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Occurrence of Fungi in Household Water Tanks in Al-Hassa, Saudi Arabia

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Some of fungi can live in scarce food and called oligotrophes. Water of household tanks provides a perfect environment especially those reservoirs that are not cleaned regularly. In Al-Hassa, Saudi Arabia, the water enter from the municipal to the tanks in every home-based system. There are two tanks in every home, the first one is inside the house on the ground floor, and the second is on the top surface of the building. A motor lifting water from the lower reservoir to the upper reservoir, which in turn distributes the water to each water inlet. In Saudi Arabia, there is no enough studies concerning fungi found in household tank water. This research aims to Shed light on the existence of a certain types of fungi in household water tanks in the city of Hofuf, Al-Hassa, Saudi Arabia. Filamentous fungi were isolated from 15 of 15 (100%) samples, yeasts from 14 (93%) and both from 14 (93%) water samples. The mean number of colony-forming units of filamentous fungi was 20-57 and of yeasts 7-16. Prevailing species were *Candida parapsilosis* and *Candida guilliermondii* each isolated from 12 samples (80%), *Phialophora verrucosa* from 11 samples (73.3%), each of *Aspergillus niger*, *Candida tropicalis* and *Rhodotorula mucilaginosa from 10 samples (66.7%), Penicillium chrysogenum* from 8 samples (53.3%), *Sterile mycelium A from 7 samples (46.7%),* each of *Cladosporium cladosporioides* and *Penicillium citrinum* from 6 samples (40%), each of *Mucor mucedo*, *Mucor mucedo* and *Penicillium nigricans* from 5 samples (33.3%), *Acremonium strictum* from 4 samples (26.7%), each of *Acremonium alabamensis*, *Alternaria alternate*, *Stachybotrys chartarum and Sterile mycelium B* from 3 samples (20%), and *Sterile mycelium C* from 2 samples (13.3%).

Key words: Household water tanks, Filamentous fungi, Yeasts, Al-Hassa, Saudi Arabia.

Fungi have true-nucleous microorganisms, facultative parasitic organisms, including both single-celled yeasts and multi-cellular filamentous fungi[1]. Many of these species can live in media of scarce nutrients (oligotrophic). They can establish their selves in water by catching nutrients from the surrounding air or the diluted materials which may be found in the water. These organisms produce secondary metabolites, some of which are toxins. Some of the fungal species and their metabolites might be considered as human pathogens or allergens[13].

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Water house tanks can be infested by fungi via many ways such as treatment breakthrough, bad in stored water facilities, leak in pipes and deficiency of good maintenance. Upon entering, these fungi spread and form a film (Biofilm) on the inner surfaces of pipes and tanks and then spread to the whole water.

Previous studies show more fungi in sample analysis of water after treatment and silted in tanks than prior to distribution to hoses. Increases of fungi through the distribution system could be due to two reasons: i) fungal spores multiply within the system or the active fungi became activated later and recover, and ii) infestation by fungi was done after entering tanks. addition of fungi in tank water at the user end, has also been shown[12]. There is no enough studies concerning fungi found in household tank water. Fungi found in the reviewing studies range from 1 CFU per litre to 5000 CFU per litre. Most commonly isolated genera in previous studies were *Penicillium*, *Cladosporium*, *Aspergillus*, *Phialophora* and *Acremonium*[6,7].

There are many factors affect occurrence of fungal taxa in household tank water. Amounts of organic matter increases the number of fungal units. Differences in acidity and calcium content may also account for some of the variation. Cold water support more fungal units than hot water, although this depends on the species considered and their optimum temperature range.

Many of the fungi that have been previously isolated from household tank water are known to be pathogenic, particularly *Aspergillus* and *some* Deuteromycota. Although healthy individuals may partially suffer from fungal infections present in household tank water. Immune deficiency persons are subject to fungal diseases for example HIV/AIDS, chemotherapy, immunosuppressive therapy or diabetes mellitus[3].

Some fungi known to accelerate of allergic disease, asthma, hypersensitivity pneumonitis and skin irritation. Fungi of *Alternaria* spp., *Aspergillus*, spp., *Cladosporium* spp. and *Penicillium* spp., have been previously isolated from drinking water, known to stimulate allergic responses in susceptible persons[3].

Fungi, especially, some species of *Penicillium*, *Aspergillus* and *Fusariam* are famous to produce mycotoxins. Concentrations of these mycotoxins in household tank water are low due to being diluted. Presence of such fungi in household tank water is a risk factor, however, no documented researches were available concerning this case[6,7,12,13].

Because of deficiency of information concerning housewater tanks in Saudi Arabia, there are many points that remain weakly understood. More research is needed to determine the quality of drinking water as a source of fungal diseases. The implications of fungi in drinking water in house tanks in Al-Hassa, Saudi Arabia were done.

Usually, the household water is not used in home drinking but used in bathing and washing purposes, kitchenware washing, ablution and others. This water represent an important source of potentially hazardous especially with daily use and the cascade of those waters and friction flog rights and the possibility of entering into stomach and respiratory channel of persons is high.

The Objective of this research was to throw the light on the occurrence of fungi in household water in tanks of 15 houses distributed in Al-Hassa, Saudi Arabia.

MATERIALS AND METHODS

Sampling places

Water samples were collected from 15 water tanks from the city of Hofuf in Al-Hassa, Saudi Arabia during March, 2013, as in the following table:

No. of sample	Site quality	Reservoir quality	Reservoir age
1	Residential home	Ceramic + cement	15 years
2	Residential home	Ceramic + cement	18 years
3	Farm	Cement	10 years
4	Farm	Cement	10 years
5	Residential home	Ceramic + cement	22 years
6	Residential home	Ceramic + cement	8 years
7	Farm	Cement	10 years
8	Residential home	Ceramic + cement	20 years
9	Residential home	Cement	4 years
10	Residential home	Cement	5 years
11	Farm	Cement	25 years
12	Residential home	Cement	6 years
13	Ain Alhewerat area	Cement	unknown
14	Ain Alhara area	Cement	unknown
15	Ain Sewedera area	Cement	unknown

Chemical analysis of the samples

Preparation of samples / standard solution and calibration curve

Fifteen samples were collected from places mentioned in the previous indicated table. All samples were filtered through Whatman filter paper no 1. Then the pH and conductivity of water samples were measured directly. Four different concentration standards were prepared with rang of linearity (average to each element) with a volume of 50 ml. The emission was measured for potassium sodium and calcium standards using flame photometer (Jenway, model PFP7, England) then the emission was plotted against the concentrations in excel to get the calibration curve. Samples were diluted in different manner. While for Cu, Zn, Fe, Co, Al and Mg, the absorbance was measured using atomic absorption (spectrometer: iCE 3000 C113300264 v1.30 from thermo). Their calibration curves were obtained by plotting standards absorbance against the concentrations in excel. For analysis of these elements samples were pr-concentrated 10 times by tacking 100 ml of sample and genteelly evaporated on hotplate to reduce volume to 10 ml. One ml of concentrated nitric acid was added to each sample for digestion process. However for analysis of Mg all samples were diluted 100 times except No 8 and 9 which were diluted five times. The concentration of the elements in each sample was calculated using the equation from its calibration curve (World Health Organization (WHO) Guidelines for Drinking Water Quality, 1-2, 1983.

Isolation procedures

Fungal occurrence was isolated from 15 water samples of household water tanks. Water was collected from a depth of 30 cm beside walls of each tank in 330 ml bottle of commercial mineral water after it was opened immediately and get rid of the water next to the reservoir and then filled it directly[9].

At the laboratory, 30 ml tank water was poured in sterilized glass Petri-dishes. Gloves and long sleeves were worn when collecting the samples to prevent skin microorganisms from contaminating the samples. The membrane filter technique was used for isolation of yeasts and fungi (American Public Health Association 1995). A hundred ml of each water sample was passed through 0·45 mm diameter membrane filters. Membranes were then placed on Sabouraud's dextrose agar (SDA) supplemented with chloramphenicol and on SDA with a chloramphenicol-actidione mixture. Plates were incubated at 28ºC for 3–4 weeks and examined every day. The mixed cultures were purified in SDA for identification and preservation. For the identification of yeasts, Physiological tests (germ-tube formation), biochemical characteristics, including carbohydrate assimilation (API 20C AUX; Bio Merieux SA, Lyon, France), and morphology (Dalmau plate technique on cornmeal-Tween 80 agar) were applied. Fungal identification was done with the aid of macroscopic and microscopic characters using different synthetic and semi synthetic culture media[2].

Statistical analysis

Treatment means were separated using Waller-Duncan *K*-ratio t-test (with some modification) when significant differences of each of ($P \leq 0.05$; $P \leq 0.01$; $P \leq 0.001$) were detected[14]. All of the experiments were repeated twice and data of one of set were subjected to the above mentioned statistics.

RESULTS

Physical and chemical properties of the samples

The main physical and chemical properties of the drinking water samples including pH, electrical conductivity, potassium, sodium, calcium, zinc and magnesium from water tanks of Hofuf, Al-Hassa, Saudi Arabia were given in Table 2. The pH values were in the range of 7.53-8.11 (lowest in sample No. 5, highest in the sample No. 9). The ranges for electrical conductivity were 0.128 to 2.695 ìs/cm. Potassium, sodium, calcium, zinc and magnesium in the investigated water samples were found in the range of 1-46.2 mg/l, 16-566 mg/l, 14.3-325.5 mg/l, ≤1.0-132.96 mg/l and 3.6-140.6 mg/1, respectively (Table 2). Results were compared with a sample of mineral water sold in the market (control).

Absorbance of all samples are less than the absorbance of the lower standard, so and after pre-concentration ten times the concentration of cupper in all samples are less than 10 ppb and cobalt in all samples are less than 5 ppb and the concentration of Al in all samples are less than 1 ppm.

Filamentous fungi were isolated from 15

of 15 (100%) samples, yeasts from 14 (93%) and both from 14 (93%) water samples. Mean fungal densities for each of the 15 samples are shown in Table 3. The mean number of colony-forming units of filamentous fungi was 20-57 and of yeasts 7-16. Yeast densities were obviously lower than those for filamentous fungi. Water of sites 5, 13, 14 and 15 showed higher densities while sites 8 and 9 showed less intensity of both filamentous fungi and yeasts.

Table 2. Mean values of physical and chemical properties of the household water samples from Hofuf, Al-Hassa, Saudi Arabia

Sample No.	pH	EC (μs/cm)	K (ppm)	Na (ppm)	Ca (ppm)	Zn (ppb)	Mg (ppm)
Mineral water sold in the market (as a control)	7.2	N.D.	1.34	33.3	7.15	0	2.7
1	7.75	1.087	21.8***	258***	131.5***	21.426***	71.2***
2	7.69	1.50	22.2***	232***	292.3***	21.426***	135.0***
3	7.80	1.289	22.8***	263***	135.1***	9.686**	73.3***
4	7.89	1.067	24.7***	309***	129.9***	3.816**	78.9***
5	7.53	1.52	22.3***	242***	325.5***	132.96***	140.6***
6	7.69	1.543	22.5***	201***	160.0***	36.102***	88.4***
7	7.79	1.168	22.4***	258***	78.5***	3.816**	49.6***
8	8.03	0.128	1	16	14.3**	< 1.0	03.6**
9	8.11	0.162	1	20	14.8**	12.621***	05.0**
10	7.87	1.316	23.4***	247***	94.1***	15.556***	55.6***
11	7.88	1.971	36.5***	422***	147.0***	< 1.0	81.6***
12	7.77	1.732	22.9***	191***	161.0***	18.491***	88.6***
13	7.92	2.695	46.2***	566***	171.9***	< 1.0	85.9***
14	7.95	2.037	28.7***	319***	111.7***	< 1.0	66.7***
15	7.95	1.324	32.3***	242***	81.7***	< 1.0	41.3***

[c] Means in a column followed by : *= significant at p < 0.05, ** = highly significant at p < 0.01, *** = very highly significant at p < 0.001, according to Waller-Duncan K-ratio *t*-test (with some modification).

Table 3. Fungal biota and mean densities of household water tanks of Hofuf, Al-Hassa, Saudi Arabia

Site	CFU cm^{-2}	
	Filamentous Fungi	Yeasts
1	29	14
2	28	14
3	31	14
4	28	13
5	57	14
6	29	12
7	30	13
8	20	7
9	21	7
10	32	14
11	28	7
12	33	10
13	56	15
14	55	16
15	57	16

Prevailing species were *Candida parapsilosis* and *Candida guilliermondii* each isolated from 12 samples (80%), *Phialophora verrucosa* from 11 samples (73.3%), each of *Aspergillus niger*, *Candida tropicalis* and *Rhodotorula mucilaginosa from 10 samples (66.7%)*, *Penicillium chrysogenum* from 8 samples (53.3%), *Sterile mycelium A from 7 samples (46.7%)*, each of *Cladosporium cladosporioides* and *Penicillium citrinum* from 6 samples (40%), each of *Mucor mucedo*, *Mucor mucedo* and *Penicillium nigricans* from 5 samples (33.3%), *Acremonium strictum* from 4 samples (26.7%), each of *Acremonium alabamensis*, *Alternaria alternate*, *Stachybotrys chartarum and Sterile mycelium B* from 3 samples (20%), and *Sterile mycelium C* from 2 samples (13.3%) (Table 4). A total of 15 species belonging to 9 genera of filamentous fungi and four of yeasts was isolated (Table 4).

Table 4. Filamentous fungi and yeasts identified from household water tanks of Hofuf, Al-Hassa, Saudi Arabia

Fungal species	Site (s)	CFU cm^{-2}
Acremonium alabamensis	2,13,14	7.5-9.4
Acremonium strictum	1,2,14,15	3.9-5.2
Alternaria alternata	7,11,12	6.4-6.9
Aspergillus niger	1,2, 5,6,10,11,12,13,14,15	4.9-6.7
Candida guilliermondii	1,2,3,4,6,7,8,9,10, 13,14,15	3.8-5.0
Candida parapsilosis	1,2, 3,4,6,7,10,11,12,13,14,15	4.7-6.2
Candida tropicalis	1,2,3,4,7,10, 12,13,14,15	2.9-3.9
Cladosporium cladosporioides	1,2,5,6,14,15	9.5-10.3
Mucor mucedo	3, 5,6,14,15	6.5-8.1
Mucor racemosus	3, 5,6,14,15	5.1-6.4
Penicillium chrysogenum	3,7, 8,9,10,11,13,15	3.8-5.0
Penicillium citrinum	3,4,5,7,13,14	8.0-9.2
Penicillium nigricans	1,4,10,13,15	7.9-8.8
Phialophora verrucosa	3,4,5,7,8,9,10,12,13,14,15	10.0-11.8
Rhodotorula mucilaginosa	1,2,3,4,6, 8,9,13,14,15	3.6-4.7
Stachybotrys chartarum	11,13, 15	2.8-3.9
Sterile mycelium A	5, 8,9,10,11,12,13	4.3-6.0
Sterile mycelium B	5,12,13	2.8-3.9
Sterile mycelium C	11,12	3.1-4.5

The results showed that the population of fungi as CFU cm^{-2} in water samples was more intense than yeasts. The highest rate was of *Phialophora verrucosa* (10-11.8 CFUcm^{-2}), *Cladosporium cladosporioides* (9.5-10.3 CFUcm^{-2}), *Penicillium citrinum* (8-9.2 CFUcm^{-2}), *Penicillium nigricans* (7.9-8.8 CFUcm^{-2}), *Acremonium alabamensis* (7.5-9.4 CFUcm^{-2}), *Mucor mucedo* (6.5-8.1 CFUcm^{-2}), *Alternaria alternate* (6.4-6.9 CFUcm^{-2}), *Mucor racemosus (5.1-6.4* CFUcm^{-2}), *Aspergillus niger* (4.9-6.7 CFUcm^{-2}), the yeast of *Candida parapsilosis* (4.7-6.2 CFUcm^{-2}), CFUcm^{-2}), *Sterile mycelium A (4.3-6.0* CFUcm^{-2}), *Acremonium strictum* (3.9-5.2 CFUcm^{-2}), each of *Candida guilliermondii and Penicillium chrysogenum* (3.8-5.0 CFUcm^{-2}), *Rhodotorula mucilaginosa (3.6-4.7* CFUcm^{-2}), *Sterile mycelium C* (3.1-4.5 CFUcm^{-2}), *Candida tropicalis* (2.9-3.9 CFUcm^{-2}), and each of *Stachybotrys chartarum and Sterile mycelium B (2.8-3.9* CFUcm^{-2}) (Table 4).

DISCUSSION

There is a certain species of fungi live in media with scarce nutrients called oligotrophics. These fungi can feed on small amount of nutrients that hunt from the air, or that which is obtained by somehow. The household water tanks represent a perfect environment for those types of fungi. Fungi found in household water tanks can get nutrients from the surrounding air or from decaying small animals and protozoa in those waters. Because of the length of staying the water in the reservoirs and lack of regular cleaning systems, fungi increase day by day. Logically, it can predicted the existence of a certain quality of fungi in such environments[7].

By examining the previous reviewers, it was found that the major fungi occurred in public potable water were dematiaceous taxa (63%), and more particularly *Cladosporium* (27%), *Phoma* (9%), *Alternaria* and *Exophiala* (each 7%)[15]. In France, filamentous fungi have been recorded from 81% and yeasts from 50% of the examined water samples[8] whereas, in Finland, the most commonly isolated fungus from supply waters was *Aspergillus fumigatus*[11]. Frankova and Horecka (1995)[5] isolated 39 genera and 64 species of filamentous fungi from 44% of drinking water samples with *Cladosporium*, *Penicillium* and *Fusarium* being predominant. Species of the genera of *Alternaria*, *Acremonium Aspergillus*, *Cladosporium*, *Exophiala*, *Penicillium*, *Phialophora* and *Candida* have previously been isolated from drinking water[7].

In this research, 15 filamentous fungi of *Acremonium alabamensis*, *Acremonium strictum*, *Alternaria alternate*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Mucor mucedo*, *Mucor racemosus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium nigricans*, *Phialophora verrucosa*, *Stachybotrys chartarum*, *Sterile mycelium A*, *Sterile mycelium B Sterile mycelium B* and *Sterile mycelium C*, and 4 yeasts of *Candida guilliermondii*, *Candida parapsilosis*, *Candida tropicalis* and *Rhodotorula mucilaginosa*, were isolated. Most of these isolated fungi were previously obtained from potable Stored water for drinking and household use.

Results here showed that there was no clear relationship between variations in the chemical composition of the tested 15 water samples and amount of isolated fungi. This could be partially related to the concentration of metallic elements located mostly in the permissible limits. However, water of sites 5, 13, 14 and 15 showed higher fungal densities (pH values less than 8) while sites 8 and 9 (pH values more than 8) showed less intensity of both filamentous fungi and yeasts.

With all this, the results which compatible with many of the previous studies, appear the extent of the presence of fungi in the domestic water tanks at 15 sites in Hofuf, Al-Hassa, Saudi Arabia.

Presence of these fungi represents dangerous to consumers of water reservoirs and those fungi were registered as toxin producers and causing agents of a lot of skin, allergies and respiratory diseases[4].

Further studies are needed to find safe and effective ways to prevent the presence of these fungi waters used in households.

CONCLUSION

From the results of this research, it can be concluded that 15 species of filamentous fungi and 4 species of yeasts have a wide distribution in household water tanks in Hofuf city, Al-Hassa, Saudi Arabia, which can have a potential risk on public health. Many of these fungi with a great reputation as cause of many diseases, especially in people with weakened immune systems. Further studies on the severity of these fungi and ways to eliminate and get rid of their sources should be studied extensively.

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