Original Article

Fungal contamination of the white agricultural fungus Agaricus bisporus taken from the local markets of Baquba city

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Abstract

The current study aimed to detect fungi contamination in agricultural mushroom samples Agaricus bisporus which was taken from some areas of Baqubah district in Diyala governorate. It was shown that all the samples were contaminated 100% , total colony formation ranged 3.264×10-3 to 5.596 ×10-3 , the results recorded 253 fungal isolates . The Canaan region recorded the highest number of fungal isolates, which was 84 isolates , followed by the regions of Buhruz, Khan Bani Saad and Baqubah, which were 66, 54 and 49 fungal isolates, in that order. The results of the study also showed that the fungal isolates that were isolated from the white agricultural fungus belonged to 3 fungal genera, called by Aspergillus, Rhizopus and Penicilium. The genus Aspergillus was superior in the number of species belonging to it since 6 species were recorded at a rate of 75% compared to the genus Rhizopus for which one species was recordedonly Rhizopus spp. The genus Penicillium recorded one species with a rate of 12.5 % each. The total species that were isolated and diagnosed were 8 fungal species: Aspergillus niger, A. parasiticus, A. nidulans, A. tamarii ,A. fumigatus, A.ochraceus, , Rizopus spp. and Penicillium verrucosum.

Keywords: Agaricus bisporus, Fungal contamination, Aspergillus, Penicillium verrucosum, Rhizopus.

INTRODUCTION

The white agricultural mushroom, Agaricus bisporus, is one of the large fungi that is characterized by its fruity body, which is fleshy . It is also characterized by its high nutritional value and economic, commercial and medicinal importance (Safwat and Al-Khouli, 2006). The white agricultural fungus A. bisporus is considered one of the most important food and medicinal sources in recent years (Bhushan et al. 2018,), as it is characterized by its high nutritional value due to its high content of protein, amino acids, vitamins, dietary minerals and dietary fiber. Therefore, it is called an integrated health food (Rathore et al., 2017). . Muszynska et al. (2018) revealed in his study that A.bisporus mushroom has many biological properties such as antioxidant, anti-inflammatory, tumor, bacterial and immune activities. In addition to its great importance in destroying cancer cells, eliminating viruses, enhancing human immunity and regulating weight (Lau et al., 2017)

Fungi Contamination is one of the affecting factors of the quality of many food crops, including edible fungi that are susceptible to contamination with filamentous fungi either during cultivation or storage from. These fungi include: Trichoderma spp., Mycogone spp. , Lecanicillium spp., Cladobotryum spp. , Sependonium spp. , Sclerotium spp. , Coprinus spp. , Penicillium spp, Aspergillus niger, Cladobotryum spp and others, which considered a polluting fungi of the large fungi which are edible ones. (Biswas and Kuiry, 2013; Ghimire et al., 2021)

Food pollution results food spoilage as an outcome of its contamination with microbes, toxins, heavy metals and other environmental pollutants. Food pollution occurs at any stage of production, storage, transportation, marketing and handling (AL-Hisnawi, 2010; Sharma et al., 2020). Because the white agricultural fungus Agaricus bisporus is one of the important food sources with high consumption for its nutritional and medicinal value, the current study aimed to detect the fungal contamination of the agricultural fungus Agaricus bisporus taken from the local markets of some areas of Baqubah city, Diyala governorate center, Iraq.

Materials and working methods:

• Samples collecting :

The white agricultural fungus Agaricus bisporus was collected from the local markets of Baqubah district, the center of Diyala governorate. The study included four areas belonging to Baqubah district, which are Baqubah, Buhruz, Canaan and Khan Bani Saad. 5 samples were collected from each one of the areas at a rate of 1 kilo for each sample. As it illustrated in Figure (1), which shows the mushroom samples collected from the local market. The samples were placed in paper bags on which all information was recorded, including the place of collection, the date of collection and the weight of the sample. The samples were transferred to the laboratory for laboratory testing. The samples were dried using an electric oven at a temperature of 70°C until the weight was stable (Al-Sahaf, 1989). Then the samples were crushed using an electric grinder . After that been done, the samples were transferred to a glass bottles, which were kept in and to the refrigerator until the required tests were performed.

Figure (1) The white agricultural fungus Agaricus bisporus which was collected from the local markets of Baqubah district, the center of Diyala governorate

• Detection of fungal contamination:

The decimal dilution method was used to detect the dried contaminated white agricultural mushroom Agaricus bisporus samples with fungi, weighing 1 gram of the sample and placing it in a glass beaker, then adding to it 9 ml of sterile distilled water. After that a medium of SDA is poured and the plate is moved with a light circular motion so that the sample is homogeneous with the culture medium, with three replicates for each one of the plates. Then the dishes were incubated in the fungi incubator at a temperature of 25 ± 2 °C until the fungal growth is appeared. (Al-Shtayeh et al., 1998).

• Diagnosis of fungal isolates:

In order to diagnose fungal isolates isolated from white agricultural mushroom samples, the phenotypic examination of the samples was carried out based on the phenotypic characteristics of the fungal colony, including the external shape of the colony, the color of the colonies, the nature of the colonies, examining the opposite side of the colony and the diameter of the colony growth, in addition to the microscopic characteristics that were studied by taking a portion From the newly grown fungal colony, it was placed on a glass slide containing the dye Stain Lactophenol cotton blue, and left for three minutes, then dried on a light flame and left to dry. The size and color of the conidia, the type of mycelium, and conidia and vesicles carriers. The classification keys which were based on were given by (de Hoog et al., 1995; Rowe et al., 2009).

Results and discussion:

Detection of fungal contamination:

The results in Table (1) showed that all samples of the white agricultural fungus Agaricus bisporus were contaminated with fungi by 100% for all regions, and samples taken from the Canaan region recorded the highest rate of total fungi numbers,

which was 5.596 × 10-3, followed by the regions of Buhruz, Khan Bani Saad and Baqubah, which they were 4.4 x 10-3, 3.6 x 10-3, and 3.264 x 10-3, in that sequence . The results also show that the total number of fungal isolates were 253 and the Canaan region recorded the highest number of fungal isolates, which were 84 fungal isolates, followed by the regions of Buhruz, Khan Bani Saad and Baqubah, which were 66, 54, and 49 fungal isolates, respectively. As we find from the results shown in Table (1) that the total numbers of fungi were higher than the permissible limit, which constitutes a source of danger to the health of the consumer. The results of the current study agree with some studies that indicated the presence of fungi contamination of the white agricultural fungus Agaricus bisporus, including (Mantu and Rana, 2000; Fletcher and Gaz, 2008).

Table (1) Total numbers of fungi in samples of agricultural fungus Agaricus bisporus taken from local markets for some areas in Baqubah District - Diyala Governorate:

Total	Total NO. of isolates	Total	Total colony formation	Location
49	7	$^{3-}10 \times 3.264$	$^{3-}10 \times 2.33$	Baqubah1
	15		$^{3-}10 \times 5$	Baqubah2
	7		$^{3-}10 \times 2.33$	Baqubah3
	7		$^{3-}10 \times 2.33$	Baqubah4
	13		$^{3-}10 \times 4.33$	Baqubah5
84	28	$^{3-}10 \times 5.596$	$^{3-}10 \times 9.33$	Canaan1
	26		$^{3-}10 \times 8.66$	Canaan2
	12		$^{3-}10 \times 4$	Canaan3
	10		$^{3-}10 \times 3.33$	Canaan4
	8		$^{3-}10 \times 2.66$	Canaan5
66	12	$^{3-}10 \times 4.4$	$^{3-}10 \times 4$	Buhruz1
	12		$^{3-}10 \times 4$	Buhruz2
	12		$^{3-}10 \times 4$	Buhruz3
	12		$^{3-}10 \times 4$	Buhruz4
	18		$^{3-}10 \times 6$	Buhruz5
54	12	$^{3-}10 \times 3.6$	$^{3-}10 \times 4$	Khan Bani Saad 1
	12		$^{3-}10 \times 4$	Khan Bani Saad 2
	9		$^{3-}10 \times 3$	Khan Bani Saad 3
	6		$^{3-}10 \times 2$	Khan Bani Saad 4
	15		$^{3-}10 \times 5$	Khan Bani Saad 5
253	**Total**			

Fungi isolated from the agricultural fungus Agaricus bisporus

The results of isolating fungi from agricultural mushroom samples Agaricus bisporus showed that the appearance of the genera Aspergillus, Rhizopus and Penicillium, and the superiority of the genus Aspergillus in the number of its species, as 6 species were recorded at a rate of 75% compared to the genus Rhizopus, for which only one species. Rhizopus. spp and the genus Penicillium, which recorded one species at a rate of 12.5% for each of them, as it shown in Table (2). It can be noted from the table that some species appeared in all samples for all the regions under study, which are: A. niger, Rizopus spp. , A. tamari. While there are species that appeared in the samples of one region only, which is P. verrucosum fungus , while the fungus A. ochraceus was recorded in two of the study regions. As for the table (4-3), it shows the total number of isolates and the percentage of appearance for each type of the fungi that were isolated from samples of the agricultural fungus Agaricus bisporus. The fungus A. tamarii, which recorded an appearance rate of 17.787% and the number of its isolates was 45 fungal isolates, followed by the fungus A. fumigatus, which had a rate of appearance of 13.834% and the number of its isolates was 35 fungal isolates, while the fungus P. verrucosum was recorded in only one sample and the number of Isolates 12 isolates and the incidence rate is 4.743%, which is the lowest among all fungi that were isolated during the study. The current results confirm that the dominance of Aspergillus species as it is a widespread fungus in all environments and environmental conditions and in various foodstream for its ability to produce a wide range of digestive enzymes as well as its production of huge numbers of asexual reproductive units (Klich, 2002).

Table (2) Fungi isolated from agricultural mushroom samples Agaricus bisporus taken from local markets for some areas in Baqubah district - Diyala governorate.

No. of isolates Fungus	Isolated types and species	Location
6	*Aspergillus niger*	Baqubah
5	*Rizopus spp.*	
3	*Aspergillus parasiticus*	
5	*Aspergillus nidulans*	
11	*Aspergillus tamarii*	
19	*Aspergillus fumigatus*	
26	*Aspergillus niger*	Canaan
12	*Aspergillus parasiticus*	
8	*Aspergillus nidulans*	
6	*Rizopus spp.*	
16	*Aspergillus tamarii*	
16	*Aspergillus fumigatus*	
10	*Rizopus spp.*	Buhruz
12	*Aspergillus niger*	
13	*Aspergillus parasiticus*	
6	*Aspergillus tamarii*	
14	*Aspergillus nidulans*	
11	*Aspergillus ochraceus*	
9	*Rizopus* spp.	Khan Bani Saad
11	*Aspergillus niger*	
12	*Aspergillus tamarii*	
10	*Aspergillus ochraceus*	
12	*Penicillium verrucosum*	

Table (3) Percentage of appearance of fungi isolated from agricultural mushroom samples Agaricus bisporus

%Emergence	No. of isolates	Fungus isolates
21.739	55	*Aspergillus niger*
11.858	30	*Rizopus spp.*
11.067	28	*Aspergillus parasiticus*
10.672	27	*Aspergillus nidulans*
17.787	45	*Aspergillus tamari*
13.834	35	*Aspergillus fumigatus*
8.300	21	*Aspergillus ochraceus*
4.743	12	*Penicillium verrucosum*
100	253	Total

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