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Isolation and Identification of Wheat Seed Fungi and Effect of Microwave on Fungi Loads in Salah Al-Din Governorate in Iraq

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Abstract: ***This study was conducted to determine the seed borne fungi of wheat cultivars in the seed production stores companies in Salah AL-Din Governorate, Iraq, 2021, and then evaluate the efficiency of Microwaves to kill or eliminate the fungal pathogens of wheat. The results of the study indicate that all samples of wheat seeds were contaminated with fungi in different percentage. 318 isolates of fungi were isolated from the studied wheat seed samples.They were identified culturally and microscopically according to approved taxonomic keys. The fungus A. flavus appeared with a percentage of 100 %, as it was present in all samples of the studied wheat seeds, as well as the species of A.niger. Then the species of Alternaria (A.alternata, A.tenuissima , A.saponaria) and the species of Aspergillus (A.flavus , A.fumigatus, A.terrus, A.niger), Rhizopus oryzae, Rhizoctonia solani, Penicillium spp, Aureobasidium pullulans, Phoma spp, Curvularia lunata, Stemphylium spp, and Mucor spp appeared with different percentages. an investigation into the effect of microwaves on fungal pathogens of wheat varieties was undertaken .This was achived by microwaving seeds at 0 , 10 , 20 , 30 and 40 Seconds . Agar plate tests were used to ass's pathogen loads. Pathogen levels were steadialy reduced at all treatment times and by 30 S treatment. All pathogens were consistently destroyed. In comparison, at 40 S the levels of saprophytic fungi were increased because it thermotolerant. The use of microwave radiation was a valuable tool for crop improvement, it was rapid, economic, efficient and safe to eradicating the pathogens.***

Keywords: Wheat, Seeds, Fungi, Microwave.

1. INTRODUCTION

Improving and maintaining crop yields of wheat grain is severly important in feeding world population that's continually growing (Satore and Slafer.1999, Oliver et al.2013).As it is thought high density cultivation of crops may have encourages growth of fungi, and the natural defense mechanisms may have reduces because use of agrochemicals. It have been proven that the microwave have to be effective for use in seed drying methods (Wesley et al.1974), as it has

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benefits of being developed technology that is safe, reliable and cheap(Oliveret al.2013).Microwave are a part of electromagnetic radiation spectrum, frequencies ranging from (300 MHZ to 300 GHZ), and wavelengths between (1m-1mm) according to countries and regions, includes five frequencies with exclusive frequency for home applications (2450 MHZ), besides they have been works through absorption at the molecular level. (Pakhomov et al ;1998).The microwave are one of the new technologies for seed treatment (Adhikari et al.,2020, Saeedeh Taheri et al., 2020) Triticum aestivum L. return to family Poaceae and it is one of the most important strategic crops for human food security and an important source of fodder due to its high nutritional value(Shewry,2009).The wheat crop is considered one of the main cultivated grains in Iraq followed by barly Hordeum vulgare L.(Soppe and Saleh,2012).Its cultivation is concentrated in China, Canada, European Union, Australia, Russia, Pakistan, India, United states, Turkey and Ukraine accounting for over 80%of the total production (Senpati and Semenov,2019). It is considered as an excellent health building food,rich in protenis 9.2-11.3, dietary fibers1.8-1.9% ,B-group vitamins, moisture content 8.4-9.8%, and a blend of minerals like Zn, Cu, Fe, Pand Mg (Johansson et al,2020). Wheat also enters the food and industry sectors it is consumed in the form of confectionary products, bread, biscuits, noodles, ethanol, cosmetics, and seitan or vital wheat gluten (Cappelli et al.,2020).The volume of Iraqs production of the wheat crop during the winter season of 2021 was amounted to 4 million and 234 thousand tons, according to (CSO, Iraq).Various seed characteristics such as germination, nutritional value, moisture content and discoloration are influenced by a number of biotic and a biotic factors during seed storage. The stored seeds are more prone to be attaked by the fungi making them un acceptable as food and feed (Chatha et al, 2016). Wheat seeds are susceptible to attack by different tybes of fungi at harvest and during storage, the first type of fungi represented by field fungi and it is a group of fungi that alter the composition and quality of seeds before harvesting (Chaladurai et al,2010).These damages can be detected through routine evaluations or periodic examinations, in general field fungi cannot appear in storage if the grain has been stored in suitable temperature and moisture contents (Christensen and Kaufman,1995). As for a second type wich is represented by storage fungi, they are those fungi that cause damage to grain during storage,and usually do not occur at a critical level prior to harvest(Muri and White,2000), The fungal flora of stored grains is mostly composed of wide spread mold species of genera Rhizopus spp, Mucor spp, Fusarium spp, Cladosporium spp, Alternaria spp, Aspergillus spp and Penicillium spp (Mathew et al, 2010). These genera are usually found in grain stored in the form of spores, in accurate quantities during transportation and storage and could be survive for several years (Ulziljargal et al, 2019), it affects grain yield as some of them cause rotting roots of wheat especially fungi Fusarium culmarum, Bipolaris sorkoniana, Rhizoctonia solani (Alrashidi , 2011) .

2. MATERIALS AND METHODS

Sample collection: The studied wheat grain samples were collected from the storages of the seed production companies in Salah-al-Deen Governorate, Iraq, for three regions Baiji , Samarra, and Sherqat for the (2020 -2021) Agricultural season, wich includes eight samples distributed into six varieties (Ibaa, Adenah, Boro, Baiji, Diglah, Cham 6 Samarra, Cham 6 Sherqat, and Alafia). samples were collected in September ,2021 and the samples were taken according to the rules and regulations of the International Organization for Examination of

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Seeds (ISTA .2018) and placed in Polyethylene bags and sealed with holes for ventilation to avoid the death of embryos, then they were transferred to the laboratory and placed in refrigerator at a temperature 4°C until the tests are carried out (Maryam et al. ,2017) .

Microwave treatment
Microwave treatment were carried out using an 800 W, LG microwave, at 2450 MHZ. For each treatment, 204 seeds were randomly taken and placed in a 90 mm diameter glass petri dishes that was placed in the centre of microwave on the rotating plate. Heating at full power was applied for 0,10,20,30 and 40 S respectively (Oliver ET al.2013).

Agar plate tests:
To assess both background and seed infection levels, agar plate tests were undertaken. These were based on Science and Advice for Scottish Agriculture (SASA) guidelines, wich adhere to the official seed health testing methods of the International Seed Testing Associations (ISTA). 200 seeds were sterilized in 1% (v/v) sodium hypochlorite solution for 1 min followed by three washes in deionized water. Seeds were aseptically transferred to Potato Dextrose Agar (PDA) (Potato extract 4.0 g/l; Glucose 20 g/l; Agar 15 g/l) with streptomycin (30 µg/ml agar) in 90 mm diameter Petri dishes. Five seeds were placed on each plate. Plates were sealed with parafilm and incubated for seven days at 25°C (Fandohan et al, 2005). After four days, the growth of the fungi was monitored and the fungi were visually identified. Followed by purification of the fungi isolates on the food medium (PDA). Seeds with no fungal growth were also recorded.

Conditions for keeping isolates
The different isolates of the fungi that were isolated from the seeds of the wheat samples after their cultivation on the medium of potato dextrose agar PDA were kept in an oblique from inside the Slants test tubes in the incubator and kept in the refrigerator at a temperature of 4°C.

Microscopy of fungal cultures and their identification
After the growth of the fungal cultures isolated from wheat seeds from the Varieties under study, they were examined microscopically by taking part of the fungal growth with a sterile inoculation needle and placing it on a glass slide containing a drop of solution lactophenol, and the sample was then spread out in a droplet of loading the slide cover was placed and examined microscopically; or by using Scotch No.800 transparent tape by taking 4 cm of the tape , so that, the adhesive side was facing the surface of the mycelium and attached with great care and caution ,where it is placed . The tape was between the thumb and forefinger, after the fungal growth stick on the tape, it was lifted gently remove from the plate and put on a slide containing a drop of lactophenol-aniline blue and then examined microscopically (Al-Khalil Abdullah, 2011), to identify spinning characteristics mycelium and spores, according to the approved taxonomic keys (Ellis, 1971; Domach et al, 1980; Nelson et al, 1983 and Pitt and Hocking, 2007).

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Purification of fungi by single spore method
The studied fungi were purified by the single spore technique by mixing a sample of 0.5 cm in diameter from the fungal colony with 10 ml sterile distilled water. The dilution was continued until the number of spores ranged between (1-10 spore microscopic field) at power 10 X, after wich 0.5 ml of The spore suspension and spread on the surface of a plate containing a medium consisting of 2 % agar and water. The dishes inoculated with the fungi were incubated in the incubater at 25 ± 2°C for 48 hours, after that fungal colonies resulting from the growth of the single spore were transferred to a new PDA medium by means of a sterile inoculation needle (Nelson et al, 1983).

Calculate the percentage of fungal appearance%
The percentage of fungal appearance was calculated as follows:
Percentage of appearance = the number of fungus appeared in the samples ⁄ the total number of samples (Mohammed Hamza Abass, 2021).

Statistical analysis:
Statistical analysis was done using Statistical Analysis System version 9.3 (SAS) . Complete Randomized Designe (CRD) by use Duncan multible level test (Duncan, 1955). P values of 0.05 were considered significant (Alrawi and Abd al aziz, 2000). Results, represented an average of three replicates per treatment.

3. RESULTS AND DISSCUSIONS

Isolation and identification of fungi
318 isolates of different fungi were isolated from grain samples of wheat varieties, results of the current study indicated in the table (1) the presence of 15 fungal species, which belongs to 9 genera of fungi associated with grain samples, which were collected from grain stores in Salah al-Din Governorate, which were exposed to human consumption and animal. These species were diagnosed after conducting a surface sterilization that affects the fungi carried out on the surface of the seeds and leads to a reduction in their presence for getting rid of contamination by using sterilized sodium hypochlorite, wich effect was limited to fungi carried out on the outer cover of seeds and does not affect fungi that infected seeds from inside or that affects the embryo of the seed, this was consistent with Sarhan (1995), (Saadoun 2005). The seeds of various crops were susceptible to invasion of pathogens during the stages of plant development, before and after harvested period by a wide range of fungal flora, seeds that carry out pathogen ,that's became as primary source of infection and that's had been an important role in pathogenicity of pathogens. As fungal flora present on Seeds or within their may lead to longer seed dormancy period and reduced germination and percentages of surviving seedlings (Christensen and Lopez, 1963) and (Hansraj et al., 2018)

The incidence of fungi
The table (1) indicates the rates of appearance of fungi isolates in different wheat cultivars. Highest percentage of occurrence was recorded for two species A.flavus and A.niger with percentage of 100 % for each as, then percentage occurrence of 75% was recorded for each of

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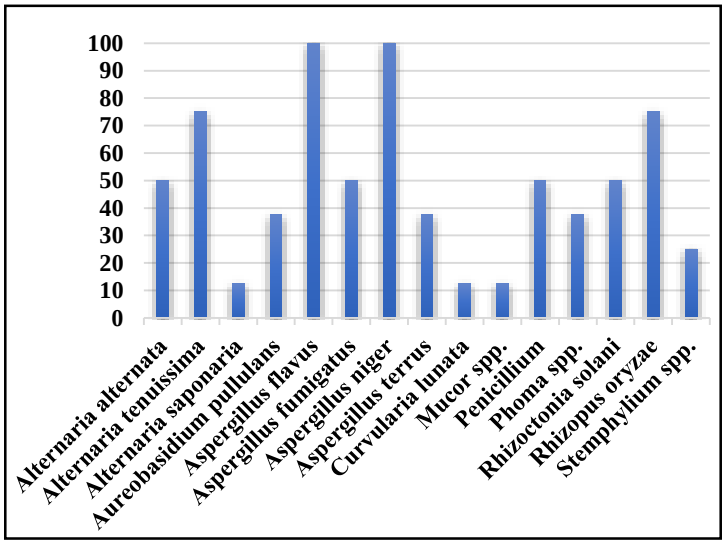
fungal species Alternaria tenuissima and Rhizopus oryzae, followed by the fungal species Alternaria alternate, Aspergillus fumigatus and Penicillium spp, then species of Aureobasidium pullulans, Aspergillus terrus and Phoma spp were recorded with an incidence of 37.5% to each as, respectively, then species of Stemphylium spp recorded an incidence of 25 %, then species of Alternaria saponaria, Curvularia lunata and Mucor spp were recorded with the lowest incidence Which amounted to 12.5 % for each of them, respectively.

Table (1): The percentage of appearance of fungi isolates in all wheat cultivars

No.	Isolates	Appearance %	No.	Isolates	Appearance %
1	Alternaria alternata	50 %	9	Curvularia lunata	12.5 %
2	Alternaria tenuissima	75 %	10	Mucor spp.	12.5 %
3	Alternaria saponaria	12.5 %	11	Penicillium	50 %
4	Aureobasidium pullulans	37.5 %	12	Phoma spp.	37.5 %
5	Aspergillus flavus	100 %	13	Rhizoctonia solani	50 %
6	Aspergillus fumigatus	50 %	14	Rhizopus oryzae	75 %
7	Aspergillus niger	100 %	15	Stemphylium spp.	25 %
8	Aspergillus terrus	37.5 %			



Fiure (1): The percentage of appearance of fungi

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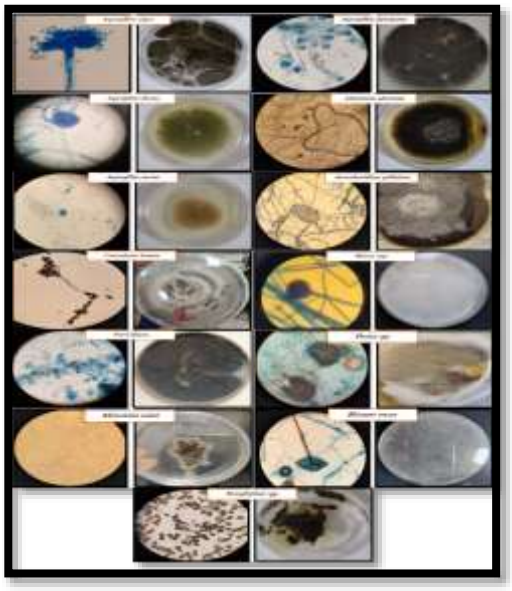

Figure (2): Culture and microscope picture for fungi isolate, culture picture using single spore technique and microscope picture with scotch tape technique solates in all wheat cultivars

Effect of microwave on contamination load:

The levels of fungal loads reported as 0 S microwave expousure the majority of fungal groups were of the species Aspergillus and Penicillium, wich could be active at moisture contents abaut (65-90 %) and that's were agreements with a study concluded that wheat seeds in Bulgaria were infected with a group of fungi belongs to the genera Alternaria, Aspergillus, Mucor, Fusarium, Rhizopus and Penicillium. (Borrisova et al.2000, Ban et al.2016, Ahmed Y.Ahmed et al., 2021) . At 10 S recorded that emergence of pathogenic fungi and their dominance over saprophytic fungi species may be due to the secondary metabolic compounds that inhibitory to other fungal species, as it were used in biotechnology. At 20 S results indicate that some of fungal species that were recorded in the treatments 0, 10 S did not appear, as well as decreases in appearance of pathogenic fungi like penicillium spp. Exposure of seeds to microwave radiation for 30S led to eliminated of pathogenic fungi and these results were agreements with (Oliver et al. 2013, Martinez et al., 2014) as they concluded that 30 S showed decrease in fungal infection. The decrease in pathogenic fungi may be due to thermal damage from microwave ovens, wich change the nature of fungal proteins, leading to cell death and loss of vitality (Daniel et al.1996). Because of, microwave radiation kills fungi that carried out by seeds through heat and desiccation (Oliver et al., 2013). The spores form was more resistant to microwaves radiation than the mycelium of pathogens wich were easily eradicated (Cavalante &Muchovej, 1993, Martinez solis et al.2014). At 40 S the saprophytic fungi were continued to appear and this may be due to the higher tolerance of theirto microwaves, especially the species of Aspergillus. fumigatus as it was thermotolerant and grows at a temperatures up to 55° David et al., 2007), as well as the species A.niger that was tolerant and resistant to high temperatures, then followed by A.flavus in appearance at high temperatures and Rhizopus oryzae. That grows at 40°C (David et al., 2007). The decrease in pathogenic fungi such as Alternaria spp, Rhizoctonia solani may be facilitated the growth of saprophytic fungi to be present at higer rates. Thus, the

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microwave oven is a promising tool, as it was eliminated pathogenic fungi at higher temperatures, and it had been proven that its use can reduces the invasion of microorganisms in wet soil (Ferris, 1984).

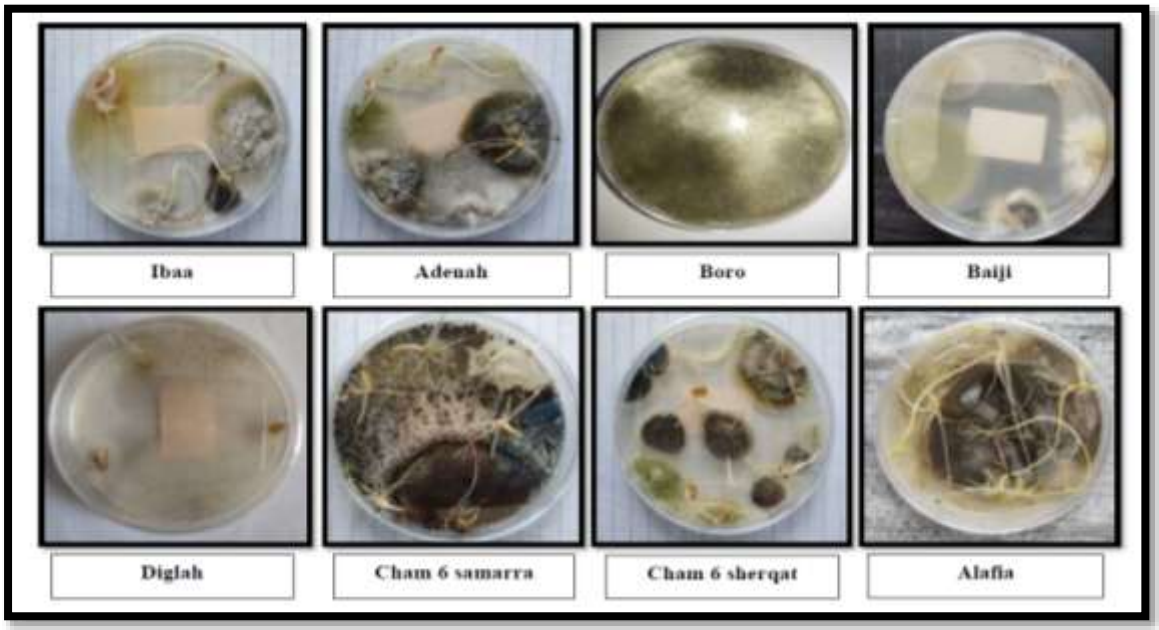

Figure (3): The effect of microwaves on the fungi associated with the seeds of the eight wheat varieties at the thermal coefficient (0) seconds.

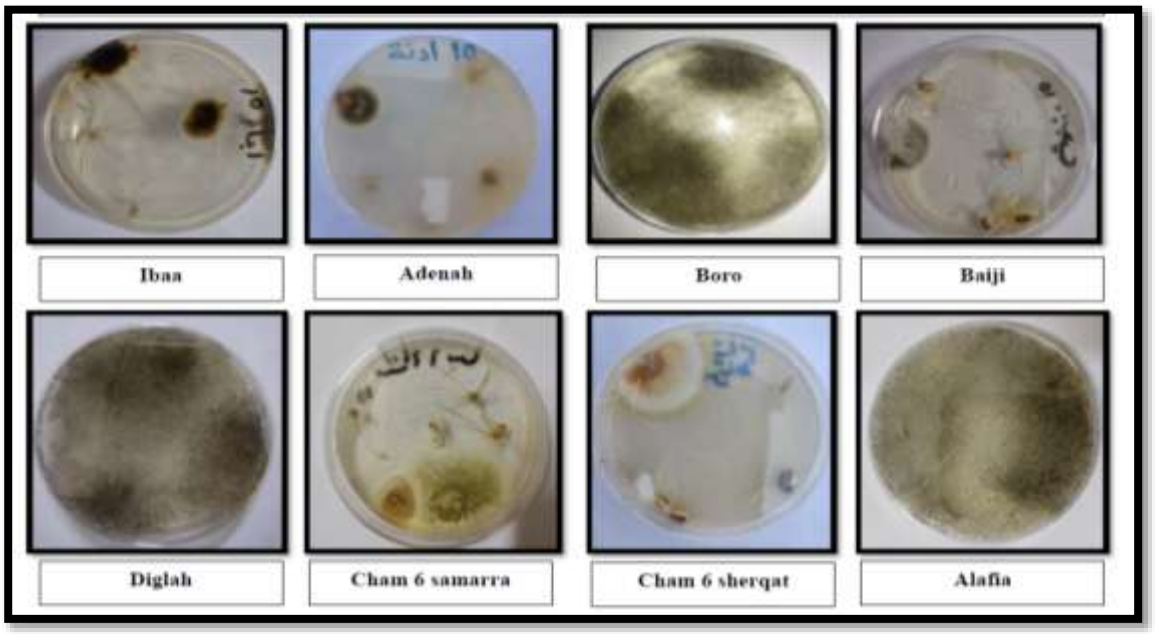

Figure (4): The effect of microwaves on the fungi associated with the seeds of the eight wheat varieties at the thermal coefficient (10) seconds

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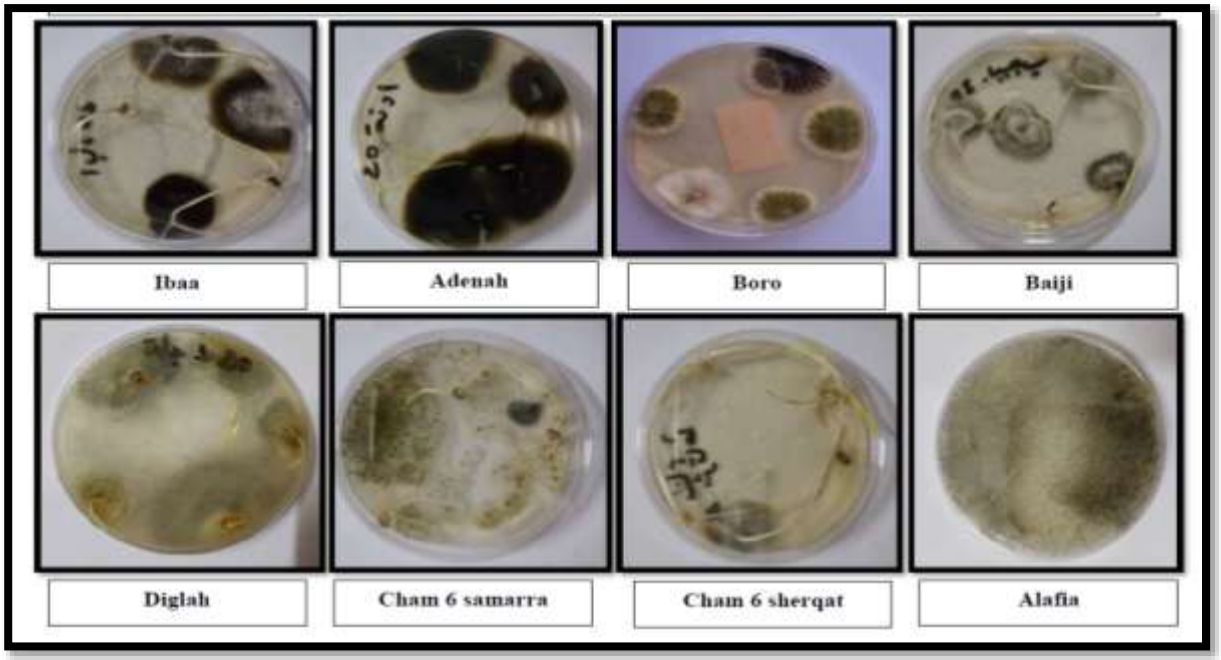

Figure (5): The effect of microwaves on the fungi associated with the seeds of the eight wheat varieties at the thermal coefficient (20) seconds

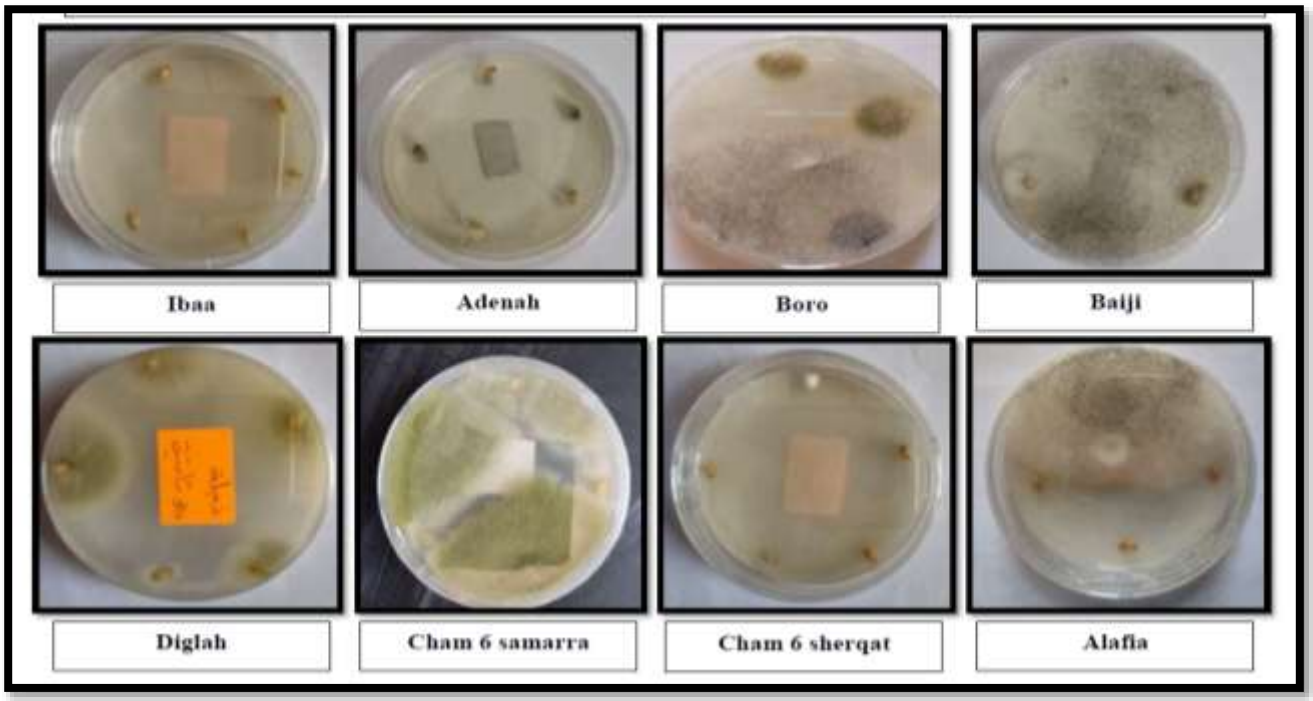

Figure (6): The effect of microwaves on the fungi associated with the seeds of the eight wheat varieties at the thermal coefficient (30) seconds

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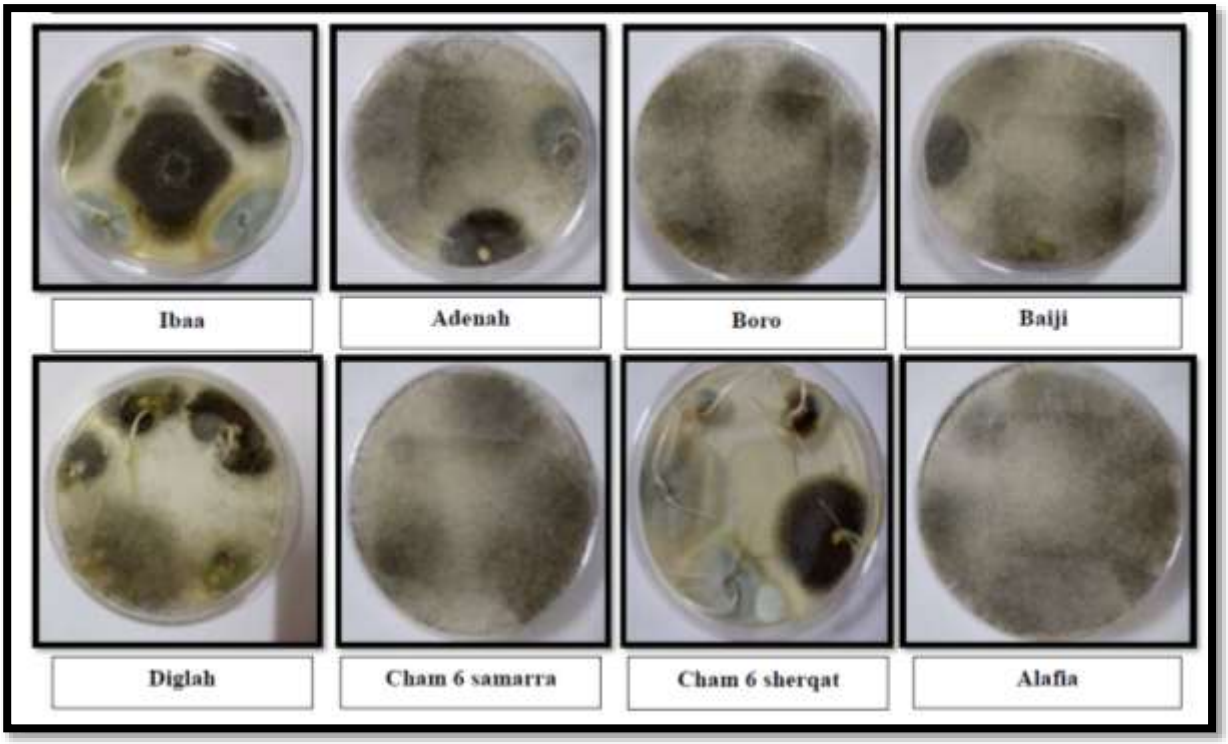

Figure (7): The effect of microwaves on the fungi associated with the seeds of the eight wheat varieties at the thermal coefficient (40) seconds

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No.	Fungi isolates	The percentage frequency of fungi in relation to the time of exposure to the microwave				
		0 S	10 S	20 S	30 S	40 S
1	Alternaria alternata	6.553 I J K L	15.833 F G	9.052 I	0 R	0 R
2	Alternaria tenuissima	7.636 I J K	13.303 G H	22.792 D E	0 R	0 R
3	Alternaria saponaria	2.083 P Q R	0 R	0 R	0 R	0 R
4	Aureobasidium pullulans	6.826 I J K L	5.972 J K L M N	1.637 P Q R	0 R	0 R
5	Aspergillus flavus	20.895 E	18.243 F	26.621 C	40.173 B	13.213 G H
6	Aspergillus fumigatus	0 R	0 R	0 R	0 R	26.458 C
7	Aspergillus niger	25.470 C	18.025 F	12.450 H	24.503 C D	44.616 A
8	Aspergillus terrus	0 R	8.819 I	1.389 P Q R	0 R	0 R
9	Curvularia lunata	1.116 Q R	0 R	0 R	0 R	0 R
10	Mucor spp.	0 R	2.718 O P Q R	1.983 P Q R	0 R	1.637 P Q R
11	Penicillium spp.	3.627 M N O P Q	6.250 I J K L M	3.264 N O P Q	0 R	0 R
12	Phoma spp.	4.990 K L M N O	0 R	0 R	3.646 M N O P Q	0 R
13	Rhizoctonia solani	5.552 J K L M N	6.667 I J K L	7.242 I J K	1.042 Q R	0 R
14	Rhizopus oryzae	14.721 G H	4.166 L M N O P	5.406 J K L M N O	5.635 J K L M N	14.071 G H
15	Stemphylium spp.	1.116 Q R	0 R	8.159 I J	0 R	0 R

Table (2): Fungal frequency percentage relative to microwave exposure time for all wheat cultivars Means with the same letter are not significantly different.

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4. CONCLUSION

The studied wheat seeds samples, in Salah AL-Din, Governorate, were all contaminated with fungi, the fungal species Aspergillus flavus and A.niger were the most frequently occurring species. The results showed possibility of recorded a specific time period to eliminate pathogenic fungi and it was 30 seconds.The saprophytic fungi cannot be removed by microwaving, because, these species were thermotolerant, so we could support this physical tretment with a chemical treatments to get the best results and could kills all fungal loads in stored seed wheats.

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