Septate conidiophores mounted biserriate sterigmata and Asymmetrical. Bar 10 µm in the pictures (2) are the

same of picture No. 3. (4) Scanning electron microscopy of *Penicillium digitatium*.

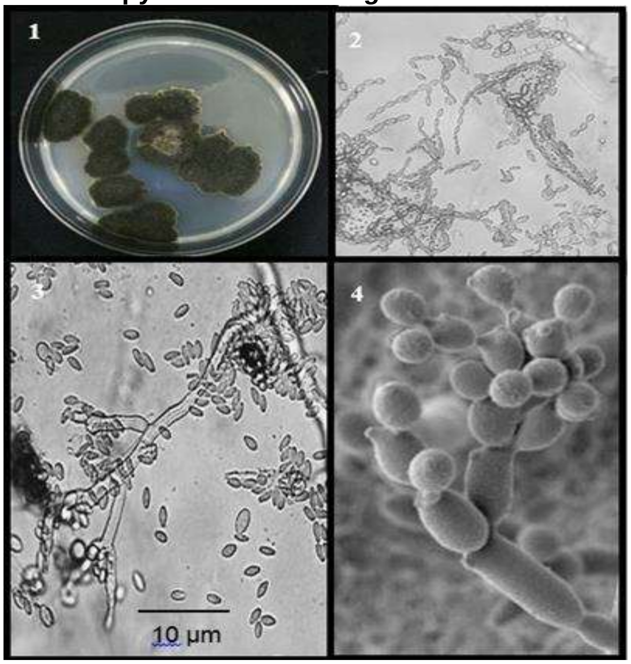

Figure 7: (1) Mycelial growth of *Cladosporium cladosporioides* on PDA at 27ºC in the dark, (2-3) were taken using a normal compound microscope. Unbranched conidiophores with branched chains of conidia are "lemon-shaped". Bar 10 µm in all pictures. (4) Scanning electron microscopy of *Cladosporium cladosporioides.*

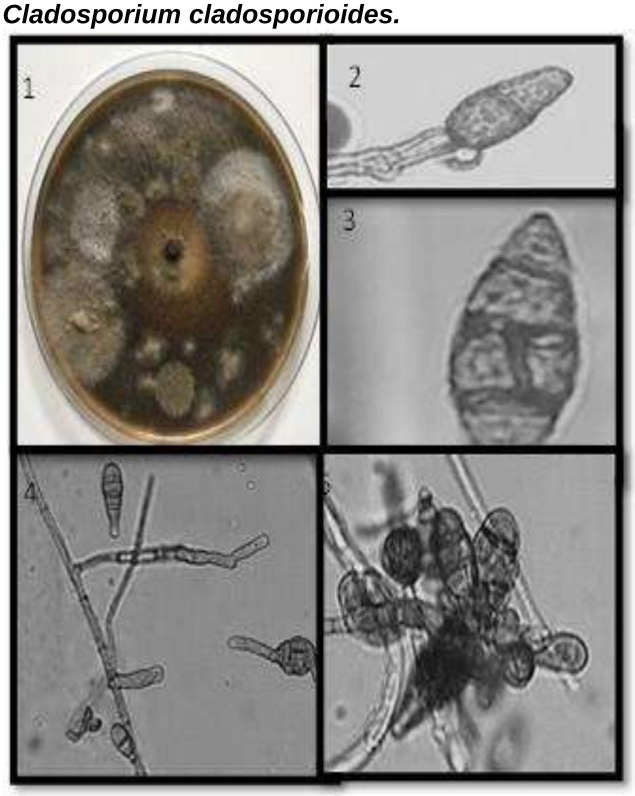

Figure 8: (1) Mycelial growth of Alternaria alternate on PDA at 27ºC in the dark. (2 – 3) were taken using normal compound microscope. Conidiophore carrying ellipsoid Conidia, formed in branched, long chain conidia. Bar 10 µm in all Figs.

The impact of various doses of lemon leaf extract and clove oil on the development of an isolated fungus' mycelium

The antifungal activity of each of the studied species was assessed using their capacity to suppress fungal growth. The greatest biological activity of all treatments was indicated by clove essential oil, which completely inhibited the growth of the examined species. As clove essential oil was used to treat all fungal isolates, as indicated in Table 2, the biological activity increased according to the increase in concentration.

From the previous table, it is clear that Clove oil inhibits the growth of all fungal species that are isolated from domestic birds, and the ability to inhibit growth increases with an increase in the oil concentration up to 25 mg/ml. The highest inhibition of growth in the fungus *Alternaria lternate* was 62.7 mm, and the lowest inhibition of growth was for the fungus *Cladosporium cladosporium* 7.7 mm at an oil concentration of 5 ml/mg. As for the lemon leaves extract, the low concentrations of the extract (5 and 10 mg/ml) did not give any effect on the growth of any of the isolated fungi, while the effect was weak at the concentration of 15 mg/ml, and the growth inhibition effect increased with an increase in the concentration up to 25 mg/ml. The highest inhibition for the fungus *Rhizopus solani* was 42.3 mm when it was treated with a concentration of 20 mg/mm, and the least inhibition was for the two fungi *Cladosporium Cladosporium* and *A. niger* with an area of 10.7 and 12.3mm, respectively.

As an average of the effect of lemon leaf extract concentrations on different fungi, *Rhizopus solani* was the most affected (19.1 mm), followed by *P. digitatium* and *A.versicolor* with an area of 15.7 and 15.6mm, respectively.

Table 2: Zone of growth inhibition (mm) as regards the clove oil and lemon leaves concentration.

Fungal species	Clove oil					Means	Lemon leaves extract					Means
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml		5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml	
Alternaria lternate	11.0*	20.0	46.0	62.7**	62.3**	40.4	0	0	12.5	27.0	31.3	14.1
A.versicolor	11.0*	22.0	46.3	54.7	55.0	37.8	0	0	16.0**	29.0	33.0*	15.6
A. niger	11.3	23.0*	45.0	55.0	59.7*	38.8	0	0	12.3	27.7	22.7	12.5
Cladosporium cladosporium	7.7	21.3	46.7*	52.3	53.0	36.2	0	0	10.7	27.3	25.7	12.7
P. digetatium	10.3	23.3**	47.0**	58.0	56.3	39.0	0	0	14.0*	33.7	30.7	15.7
Rhizopus solani	11.7**	22.3**	45.3	62.3**	50.0	38.3	0	0	12.7	42.3**	40.7**	19.1
Mean	10.5	22.0	46.1	57.5	57.3		0.0	0.0	13.0*	29.4*	29.4	

Diameter of inhibition zone (mm) = Average zone of growth inhibition diameter± standard deviation (n=3)

Results appeared that all six tested fungi were inhibited by using Cloves oil at all concentrations while, the Lemon leaves extract at 15, 20, and 25 mg/ml. On the other hand, 5 mg/ml did not appear any effect on the growth and sporulation of the tested fungi. Clove oil (20 mg/ml) recorded the highest antifungal effect than lemon leaf extract (20 mg/ml). The measuring of Inhibition zones was about 60.7 mm (in diameter) in Clove oil but in lemon leaf extract was 40.7 mm, as shown in (Table 2, Fig 9 and 10).

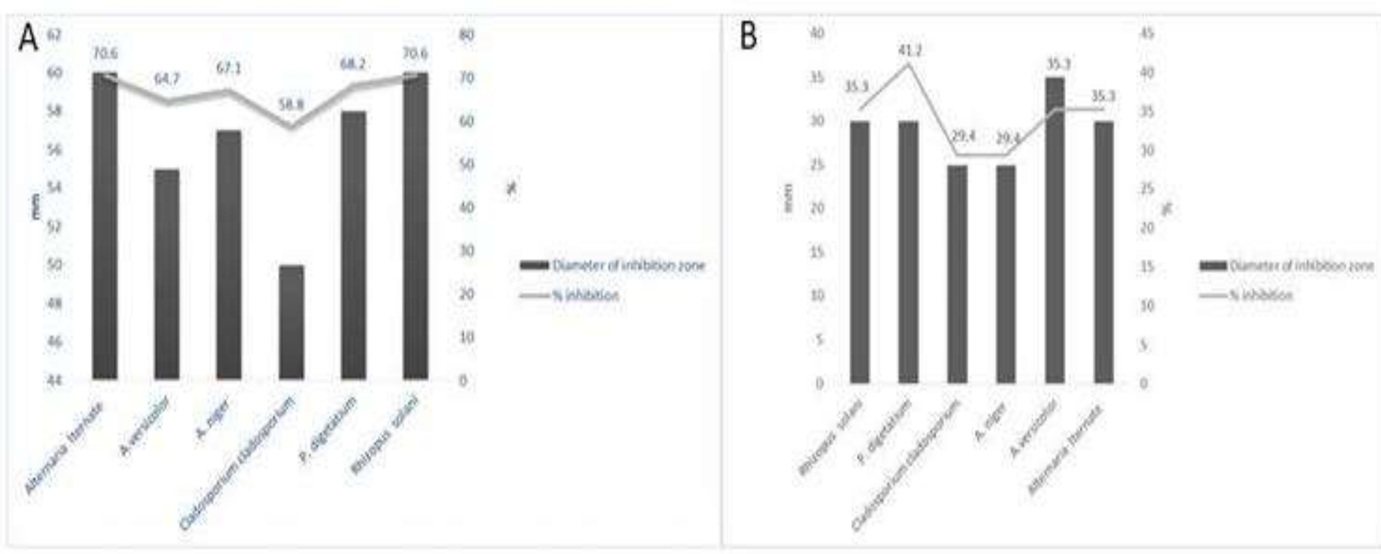

Figure 9: (A) Diameter of mycelial growth (DCM) of tested fungi and inhibition (%) presented by 20mg/ml of Clove oil. (B) mycelial growth (DCM) of tested fungi and inhibition (%) presented by 20mg/ml of lemon leaves extract.

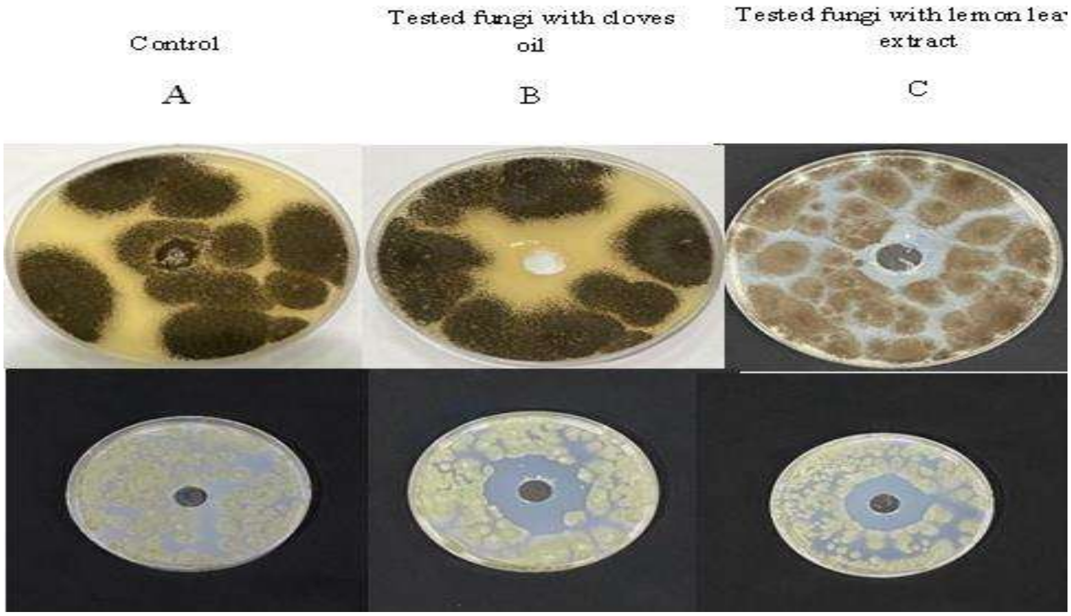

Figure 10: Effect of cloves oil and lemon leaves extract on growth of isolated fungi cultivated in PDA medium after 6 days at 28˚C in the dark. Inhibition zones represent the influence. (A) well embedded with ethanol (control), (B) well embedded with lemon leaf extract (20mg/ml), before culturing. (C) Well-embedded with clove oil (20mg/ml), before culturing.

Determination of the chemical composition of clove E.O by GC-MS

The identification and quantification of the vegetable oil ingredients were examined using the essential oil extracted from cloves using GC-MS technique. By comparing the retention time with that for typical compounds mentioned in the literature, 40 components were found. The composition of the essential oil and its percentage are shown in the table. 3.

Table 3: Chemical composition of clove E.O

No.	Rt (min)[a]	Components	Percentage %
1	4.983	Furfural	0.08
2	5.263	Maleic anhydride	0.08
3	6.974	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.04
4	8.794	1,6-Octadien-3-ol, 3,7-dimethyl-	0.06
5	9.773	Acetic acid, phenylmethyl ester	0.10
6	10.27	Methyl salicylate	0.14
7	11.059	2H-1-Benzopyran, 7-methoxy-2,2-dimethyl-	0.11
8	11.389	Phenol, 4-(2-propenyl)-	0.66
9	11.669	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	0.52
10	12.986	Phenol, 2-methoxy-4-(2-propenyl)-	71.05
11	13.832	Caryophyllene	14.73
12	13.96	1,2,3-Benzenetriol	0.17
13	14.195	Phenol, 2-methoxy-4-(1-propenyl)-	0.30
14	14.309	.alpha.-Caryophyllene	2.92
15	14.863	.alpha.-Farnesene	0.44
16	15.092	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	0.10
17	15.226	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	3.90
18	15.366	Homovanillyl alcohol	0.37
19	15.506	.alpha.-Calacorene	0.05
20	15.69	1,Z-5,E-7-Dodecatriene	0.26

21	15.957	Caryophyllenyl alcohol	0.10
22	16.129	Caryophyllene oxide	0.90
23	16.453	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	0.20
24	16.74	Trans-Z-.alpha.-Bisabolene epoxide	0.40
25	16.803	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol	0.49
26	16.988	.alpha.-Cadinol	0.04
27	17.261	Cyclododecane	0.25
28	17.389	2',3',4' Trimethoxyacetophenone	0.07
29	17.936	Benzeneacetic acid, .alpha.-hydroxy-4-methoxy-, methyl ester	0.15
30	18.267	Benzyl Benzoate	0.22
31	18.33	Ethyl-.beta.-(4-hydroxy-3-methoxy-phenyl)-propionate	0.09
32	18.877	Isoaromadendrene epoxide	0.27
33	19.431	Benzoic acid, 2-hydroxy-, phenylmethyl ester	0.12
34	20.544	Octadecanoic acid	0.05
35	21.543	9,12-Octadecadienoic acid, methyl ester	0.07
36	22.669	6-Octen-1-ol, 3,7-dimethyl-, acetate	0.08
37	23.413	Farnesol (E), methyl ether	0.09
38	25.265	1-Benzothiepin, 2,3,4,5-tetrahydro-	0.21
39	25.634	3-Allyl-6-methoxyphenol	0.05
40	26.474	Eicosane	0.06

[a]Rt: retention time (min)

Table 4: Number and percentages of Parasites isolate from pet birds, according to the source of their isolation.

Types of Birds	Sources of isolates	Parasites species	Number of appearances of isolates*	Percentage of appearance of isolates %
Melopsittacus undulates	feather	*Cnemidocoptes gallinae*	20.00	97.5%
		C mutans family Sarcopiidae	18.00	85%
	feed	*Isospora serinuse n. sp.*	15	90%
	feces	*Isospora serinuse n. sp* oocyst	10	80%
Serinus canaria	feather	*Cnemidocoptes gallinae*	16.00	70%
		C mutans family Sarcopiidae	20.00	97.5%
	feed	*Isospora serinuse n. sp.*	12.2	80.6%
	feces	*Isospora serinuse n. sp* oocyst	11.5	70.5%

Clove essential oil contains 40 compounds, ranging from 0.05 to 71%, as shown by the chemical composition analysis. The essential oil is mainly composed of eugenol with a content of 70%, followed by caryophyllene at 14.73%, eugenyl acetate 3.9% and alpha caryophyllene 2.92%.

Identification of parasites

Results appeared that *Cnemidocoptes gallinae* and *C mutans* family *Sarcopiidae* recorded 97.5%, and 85% respectively in *Melopsittacus undulates.* While in *Serinus canaria* they recorded 70% and 97.5% respectively. On the other hand, *Isospora serinuse* n. sp. recorded 90% in *Melopsittacus undulates* while in feces *Isospora serinuse* n. sp oocyst recorded 80% compared with *Serinus canaria* which recorded 70.5%, while by feeding recorded 80.6% *Isospora serinuse* n. sp.

DISCUSSION

The results of this study demonstrate *Penicillium glabrum* was the most frequent fungus (79 out of 5 samples) and represented 39.5% of the total count, followed by *Cladosporium cladosporioides* that was recovered from 4 samples matching 30% of the total count of fungal isolates. *Aspergillus* species were represented by *A. niger* and *A. versicolor* sharing 1.5 % and 3%, respectively. *Rhizopus solani* represented 21%

of the total count, while *Alternaria alternate* gave 16% of the total count but with low occurrence in *M. undulates*. There are several studies conducted to investigate fungi in the feces of some birds, 177 fungal isolates have been found, including *Candida spp* (35.02%), and *Rhodotorula spp*. (15.81%), and filamentous genera are *Aspergillus* (28.24%), Mucor (14.68%), and *Penicillium* (6.21%). In another study conducted to find out the extent of contamination of bird food with fungi, it was found that the dominance was *Aspergillus*, followed by *Alternaria*, then *Fusarium,* and *Penicillium* respectively (Josiara et al*.* 2014). Moreover, Arenas-Castro et al*.* (2016) isolated *Aspergillus* and *Penicillium* from feathers, the appearance of *Aspergillus* was more than *Penicillium*. Similarly, in a study on fungal species isolated from birds in Spain, *Penicillium sp* was isolated 21 (48.84%), followed by *Aspergillus sp* 13 (30.23%), then *Alternaria sp* appeared 4 (9.3%). Finally, *Mucor sp* is only twice (4.65%).

Whether it is a house area or shops, pet birds were everywhere. In the past, controlling the disease of pet birds with pathogenic fungi was done by using synthetic fungicides. However, all such synthetic fungicides are highly toxic and present for longer periods in the form of residues and sometimes kill non- target organisms. So, it must be banned, and its new alternatives are discovered in the form of natural fungicides. The phenolic hydroxyl groups in clove essential oil have significant antimicrobial activity (Natália, et al. 2020; Ramsdam, et al. 2021; Tian et al. 2022) due to the influence of the biophysical and biological properties of the main constituents of the essential oils (Zorzi-Tamazoni et al*.* 2018). However, a significant amount of eugenol (70%) is linked to the biological activity of clove essential oil. Vinay, et al. (2022) demonstrated that phenolic compounds, such as eugenol, can damage proteins and interact with phospholipids in the cell membrane, changing the permeability of the membrane. This is due to the acidic nature of the hydroxyl group, which forms a hydrogen bond with the active enzyme core. Clove essential oil had the highest biological activity in the current study's treatments, completely inhibiting the growth of the evaluated species. Hee and Min, 2007 showed that vapor volatilization from clove essential oil is a potent antifungal agent against the majority of human pathogenic fungi. They found that the biological activity was proportional to the increase in concentration against all fungal isolates. As an antifungal agent, volatile essential oils may therefore have a wide range of applications, including the treatment of athlete's foot. Clove oil was shown to be an effective inhibitor against *Collelotrichum gloeosporioides* when tested in vitro and in vivo using various treatments (Wang et al*.* 2019). Its toxic effect on fungi may be caused by its destruction of the cell membrane and wall as well as the leakage of intracellular components. Lower EC50 and MIC values showed that EO would be more effective in inhibiting fungal infection when used as a contact gas. All of the gram-positive and gram-negative bacteria linked to dental caries were suppressed by clove oil and clove extract, which both exhibited broad-range antibacterial action. Clove oil, as opposed to clove extract, produced a zone of maximal inhibition diameter (IZD) against the major dental plaque-causing bacteria. According to Gupta and Dhan (2021), clove oil had a greater inhibitory zone diameter (IZD) than clove extract did against the tested fungus species. In this study, the results showed that the six tested fungi were inhibited using clove oil and lemon leaf extract at 5, 10, 15, 20, and 25 mg/ml, but at 5 and 10 mg/ml, no effect was shown on the growth and spores of the tested fungi. Clove oil (20 mg/ml) recorded the highest antifungal effect than lemongrass leaf extract (20 mg/ml). The zones of inhibition measured approximately 60.7 mm (in diameter) in clove oil but in lemon leaf extract was 40.7 mm. However, the evaluation and efficacy of botanical products for use as antiseptics need future research efforts so that they can be converted into a viable form for daily use in sterilization and disinfection.

More research is needed to determine if these products are safe and beneficial for people with fungal diseases either alone or in combination with traditional treatments that can help reduce fungal infections worldwide. YANG, et al (2023) found that *Isospora serinuse* n. sp. from a domestic pigeon (*Columba livia domestica*) in Japan showed 97.5% similarity to *Isospora* sp. Tokyo. The Australian red wattlebird (*Anthochaera carunculata*) and *I*. *serinuse* n. sp. shared 94.9% of the same genes at the 28S locus. We concur with (Schrenzel et al*.* 2005; Berto et al. 2011; and Schoener et al. 2013) that passerine birds have been used to characterize a variety of *Isospora* species across the globe. The passerine coccidia is considered the most genetically related species to Eimeria, these parasites, from fecal oocysts (Schoener et al*.* 2013). Infection with mits is one of the commonly encountered parasite infestations in pet and wild birds causing itching and discomfort and, on several occasions, associated with a secondary bacterial infection. The present study describes the degree of mite infestation in a budgerigar bird *(Melopsittacus undulatus)* presented with clinical signs of pruritus, loss of feathers, scales, and crust on eyes, leg, and feet region, overgrowth of the beak, decreased appetite the percentage was 97.5%,85%. Microscopic examination of alkali-digested deep and superficial skin scraping revealed severe infestations with *Cnemidocoptes* spp. Our results also agree with (Sam et al. 2014), the deep skin scrapping from the same sites revealed an absence of mites.

These birds are more susceptible to *Cnemidocoptes* species which are transmitted between individuals through either direct or indirect contact (Maria et al*.* 2014). The mite *Cnemidocoptes gallinae* and *C mutants* of the family *Sarcopiidae* is widely prevalent among domestic and wild birds in India.

The identifying features of these mites include a globose body, interrupted stance to form scales, absence of spines on the dorsal surface (Rajubudeli et al*.* 2008), and two longitudinal criticized bars running from the bases of the pedipalps to the level of the legs, where they are united by a transverse bar (Soulsby, 1982).

In Mansoura Public Park, Egypt, scabies caused by *Knemidocoptes pile* (K. pile) was characterized in a caged parrot (Melopsittacus undulatus) where a study was conducted to detect the causative parasite (*Knemidocoptes pile*) by documenting the clinical findings, skin scrapings and histopathological changes in the areas infected. Where 11.5% of the sample (19 individuals out of 165) showed clear lesions of progressive dermatitis, a proliferation of scaly epithelium of different degrees, thick yellow scales, and loss of feather areas. Because of feeding difficulties and loss of appetite, most cases showed a decrease in body weight. Lameness was observed in advanced chronic cases when the feet and face were affected, and sometimes blindness was a result of crusting on the face and leg. The clinical disease of K. piles infection disappeared 7-20 days after a single subcutaneous injection of 0.02-0.1 mL of ivermectin (Abou-Alsoud and Karrouf, 2016). Early diagnosis management of mite infestation complications aids and is essential to restoring normal health to infected birds (Sam et al. 2014).

The irritating itch is caused by *Cnemidacoptes gallinae* by penetrating the skin next to the feathers of birds and causing an itchy inflammatory condition (Saif et al*.* 2003). Feathers come loose easily and are plucked out by birds (Urquhart et al*.* 1996). The lesions appear predominantly on the back and wings and rarely on the head and neck.

CONCLUSIONS

According to the study's findings, pet birds may serve as a reservoir for environmental fungal spores that can infect humans, and clove essential oil has proven to be an effective antifungal agent against several pathogenic fungi. Therefore, employing clove essential oil as an antifungal agent may have a variety of therapeutic applications, including aromatherapy to stop the spread of fungal spores. On the other hand, the outcomes mentioned above, utilizing the clove oil concentration (20 mg/ml), exhibit effective antifungal effects. Toxicology, improved formulations, and the choice of the best doses for use in clinical settings will all require additional investigation. The use of essential oils in the emulsion system is also advised as a promising way to extend and enhance their antifungal action.

Supplementary materials

Not applicable.

Author contributions

Conceptualization, Shaima M. N. Moustafa and Hallouma Bilel; methodology, Shaima M. N. Moustafa, Hallouma Bilel; Rania H. Taha, Hanan Taher, Bashayr Faris Alsabilah, Yasser M. Ahmed. software, Shaima M. N. Moustafa and Hallouma Bilel.; validation, Shaima M. N. Moustafa and Hallouma Bilel.; formal analysis, Shaima M. N. Moustafa and Hallouma Bilel; investigation, Shaima M. N. Moustafa and Hallouma Bilel.; resources, Shaima M. N. Moustafa and Hallouma Bilel; data curation, Shaima M. N. Moustafa and Hallouma Bilel.; writing-original draft preparation, Shaima M. N. Moustafa, Yasser M. Ahmed and Hallouma Bilel; Rania H. Taha, Hanan Taher, Bashayr Faris Alsabilah, Yasser M. Ahmed. writing-review and editing, Shaima M. N. Moustafa, Yasser M. Ahmed and Hallouma Bilel; Rania H. Taha, Hanan Taher, Bashayr Faris Alsabilah, Yasser M. Ahmed. visualization, Shaima M. N. Moustafa, Yasser M. Ahmed and Hallouma Bilel; Rania H. Taha, Hanan Taher, Bashayr Faris Alsabilah, Yasser M. Ahmed. project administration, Shaima M. N. Moustafa.; funding acquisition, Non. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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