

Effect of *Tribolium castaneum* in Qualitative and Quantitative Contamination with Fungi

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ABSTRACT

The objective of this study was to estimate the quantitative contamination fungi in sound whole flour that infested with red flour beetle *Tribolium castaneum* under the effect of different numerical levels of insect, which are (5, 10, 20) pairs in the level of contamination. The study was accomplished under a temperature 30 ± 2 °C and humidity $70 \pm 5\%$. The result showed the following. 13 species of fungus which belong to five genera were isolated from sound and infested whole flour samples with three numerical levels of the insect. These were (5, 10, 20) pairs and for two generations. The species isolated were: *Aspergillus flavus*, *A. niger*, *A. terreus*, *Mucor hiemalis*, *Alternaria*, *Paecilomyces* Sp. *Penicillium* sp1, *Penicillium* sp2. Whereas at the two numerical levels (10, 20) pairs of insects, the fungus species *Sterils mycelia*, *A.ustus*, *Emericella* sp, *Sepedonium* sp. were isolated.

The more frequently fungus in the samples of infested flour in the level 20 pairs of insects was *A. flavus*. Its frequency was (47.37%), also in level 5 pairs of the fungus *A. niger*, which frequency ratio was (42, 58%). In the sound flour samples the more frequency. *A. fumigates* in level 5 pairs of insects which its frequency was (34, 48%) and in level 20 pairs of insects was the fungus *A. niger* which its frequency was (66.6%). The results showed that the rate of numerical of fungi increased with increasing levels of numbers of the insects, the highest rate of fungus numerical was recorded in infested whole flours at the numerical level 10 pairs of insects was (31.41X10²) colony/ gm. While their average numbers were (15.83X10²) colony /gm at level 5 pairs of insects. It was observed that the average number of fungus in flour insects was higher than their numbers in sound flour.

Keywords: *Tribolium castaneum*; Fungi; Contamination; Sound whole flour

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INTRODUCTION

Red flour beetle *Tribolium Castaneum* belongs to order coleopteran family flour beetle which is comedown from Tenebrionidae which was numbers pests attack stored and broken maternal as it is considered secondary pests of cereal when it is not able to penetrate in sound grains and feeding on grains which were previously attack by other insects as they could be brought up by feeding on pop corns and wheat grain (1). These insects are widely spread in the world, as adult insects and larra are feeding on grains and its products, especially, flour crumps and bran causing a bad smelly for it (2). Flour beetles of both species and red confused flour beetles pests which of high importance and of widely stored cereal product (3) in the case of the presence of insects in high large numbers, they will cause of these products in general and flour which in particular which consequently would affect the commercial value of flour because it would lose a lot of its natural properties such as change in color and flavor in addition to being kind of mold as a result of separated adult insects some violate compound as quanon. The flour infested with red flour beetle lose a lot of properties that quality for the good work bread as viscosity (4). In addition to the main damage above mentioned it to this insect's contamination and cereal and get destroying theme and making theme unfit for human consumption by the mixing of bodies and different stages of the insect as well as its eggs and phases. Where heating of grains flour and moisture transmission of insects from store to another leads to transmission of insects with it fungi spores, which cause rot of flour and change its nature and its food value (5). The fungal contamination occurs through the numerous and widespread fungal spores for the small size and through part of hyphae that were exposed (6). (7) showed that the moisture content of the stored grains and temperature and insects during storage and marketing play a major in grains

contamination. The red beetle flour beetle in fungus because of the long peered of storage associated with an increase in fungi that had increased lengthening storage time, the most fungus belong to species of genera: (*Aspergillus*, *Penicillium* and *Alternaria*)

MATERIALS AND METHOD

Cultural media used in isolated and identification fungi

A. Potato dextrose agar

This medium was prepared according to the firm that provided it by melting 39 gm of potato in 1000ml of distilled water and cleaned them sterility by autoclave added to 00.5gm of antibiotic klorimphetical often being frightened to 45 °C, then pour in Petri dished e and used to growth of fungi. (8).

B. Mait extract agar

This medium with the addition of barley powder of the production company macnur in couda.

Barley (20gm) with 1gm peptone and (20) gm for each dextrose and agar was added to previous materials a litter of distilled water and after boiling the mixture mentioned.

C. The effect of three different numerical levels of red flour beetle at the level of fungi contamination.

Three different numerical levels of the insect were released. These were (5,10, 20) pairs, of adult insect (females + male) on the whole flour. 100 gm of whole flour was placed in glass bottles with dimensions (9 * 15 cm). In each glass bottles three adult insect's new emergence were released and of mentioned levels (9) and three replication per level, then was tightly closed by pieces of gauze cloth. Then were put in the incubator at 30 ± 2 °C. Temperature and $70 \pm 5\%$ relative humidity with three replications. They were kept under watching for each containing 100g of whole flour but not infested with insects (control treatment) it was followed for

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two generations. Samples of infested and non-infested flour were taken, at following dates:

- 1- Two weeks after of infection.
- 2- Four weeks after infection.
- 3- Two weeks after emergence of first generation insects.
- 4- Four weeks after emergence of first generation insects.

Fungal contamination was known at any of the above dates.

1- The study of the structure the fungal contaminations

The total number

The total numbers of fungi was estimated in flour by weight 10 gm of each infested with insect and sound (control) then add 90 mL of serial dilution solution containing sodium chloride (0.85%). Represent dilution 10^{-1} and attended every frightening sieve 10^{-3} . 1mL of dilution was transferred to sterile petri dishes and repeat for each dilution and add to it sterilizer added chloramphenicol with medium and moved the dishes towards the clock and otherwise; it was the placed in the incubator at 28 °C for 5-7 days depending on the method (10). The percentage of frequency of isolated genera was calculated during the study and for all samples. The percentage of frequency in each sample was calculated as well as the total frequency of fungi for all flour samples during the study period by applying the following equations: Percentage of frequency = Number of fungus isolated / Number of total isolates of all fungi

Examination and identification of fungi

The dishes were examined after 5-7 days of culture for the purpose of deification the dishes using dissecting microscope. The isolated colonies were moved to a slanted medium to obtain pure isolates after growth and incubated media in the refrigerator. The purpose of studying the characteristics of isolated fungi and identification using compound microscope by preparing glass slides stained with lacto phenol containing a blue – methyl. The fungi isolated from the flour were identified by (11). The morphological characteristics of colonies shape, color and colony diameter were also adopted as well as the microscopic characteristics of colonies such as the shape fungus mycelium and size, color and conidiospores.

Methods of isolating fungus

Fungus was isolated in this study using two different methods and two types of medium were used as shown below:

1- Method of casting dishes

Spared 1gm of each sample of flour by a sterile teaspoon in a sterile glass dish 9cm diameter then add the sterile medium to the dish before hardening and moving motion for purpose of uniform distribution of the sample, then left the medium to harden. Incubated the isolated the dishes after hardening the medium under 30 ± 2 °C. Two replications were used for each sample (11).

2- The sterilization (Autoclave)

The autoclave was used to sterilize the medium MEA, PDA and solution at 121 °C for 15 mins. The method of dry sterilization of all types of glass used by electric oven was applied at 180-200 °C for 2hr.

Statistical analysis

All experiments were carried out according to factorial experiment with complete randomized design. Statistical comparisons of the results were performed by one-way

ANOVA using SPSS ver.23. Significant differences ($P < 0.05$) between the fungus and time were analyzed by L.S.D *triplicates range test (12).

RESULTS AND DISCUSSIONS

Quality contamination

Result showed in tables (1, 2 and 3) that 13 species of fungus had been isolated from sound and infested whole wheat flour samples at different numerical levels (5, 10 and 20) pairs of red beetle flour *Tribolium castaneum* and after two generation under the influence of temperature 30 ± 2 °C and relative humidity 70 ± 5 % it was clear from results that some fungi species differ depending on the level of numbers of insects and the state of flour and dates of sampling. 13 species of belonging to 5 genera were isolated another infertile fungus. The study showed that the most common species in sound and infested flour was *Aspergillus*. The two species *A. flavous*; *A. niger* recorded the highest frequency percent in sound flour at the numerical levels (10 and 20) pairs of insects respectively reaching 60.66% while in the infested flour the frequency percent was 47.37 and 42.58% at both numerical level (10 and 20) pairs of infested. This may be due to the effect of fungi infested the crop while in the field, the fungi transfer to store and when contamination are favorable for growing the fungi will grow and reproduction and increase their numbers of other genera and become infested high for their ability to endure harsh contamination compared to other genera (13) especially fungi *Aspergillus*. It can grow in low water content and large thermal range may be suitable for the growth of fungi, this was in line with his finding. The lowest frequency genera were found in the sound flour samples; *Alternaria alternate*, *Aspergillus ustus* and *Emericella* sp at the numerical levels (5 and 10) pairs of insects and reached 4.5 1.21 and 1.27% respectively. The study showed that the insects flour samples had the lowest genera frequency were *Penicillium* sp, *Sepedorium* at numerical levels (5 and 20) pairs of insects respectively. The percentage reached 3.95 and 3.03 % due to the lack of adequate water content in those samples or because storage condition were unsuitable for their growth and reproduction. The inability to produce quantities of enzymes analyzed on stored stuff. Previous studies had shown that these fungi could be from storage insects such as confused flour beetle [*Tribolium confusum*]; Rice weevil [*Sitophilus oryzae*] and peen beetle [*Callosobruchus chinensis*] (14). (15) explained that seed of yellow corn in the stores were infested with fungi with the present of maize weevil [*Sitophilus zeamais*] and damage was increasing, and the following species were isolated: *Aspergillus flavous*; *Fusarium moniliform* and *Penicillium spp.* (16) they had isolated many species of storage fungi from stored pean seeds and wheat seeds which infested saw-toothed grain beetle [*Oryzaephilus surinamensis*] which *Fusarium*; *Aspergillus*; *Alternaria* and *Penicillium*. The results also showed that the variation in percentage the frequency of fungal species by the level of insect may be due to the secretion of some fungi toxins as *Aspergillus flavou*; *Pencillium* and *Paecilomyces* spp affecting the rest of fungal species this was confirmed by. It was found that the amount of aflatoxin B1 produced increased with increasing severing of infested corn grain stored with khapra beetle [*Trogoderma granarium*]. Contamination of this maize weevil [*Sitophilus zeamais*] was also found with *Aspergillus flavou*, the infested corn seeds lead to increased contamination of aflatoxin it was preferable that the insects carry on their outer surface the spores or conidiospores fungal that were transmitted to the seeds when they are couched.

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Table 1: Percentage of fungus frequency in whole wheat flour and infested with red flour beetle *Tribolium castaneum* at numerical levels of 5 pairs for two generations.

Fungus	Type of Flour condition	Time			
		2 weeks after Infestation	4 weeks after Infestation	2 weeks after emergence of F1	4 weeks after emergence of F1
<i>Alternaria alternata</i>	Sound	0.00	0.00	4.54	0.00
	Infested	0.00	0.00	0.00	7.14
<i>Aspergillus flavus</i>	Sound	6.66	0.00	18.18	35.13
	Infested	42.42	23.80	25.75	30
<i>A.fumigatus</i>	Sound	13.30	34.48	27.27	5.40
	Infested	0.00	9.32	0.00	2.85
<i>A.niger</i>	Sound	46.70	44.82	22.22	54.05
	Infested	24.24	38.00	40.90	21.43
<i>A.terreus</i>	Sound	0.00	0.00	18.81	0.00
	Infested	15.15	14.28	4.54	0.00
<i>A.ustus</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Emericella sp</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Mucor hemalis.</i>	Sound	33.33	3.45	9.09	5.40
	Infested	15.15	14.28	0.00	20.00
<i>Paecilomyces sp</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	39.39	7.14
<i>Penicillium sp1</i>	Sound	0.00	0.00	0.00	0.00
	Infested	3.03	0.00	0.00	11.43
<i>Pinicillium sp2</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Penicillium sp3</i>	Sound	0.00	17.24	0.00	0.00
	Infested	0.00	0.00	0.00	0.00

L.S.D = 3.47

Table 2: Percentage of fungus frequency in whole wheat flour and infested with red flour beetle *Tribolium castaneum* at numerical levels of 10 pairs for two generations.

Fungus	Type of Flour condition	Time			
		2 weeks after Infestation	4 weeks after Infestation	2 weeks after emergence of F1	4 weeks after emergence of F1
<i>Alternaria alternata</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Aspergillus flavus</i>	Sound	60.00	5.17	37.77	36.36
	Infested	19.35	2.57	42.15	6.45
<i>A.fumigatus</i>	Sound	0.00	10.34	0.00	0.00
	Infested	22.58	4.28	0.00	54.50
<i>A.niger</i>	Sound	30.00	34.48	40.00	42.58
	Infested	41.94	27.14	12.39	0.00
<i>A.terreus</i>	Sound	15.00	1.72	0.00	0.00
	Infested	0.00	5.71	11.57	0.00
<i>A.ustus</i>	Sound	0.00	1.72	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Emericella sp</i>	Sound	5.00	0.00	15.56	3.03
	Infested	16.13	28.57	0.00	5.80
<i>Mucor hemalis.</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	4.13	0.00
<i>Paecilomyces sp</i>	Sound	0.00	0.00	8.89	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Penicillium sp1</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	11.43	9.09	0.00
<i>Pinicillium sp2</i>	Sound	0.00	20.60	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Penicillium sp3</i>	Sound	0.00	3.45	0.00	0.00
	Infested	0.00	0.00	0.00	0.00

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<i>Rhizopus sp.</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Sepedonium sp.</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	8.25	0.00
<i>Sterile mycelia</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	29.03

L.S.D = 1.32

Table 3: Percentage of fungus frequency in whole wheat flour and infested with red flour beetle *Tribolium castaneum* at numerical levels of 20 pairs for two generations.

Fungus	Type of Flour condition	Time			
		2 weeks after Infestation	4 weeks after Infestation	2 weeks after emergence of F1	4 weeks after emergence of F1
<i>Alternaria alternata</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Aspergillus flavus</i>	Sound	9.09	28.17	12.50	13.30
	Infested	14.28	23.66	34.26	47.37
<i>A.fumigatus</i>	Sound	31.80	1.40	2.50	6.66
	Infested	0.00	6.10	24.07	1.30
<i>A.niger</i>	Sound	31.80	56.34	27.50	6.66
	Infested	35.70	15.27	9.26	13.16
<i>A.terreus</i>	Sound	0.00	7.64	0.00	0.00
	Infested	7.14	28.24	0.00	0.00
<i>A.ustus</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Emericella sp</i>	Sound	0.00	4.22	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Mucor hemalis.</i>	Sound	0.00	0.00	57.50	13.30
	Infested	28.57	17.55	1.85	17.10
<i>Paecilomyces sp</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	17.10
<i>Penicillium sp1</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Pinicillium sp2</i>	Sound	13.64	2.80	0.00	0.00
	Infested	0.00	26.71	0.00	0.00
<i>Penicillium sp3</i>	Sound	9.09	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Rhizopus sp.</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	0.00
<i>Sepedonium sp.</i>	Sound	0.00	0.00	0.00	0.00
	Infested	0.00	0.00	0.00	3.95

L.S.D = 2.06

CONCLUSION

The most common fungi isolated from sound and infested flour cultivars with red flour beetle *Tribolium castaneum* were found to be of the genera *Alternaria*; *Mucor*; *Penicillium*; *Aspergillus*. It was found that there was a positive relationship between the number of fungi and the numerical levels of insect, where the number of fungi increased with the increase in the number of red flour beetle *Tribolium castaneum*.

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