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QUALITATIVE AND QUANTITATIVE CONTAMINATION WITH FUNGI OF SORGHUM SEEDS INFECTED WITH SITOTROGA CEREALELLA

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Abstract:

59 isolates belonging to 19 species of fungi within 13 genera, in addition (Sterile mycelia) were separated from sorghum seeds infected with the grain moth S. cerealella, after a storage period of four months. The least number of species and fungal isolates were isolated from seeds Maize was recorded after a storage period of eight months, and it was 24 isolates belonging to 8 varieties of fungi as well as sterile hyphae of fungi, and the outcomes of the chi-square (X2) statistical analysis revealed that the variation in seed storage period had a significant ($P<0.05$) impact on the total number of fungal isolates.

The number of fungal isolates isolated from dead insects that infect corn seeds for different storage periods , the difference in seed storage time had little impact on the number of isolates of fungi

Deuteromycotina and Zygomycotina comprised the majority of the fungal species that were isolated for the study. The Aspergillus genus of fungi recorded a distinct appearance and attained the highest frequency rates.

Keywords:

INTRODUCTION:

The grain moth is described as a small-sized insect, which has the ability to infect field crops such as wheat and barley (Al-Azzawi and Mahdi, 1983), as well as attacking the grain inside stores (Ismail, 2014).

The grain moth infects barley seeds, rice seeds, wheat seeds, yellow corn and sorghum seeds, causing a decrease in their economic and marketing value, due to the weight loss of those seeds, as well as the effect of infection on the germination process of seeds (Bhard waj et al., 1977; Shazali, 1987).

Insect infestation of seeds encourages both bacterial growth and fungal growth by transferring them to the spores of these microorganisms (Hansen and Hell, 2004). The grain moth may transmit the spores of different types of fungi by sticking these spores on their bodies, and Specifically, mycotoxins producing mycotoxins, such as Aspergillus flavus, which is a producer of the most important of them (CABI, 2007)).

Numerous serious health issues both in humans and animals are brought on by mycotoxins (Gardwell et al., 2001).

Therefore, the current study aimed to demonstrate the effect of the stored grain moth in causing fungal contamination on sorghum seeds infected with the insect and to study the impact of various storage times on the degree of qualitative and quantitative fungal contamination.

METHODS AND MATERIALS:

1- Insect and sorghum seed samples:

A pure culture of the insect was obtained from the Integrated Control Center of the Ministry of Agriculture in Baghdad, and the insect was diagnosed by Prof. Dr. Muhammad Salih Abdul-Rasoul/Natural History Museum/University of Baghdad. The insect was raised on a medium consisting of wheat seeds after placing it in plastic bottles with dimensions of 15 x 9 cm covered with pieces of boring cloth, then the boxes were placed in the incubator at a temperature of 28 °C and the nutrient medium was renewed from time to time throughout the research period (Abboud, (2014).

Sorghum seeds were purchased from local markets in the city of Nasiriyah and placed in sterile polyethylene bags and transferred to the laboratory. The seed samples were distributed at a quantity of 250 gm in sealed glass flasks, and sterilized in an autoclave at a temperature of 121°C and a pressure of 15 pounds/inj2. For a quarter of an hour to get rid of any insect or fungal infestation (Linera and Ewel, 1984)

2- The culture media used for isolating fungi:

To isolate the fungi found on seeds or insects after death, two types of culture media were used: potato agar and dextrose agar (PDA) medium and sabouraud dextrose agar (SDA) medium. Oxoid supplied instructions. They were sterilized according to the previous method in the autoclave, after adding 250 mg of the antibiotic Chloramphenicol) for the purpose of inhibiting bacterial growth.

Sterilized sorghum seeds were distributed in clean and sterilized plastic boxes with dimensions of 9 x 15 cm by taking a weight of 150 gm of sterilized sorghum seeds for a storage period of two months, 250 gm of seeds for a storage period of 4 months, and 500 gm of seeds for a storage period of 8 months, with two duplicates per each. storage period. Then one of them was infected with the insect, by releasing five pairs (5 males and 5 females) of the newly-appearing complete insect emerging from the virgin's role for a period not exceeding two days or less, while the other duplicate was left without injury for the purpose of control.

After the end of each storage period, the specific contamination with fungi was investigated by taking 2 gm of sorghum seeds infected with the insect and planting them on the medium of PDA, SDA, and after incubating the dishes for 5 days at a temperature of 25 °C, then diagnosing the developing fungal colonies, as the above two mediums were used. To isolate and diagnose the fungi found on the dead insects that were taken from each storage period, the following sources were relied upon in diagnosing the isolated fungi:

Domsch et al. (1980), Klich (2002), Klich and Pitt (1988) and Pitt and Hocking (1997)

3- Frequency %:

The percentage of the frequency of fungal species isolated from seeds or insects was calculated based on the following equation:

$$\text{Frequency percentage (F\%)} = \frac{\text{The number of isolates of mushrooms}}{\text{Total number of isolates}} \times 100$$

4- Statistical analysis:

The results were analyzed statistically by using the SPSS statistical program, using the Chi-Square (X2) test.

RESULTS AND DISCUSSION :

1- Fungi isolated from corn kernels infected with S.cerealella and for different storage periods:

The results of the current study showed, as shown in Table (1), a distinct fungal diversity, as the number of fungal species isolated from corn grains infected with the grain moth S.cerealella (14) was a type of fungi, in addition to sterile fungal hyphae (sterile mycelia) after a period of two months from Storage, and this fungal diversity increased after a storage period of four months, so the number of isolated fungal species was (19) in addition to sterile fungal hyphae, but the number of isolated fungal species decreased after a storage period of eight months, so the number of fungal species was (8) of fungi as well as sterile fungal hyphae (Fig. 1) It is noted from the table that during the storage period of four months, the largest number of fungal isolates was isolated and it reached 59, while the number of isolates after storage period of two months and eight months was 43 isolates and 24 isolates, respectively. Significantly by using chi-square ($p<0.05$ (x2) according to the difference in the storage period with the superiority of the storage period of four months in the number of isolates over the storage period of two months and eight months. In fungal diversity as well as an increase in the number of fungal isolates, studies indicate that The increase in the population of insects that infect stored crops is accompanied by an increase in the number of fungal species isolated from them (Abboud, 2014), while the continuation of storing crops for long periods with insects infested may lead to a clear decrease in the fungal community present on those crops. Christen (1968) Dorworth found And a decrease in the number of fungi isolated from the stored materials with the progress of the storage period, which leads to the occurrence of a state of competition between the developing fungal species for food, and the survival of the most adapted to the conditions of food shortage significantly increases and the death of large numbers of insects. t was also noted through the results that most of the isolated fungi belong to the deuteromycota and the zygomycota, and the number of their species reached 20 and 3 species, respectively, and this is consistent with what was stated by Niaz and Dawar (2009). Most of them are for the deficient fungi from stored sorghum seeds, as well as isolating other types of vaccinated fungi, but in a lesser percentage, as confirmed by Al-Awadi (2008) in A study carried out to isolate the fungi associated with stored cowpea seeds and infected with the southern cowpea beetle Callosbruchus maculates, that the majority of the fungi belong to the imperfect fungi.

The fungal genera isolated during the study varied in the number of fungal species affiliated to each genus. Both Aspergillus and Alternaria scored superiority over the rest of the genera, as four species were isolated for each of them. The species affiliated with the genus Aspergillus were A. flavus, A. fumigatus, A. .niger and A.ochraceous, as for the genus Alternaria, the species was isolated from alternate, A.chlamydospora, A.phragmospora, and A.radicina, two species were isolated from the genus Cladosporium, while one species was isolated from the rest of the fungi from sorghum seeds infected with a grain moth. The current study's findings are consistent with previous research. Niaz and Dawar (2009) by isolating the genus Aspergillus from most samples of sorghum seeds, and its appearance rate was 70%. As pointed out by Kunwar (1989) that race Aspergillus, Alternaria, Cladosporium, Mucor and Rhizopus are the most important genera of fungi associated with stored materials.

It was noticed from the results that the highest frequency rate achieved by A.niger was 18.6% and 25% during the storage period of two months and eight months, respectively, while the highest frequency after four months storage period was 15.25%, while A. flavus achieved the highest frequency. (25.5%) After four months of storage, it is one of the most common fungi on stored food due to Its production of large numbers of asexual reproductive units, as well as its ability to withstand high salt and sugar concentrations (Pentzer and Harvey, 2009).
The study also showed that other types of fungi recorded a low frequency, as in Nigrospora oryzae and Mucor sp.

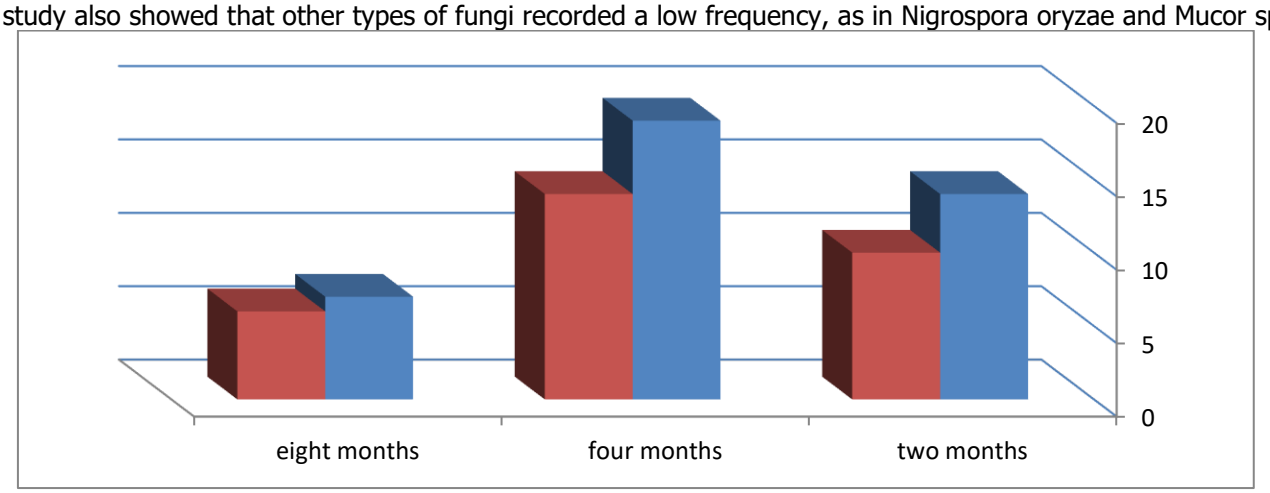

Figure (1): Number of fungal species and genera isolated from sorghum seeds infected with S. cerealella and for different storage periods.

isolated fungi	Two months		Four months		Eight months	
	number of isolates	Frequency%	number of isolates	Frequency%	number of isolates	Frequency%
***Absidia corymbifer* (Cohn)Sacc&Trotte**	**1**	**2.3**	**2**	**3.3**	**-**	**-**
***Acremonium kiliense* Grutz**	**2**	**4.6**	**3**	**5.08**	**-**	**-**
Alternaria* (F.r) kiessler *alternata	**5**	**11.6**	**1**	**1.6**	**-**	**-**
***A.chlamydospora* Mouchacca**	**2**	**4.6**	**-**	**-**	**-**	**-**
***A.phragmospora* van Emden**	**-**	**-**	**2**	**3.3**	**3**	**0.12**
***A.radicina* Meier,Drechsler**	**-**	**-**	**1**	**1.6**	**-**	**-**
***Aspergillus flavus* Link**	**7**	**16.2**	**9**	**15.25**	**5**	**0.2**
***A.fumigatus* Fres**	**4**	**9.3**	**4**	**6.77**	**-**	**-**
***A.niger* van Tieghem**	**8**	**18.6**	**5**	**11.8**	**6**	**25**
***A.ochraceus*Wilhelm**	**-**	**-**	**5**	**8.47**	**-**	**-**
***Auerobasidium pulluans*(DeBary) Arnaud**	**-**	**-**	**4**	**6.77**	**-**	**-**
***Cladosporium cladosporioides*(Fresen)**	**2**	**4.6**	**2**	**3.3**	**-**	**-**
***Cladosporium* sp.**	**1**	**2.3**	**2**	**3.3**	**2**	**8.3**
***Curvularia lunata*(Wakker)**	**-**	**-**	**1**	**1.6**	**2**	**8.3**
***Fusarium* sp.**	**-**	**-**	**2**	**5.08**	**-**	**-**
***Humicola fuscoatra* Traaen**	**1**	**2.3**	**2**	**3.3**	**-**	**-**
***Mucor* sp.**	**2**	**4.6**	**-**	**-**	**-**	**-**
***Nigrospora oryzae*(Berk&Broom).**	**1**	**2.3**	**-**	**-**	**1**	**4.1**
***Penicillium* sp.**	**5**	**11.6**	**6**	**0.1**	**4**	**16.6**
Eghrenb.ex.Fr.)	**1**	**2.3**	**2**	**3.3**	**-**	**-**

***Rhizopus stolonifer*(**						
lignicola **Pesante**	**-**	**-**	**3**	**5.08**	**-**	**-**
Sterile Mycelia	**1**	**2.3**	**2**	**3.3**	**1**	**4.1**
Press) ***Ulocladium atrum*(**	**-**	**-**	**1**	**1.6**	**-**	**-**
Total number of isolates		**43**		**59**		**24**

Table (1): Fungi isolated from sorghum grains infected with S.cerealella and for different storage periods.
X^2=5.070 p<0.05 $x^2_{tabulated}$=4.8

2- Fungi isolated from dead whole of S. cerealella which infected sorghum seeds for different storage periods:

Table (2) shows the types of fungi that were isolated from the whole dead S.cerealella grain moth and found on sorghum seeds and isolated within different storage periods. It is noted that the two-month storage period was the most fungally diverse in terms of the number of isolated fungal species and genera, Figure (2).

It is clear from the results that the largest number of fungal isolates that were isolated from dead insects that infect sorghum seeds amounted to 33 isolates during a two-month storage period, while the number of isolates started decreasing with the continued increase in the storage period, and 26 fungal isolates were isolated after four months. Fungal isolates after eight months' storage period amounted to 24 isolates, and the number of isolates was not significantly affected by the difference in storage period, as there were no significant differences p<0.05 Most of the isolated species belong to the deficient fungi, and these fungi outperformed the vaccinated fungi (Zygomycota) and the percentage of their presence on the whole dead grain moth that infects sorghum seeds for a storage period of eight months was 90%, while these fungi appeared on dead insects after two and four months with a percentage of 90%. 88.9% and 81%, respectively, Fig. (3), and the dense presence of deficient fungi may return to insects after 8 months compared to their presence During a period of two and four months, the food substance, represented by the sorghum seeds, is exhausted, and these fungi are directed to take advantage of the substances contained in the insect's body and exploit them as food It also became clear through the results that the three species belonging to the genus Aspergillus are A. flavus, A. niger and fumigatus. All of them were isolated from insects during the three storage periods, in addition to the type Rhizopus stolonifer, which is a zygotic fungus, and this is consistent with what Abdel-Razik (1986) mentioned when he isolated the two fungal sexes from all the whole of the insect Oryzaephilus surinamensis that infects wheat seeds A. flavus achieved the highest frequency rates within a period of four months, reaching 30.7%, followed by A.niger fungus, which reached 27.2% within a two-month storage period, and this is consistent with what was mentioned by Okwulchie (2004). It was found that insects belonging to the genus Sitophilus were infected with A.flavus.

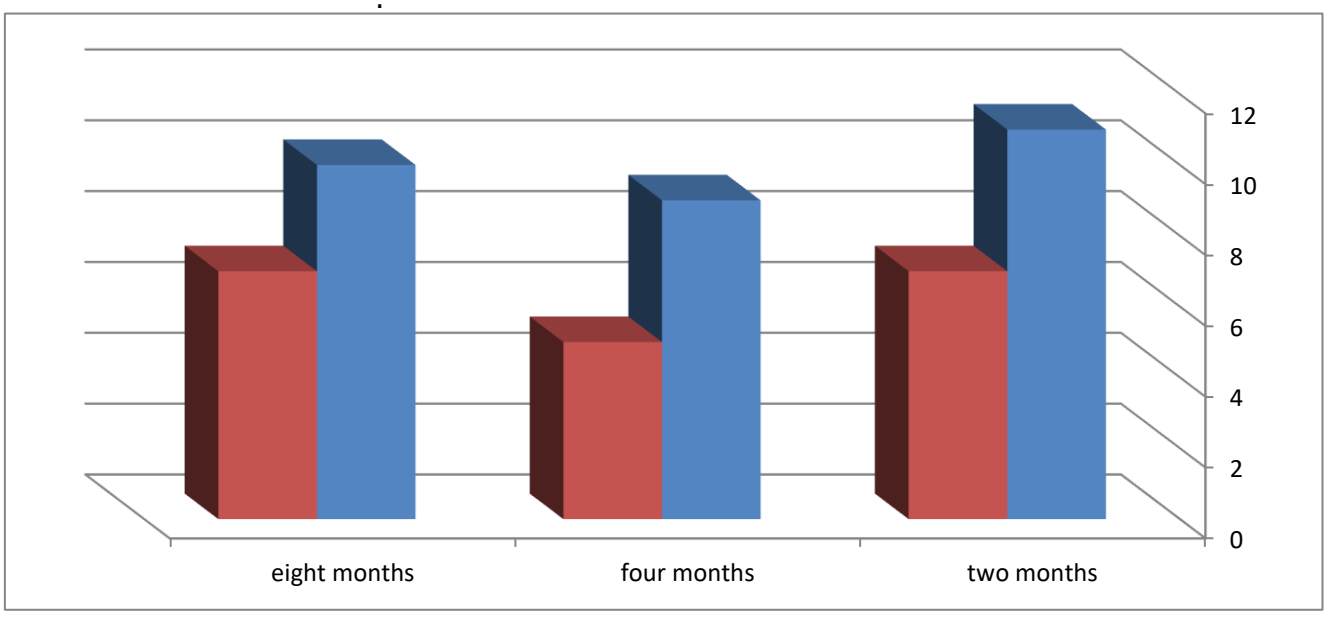

Figure (2): Number of fungal species and genera isolated from dead S. cerealella whole moth that infected sorghum seeds for different storage periods.

isolated fungi	Two months		Four months		Eight months	
	number of isolates	Frequency %	number of isolates	Frequency %	number of isolates	Frequency %

***Alternaria alternata*(Fr.)Kiessler**	**-**	**-**	1	3.8	4	16.6
***A.chlamydospora* Mouchacca**	**2**	**6.06**	-	-	-	-
***A.phragmospora* van Emden**	**4**	**12**	1	3.8	1	4.1
***Aspergillus flavus* Link**	**5**	**15**	8	30.7	2	8.3
***A.fumigatus* Fres**	**2**	**6.06**	2	7.6	2	8.3
***A.niger* van Tieghem**	**9**	**27.2**	5	19.2	5	20.8
***Cladosporium cladosporioides*(Fresen)**	**-**	**-**	3	11.5	3	12.5
***Fusarium* sp.**	**-**	**-**	-	-	3	12.5
***Mucor* sp .**	**3**	**9.09**	-	-	-	-
***Penicillium* sp1.**	**1**	**3.03**	3	11.5	-	-
***Penicillium* sp2**	**1**	**3.03**	-	-	1	4.1
(Eghrenb.ex.Fr.) *Rhizopus stolonifer*	**2**	**6.06**	1	3.8	2	8.3
Scytalidium lignicola Pesante	**1**	**3.03**	-	-	-	-
Sterile Mycelia	**1**	**3.03**	2	7.6	-	-
***Ulocladium atrum*(Press)**	**2**	**6.06**	-	-	1	4.1
Total number of isolates		**33**		26		24

Table (2): Fungi isolated from whole dead grain moth S.cerealella that infected sorghum seeds
$X^2=1.6$ $p<0.05$ $x^2_{tabulated}=4.8$

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