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Full Length Article

Impact of Wheat Flour Infestation by some Insects on its Quantity and Quality Loss, Fungal Contamination and Mycotoxins

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Abstract

Wheat flour samples were artificially infested with *Corcyra cephalonica, Ephestia kuehniella, Tribolium confusum* (separately) and with the three insect populations together. The effect of insects' infestation on wheat flour quantity and quality loss, fungal contamination and presence of mycotoxins was studied after two months of storage (25±2ºC and 65%RH). Wheat flour infested with insects was sieved into two portions (sieved and residual flour) after insects populations have been recorded. The results revealed radical changes in weight loss, population density of insects, nutritional composition, interaction between insects and fungal contamination in the flour samples. The maximum residual flour loss was recorded by *C. ceplalonica* followed by *E. kuehniella* while the minimum residual loss was induced by *T. confusum*. The flour consumption by the tested insects may be arranged in descending order as follows: three insects tested together > *C. cephalonica* > *T. confusum* > *E. kuehniella*. There was a significant correlation between the amount of flour consumed and insect population. Also, there was a significant increase in the total protein contents and decrease in the monosaccharaides and disaccharides in all infested sieved and residual flour samples tested. The average log total molds count in control flour samples was 1.28, while the average log of insect infested flour samples ranged from 2.36 to 4.34. Seven to ten fungal species belonging to five genera (*Aspergillus, Penicillium, Cladosporium, Eurotium* and *Emercilla*) were isolated from control and infested flour samples. Many fungal species were isolated from infested flour, but most of them belong to *Aspergillus* and *Penicillium.* Aflatoxins B_1, B_2 were detected in all flour samples, but at different levels. Ochratoxin A was not detected in the control samples and *C. cephalonica* flour samples while it was detected in flour samples infested with the other treatment. © 2016 Friends Science Publishers

Keywords: Fungal contamination; Flour; Insects; Mycotoxins

Introduction

From the time of the crops are harvested in the field to the moment when they are removed for consumption, they are subjected to damage by stored grain insects. It is generally considered that 5–15% of loss in different stored grains occurs as a result of insect pest infestation (Padin *et al.,* 2002).

Egypt is a subtropical state with a warm climate that corroborates the multifunction of spoilage microorganisms and pests of stored food products. Due to lack of proper warehousing facilities, stored grain insects largely damage grains in stores as well as during shipping and transportation. Insects and fungal infestation of stored food commodities is considered a very serious problem in Egypt and worldwide. These spoilage agents lead to the decay of the commodities manifested by loss of weight, nutritional value and toxicity due to the production of mycotoxins. Huge quantities of musty stored grains and flour commercialization, due to the presence of insects and insect fragments caused by bad storage are frequently recorded (Lorini, 2003). Among the most factors are insects that are directly connected to cereal quality and quantity spoil (Birck *et al.,* 2003a; b). *Corcyra cephalonica, Ephestia kuehniella and Tribolium confusum,* species are the most common pests of wheat flour. Under favorable conditions, they have a short generation time. They disperse easily by flight. Therefore, they are not dependent on humans for their dispersal (Simpanya *et al.,* 2001).

The quality of grain may decrease due to depletion of specific total nutrients content such as crude fat, total protein total carbohydrates and sugars (Jood *et al*., 1993; 1996a). Also, Jood and Kapoor (1993) reported significant increases in the total protein.

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The interaction between insect and fungal infestation of stored products is still in an area of active research in attempts to demonstrate the relation between insects and fungi in food damage. Before the grain being milled into flour, are already contaminated with a range of potentially deteriorative agents. Fungi normally accompany or follow insect invasion (Miller, 1995). Additionally, insects can act as vectors of fungi, serving as internal and external carriers of spores. This includes serving as a mobile source of fungal metabolites and mycotoxins (Barney *et al.,* 1995).

Cereals and other crops are susceptible to attacks by various genera of fungi, many of which produce toxic metabolites, the so called mycotoxins (Abbas *et al.,* 2002). The major mycotoxigenic fungi involved in the human food chain belong to the genera *Aspergillus, Penicillium* and *Fusarium*. Mycotoxins have been associated with several toxic effects on animal and human health including carcinogenic, mutagenic, teratogenic and immunosuppressive activity (Eaton and Callagher, 1994). One of the primary objectives of these studies was to investigate effect of insect infestation on weight loss, population density of insects, nutritional composition, interaction between insects and fungal contamination and mycotoxin in the flour samples.

Materials and Methods

Samples

Samples of wheat flour (72% extraction) used in this experiment were purchased from a local supermarket in Cairo.

Insects

Three stored product insects namely *Corcyra cephalonica, Tribolium confusum* and *Ephestia kuehniella* were used in this study. They were provided by the Natural Control Laboratory Department of Research Natural Products, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

Rearing Technique to Obtain Larvae

The stored product insects used in this study (*C. cephalonica, T. confusum* and *E. kuehniella*) were reared on milled wheat (*Triticum aestivum*) and dried yeast (1 gm yeast/500 gm milled wheat), in a glass jar in the darkness at 25±2ºC and 65±5% relative humidity. A stock culture was kept in the Laboratory of Natural Control. For bioassays, full grown larvae were collected from the rearing colonies and used for infestation.

Irradiation Process

Five hundred of each wheat flour samples were exposed to gamma radiation for disinfestation. The irradiation process was carried out using ^{60}Co Gamma Research Irradiator (Indian) located at the National Centre for Radiation Research and Technology (NCRRT), Nasr city, Cairo, Egypt. The dose rate of this gamma radiation source was 2.7 kGy/h at the time of the experiment. The irradiation dose used for insect disinfestation of the flour samples was 3.0 kGy. The real doses received by samples were measured with alanine dosimeters traceable to National Physical Laboratory (NPL), Uk. Three replicate samples were used.

Insect Infestation Process

Forty five uniform size larvae from each insect under investigation (*C. Cephalonia, E. kuehniella* and *T. confusum*) were taken from the rearing culture and added to 500 mL glass jars containing 500 gm of radiation disinfested wheat flour, which was used as the insects' diet. Insects were reared at normal laboratory conditions (25±2°C and 65±5% relative humidity). Three replicates of each insect treatment samples as well as control samples were used. The jars were placed in an incubator under constant condition, 25±2°C, 65±5% RH and photoperiod of 12:12 h light: dark cycle for two months. Doors of the used incubator were opened for a period of 30 min on alternate days to ensure proper aeration.

Analysis

After the period of two months, each flour sample was sieved through 0.5 mm sieve to obtain sieved and residual flour (two flour samples were obtained from each replicate). Samples were analyzed once in two months for the following progeny count. The insect populations' progeny were removed from the residual flour. The numbers of larvae, pupa and adults were counted.

Determination of Weight Loss

Sieved and residual flour were weighted for:

1- Wheat flour infested with *C. Cephalonia.*
2- Wheat flour infested with *E. kuehniella*.
3- Wheat flour infested with *T. confusum.*
4- Wheat flour infested with the three above insect populations altogether.
5- Control samples.

Determination of Monosaccharide and Disaccharide

The method used for the quantitative determination of soluble sugars was that of Homme *et al.* (1992). Twenty mL of boiling ethanol (80%) was added to 0.5 g flour and grinded in a mortar, then boiled in a boiling water bath for 15 min. and then the extract was filtered. The filtrate containing monosaccharide and disaccharides was put in an oven until complete dryness then dissolved in 20 mL of distilled water. For estimation of monosaccharide, the anthrone sulphuric acid method

according to Singh and Sinha (1977) was used. 10 mL anthrone reagent (50 mg anthrone were added to 72% sulfuric acid) was added to 0.5 mL sample or standard solution, and then boiled in a boiling water bath for 15 min, and the tubes are cooled and read at 620 nm against blank containing only water and the anthrone reagent. A calibration curve using pure glucose was carried out. For estimation of sucrose, 4.5 mL of KOH 30% were added to the 10 mL sample extract or 10 mL sucrose standard and boiled in a boiling water bath for 10 min. In a fresh test tube, 2 mL sugar solution or standard solution after hydrolysis were added to the 10 mL of anthrone reagent and incubated for 15 min. in a boiling water bath, then read at 620 nm.

Determination of Total Protein

The method used for the protein extraction was that of Laemmli (1970) as modified by Studier (1973), one gm ground in mortar with two ml extraction buffer (20 mL of 10% SDS, 10 mL glycerol, 6 mL of 1 Mtris and 0.8 mL of 0.25M EDTA dissolved in 52.4 mL distilled water).

Total Protein Assay

Protein content was determined by the Bradford method (Pandey and Budhathoki, 2007). The standard curve was prepared by dissolving one gm of bovine serum albumin in 0.15 NaCl and the volume was diluted up to 10 mL by distilled water (gm/mL) and from further dilutions serial concentrations of albumin solution were prepared.

Determination of Fungal Count

Czapek's-Dox Agar medium with adding 200 mg cycloheximide/litter was used for determination of total fungal count. Ten gm of flour were suspended in 90 mL physiological saline solution (85% NaCl). Serial dilutions were prepared from that suspension. 1 mL of each dilution (10^{-1}–10^{-4}), in duplicates, was placed in the center of petri dishes and poured with the medium. The dishes were incubated at 35ºC for 3–5 days. The number of molds was counted and calculated as colony forming unit/gm (cfu/g) (Beuchat, 1992).

Isolation, Purification and Identification of Fungi

Malt extract agar medium was used for purification of molds. Aseptically, a small part of the fungal growth grown on Czapek's-Dox Agar was taken from the surface of fungal colonies using a sterilized needle and transferred onto prepared petri dishes with malt extract. The dishes were incubated at 25ºC for 3–5 days (Pitt and Hocking, 1985). The purified fungi were identified according to their morphological and microscopical characteristics according to the description of Raper and Fennel (1965), Sansom *et al.* (1995) and Pitt and Hocking (1997).

Detection of Mycotoxins

A thin layer chromatography (TLC) method was used for the detection of aflatoxins and ochratoxin A according to Soares and Rodriguez-Amoya (1989) using standards of each mycotoxin.

Statistical Analysis

All data obtained for biochemical studies were statistically analyzed and the variance ratios were calculated. The ANOVA was used involving by using (SPSS) computer program, ver.15.0, and the significance among the samples was calculated at $P \leq 0.05$.

Results

The present investigations revealed changes in weight loss, population density of insects, nutritional composition and interaction between insects and fungi in food spoilage of flour sample when subjected to artificial infestation with *C. cephalonia, E. kuehniella* and *T. confusum* larvae for two months.

Results indicate that all flour samples suffered losses. But the maximum weight loss % was recorded in the sample infested by *C. cephalonia* 44.8%, followed by 41% for sample infested by the three insects together (*C. cephalonia, E. kuehniella* and *T. confusum*) and 33.7% for *E. Kuehniella.* The minimum weight loss (11%) was found in the sample infested by *T. confusum* (Table 1).

The total weight loss % that has been recorded was divided into two categories, (i) the first type is the residual flour that resulted from the insect infestation after sifting each sample, the maximum residual flour was 147.3 mg by *C. ceplalonica* followed by 129 mg for *E. kuehniella* and 74.7 mg by the three insects tested together, the minimum residual loss was 10.3 mg and it was induced by *T. confusum*. (ii) The second type is the flour which was consumed by the insects and may be arranged as follows: 130.3 mg were consumed by the three insects tested together, 76.7 mg by *C. Cephalonia*, 44.9 mg by *T. confusum* and *39.3* by *E. kuehniella* (Table 1).

The total insect population detected can be divided into three stages (larval stage, pupal stage and adult stage) in each sample (Table 2). Insect population varied during the 60 days of storage and can be described as follows:

a) In the samples infested by *C. cephalonia*, 487.7 larvae, 78.3 pupae and 16.7 adults were detected.
b) In the samples infested by *E. kuehniella*, 256.7 larvae, 56 pupae and 9 adults were detected.
c) In the samples infested by *T. confusum*, 522.7 larvae, no pupae and 865 adults were detected.
d) In the samples infested by the three insects tested together, 375 larvae, 46.3 pupae and 300 adults were detected.

Table 1: Laboratory estimation of weight loss caused by *C. cephalonia, E. kuehniella* and *T. confusum* after 2 months of storage

Treatments	Sieving flour (mg)	Residual flour (mg)	Consumed flour (mg)	Total weight loss (mg)	Total weight loss (%)
C. cephalonica	276 ±4^{bcd}	147.3±5^{bcd}	76.7 ±9.1^{bcd}	224 ±4^{bcd}	44.8
E. kuehniella	331.7 ±7.6^{acd}	129±3^{acd}	39.3 ±8.3^{ad}	168.3 ±7.6^{acd}	33.7
T. confusum	444 ±1.5^{abd}	10.3±1.1^{abd}	44.7 ±1.5^{ad}	55 ±1^{abd}	11
C. cephalonica E. kuehniella T. confusum	295 ±15^{abc}	74.7 ±4^{abc}	130.3 ±7.5^{abc}	205 ±15^{abc}	41

The control sample= 500 mg and there isn't any weight loss in the control sample. a significant different for *C. cephalonica* at ($p < 0.05$); b significant different for *E. kuehniella* at ($p < 0.05$); c significant different for *T. confusum* at ($p < 0.05$); d significant different for three insect tested together at ($p<0.05$)

Table 2: Total number of offspring resulted from tested insects *C. cephalonia, E. kuehniella* and *T. confusum* after 2 months of storage

Treatments	Number of progeny (Mean ±S.D.)		
	Larvae	pupae	adult
C. cephalonica	487.7 ±16.2^{bcd}	78.3 ±2.9^{bd}	16.7 ±1.5^{cd}
E. kuehniella	256.7 ±10.4^{acd}	56 ±4^{ad}	9 ±1^{cd}
T.confusum	522.7 ±5.7^{abd}	-	865 ±21.5^{abd}
C. cephalonica E. kuehniella T. confusum	375 ±10.4^{abc}	46.3 ±3.8^{ab}	300 ±9^{abc}

There isn't insect population was detected in the control sample, a significant different for *C. cephalonica* at ($p < 0.05$); b significant different for *E. kuehniella* at ($p < 0.05$); c significant different for *T. confusum* at ($p < 0.05$); d significant different for three insect tested together at ($p < 0.05$)

Table 3: Biochemical changes induced in the total protein of infested flour by *C. cephalonia, E. kuehniella* and *T. confusum* after 2 months of storage

Treatments	Total protein(mg/g) Mean ±S.D.	
	Sieving flour	Residual flour
Control	2.8 ±0.4^{bce}	
C. cephalonica	6.3 ±1.8^{acde}	40.9 ±2.9^{acde}
E. kuehniella	14.7 ±0.8^{abe}	46.8 ±1.7^{abde}
T.confusum	15.8 ±1.2^{abe}	4.8 ±0.5^{cbe}
C. cephalonica E. kuehniella T. confusum	32.9 ±2.3^{abcd}	51.7 ±2.1^{abcd}

Significant different for control at ($p < 0.05$); b significant different for *C. cephalonica* at ($p < 0.05$); c significant different for *E. kuehniella* at ($p<0.05$); d significant different for *T. confusum* at ($p < 0.05$); e significant different for three insect tested together at ($p < 0.05$)

Biochemical analysis of infested flour exhibited significant differences among different biochemical parameters (total protein and total carbohydrate) in the sieved and residual flour after infestation by *C. cephalonia, E. kuehniella* and *T. confusum.*

The results tabulated demonstrated significant increase in the protein contents of the sieved and residual flour after 2 months from infestation. Maximum increase of total protein in the sieved flour was 32.9 mg/g which was observed in the three insects tested together followed by 15.8 mg/g for *T. confusum*, 14.7 mg/g for *E. kuehniella* and 6.3 mg/g for C. *cephalonia* as compared to 2.8 mg/g with normal control (Table 3). Also, the maximum increase of total protein in the residual flour was 51.7 mg/g observed in the three insects tested together followed by 46.8 mg/g for *E. kuehniella*, 40.9 mg/g for C. *cephalonia* and 4.8 mg/g for *T. confusum* as compared to 2.8 mg/g with normal control (Table 3).

There was a significant decrease in the monosaccharaides of the infested sieved and residual flour after 2 months of storage. Maximum decrease of monosaccharaides in the sieved and residual flour respectively was 25.1 and 15.2 mg/g observed in *C. cephalonia* followed by 22.4 and 11.3 mg/g for *E. kuehniella*, 18.7 and 7.2 mg/g for the three insects tested together and 12.2 and 7.2 mg/g for *T. confusum* as compared to 29.5 mg/g with normal control (Table 4). Also, the determination of disaccharides in the sieved and residual flour recorded significant decrease in the disaccharides of sieved and residual flour respectively was 15.5 and 14.8 mg/g in the *C. cephalonia* followed by 14.6 and 10.7 mg/g for *E. kuehniella* and 12.4 and 9.9 mg/g for the three insects tested together. Finally, 7.3 and 4.7 mg/g for *T. confusum* as compared to 17.7 mg/g with normal control (Table 4).

Grains and flour are susceptible to insect infestation and contamination by different types of insects and fungi starting from the field (grain), grain storehouse, milling and flour storehouse. The interaction between insect infestation, fungal level and mycotoxins contamination in wheat flour was investigated in this part of experiments after two months of storage. The results in Table 5, show that the control (without insect infestation) wheat flour samples under investigation had a very low fungal count (log 1.28), i.e., 20 cfu/gm.

The sieved and residual wheat flour samples infested with *Corocyra cephaloneica* and those infested with *T. confusum* had log total fungal counts of 4.11, 3.52, 4.34 and 2.64 cfu/gm, respectively. Log total fungal count of sieved and residual flour infested with *E. kuehniella* was 3.39 and 2.36 cfu/gm, respectively.

Table 4: Biochemical changes induced in the total carbohydrate of infested flour by *C. cephalonia, E. kuehniella* and *T. confusum* after 2 months of storage

Treatments	Mono. (mg/g) Mean ±S.D.		Di. (mg/g) Mean ±S.D.	
	Sieving flour	Residual flour	Sieving flour	Residual flour
Control	29.5 ±0.9^{bcde}		17.7 ±0.4^{bcde}	
C. cephalonica	25.1 ±1^{acde}	15.5 ±1.2^{acde}	15.5 ±1.4^{acde}	14.8 ±0.9^{acde}
E. kuehniella	22.4 ±1.1^{abde}	11.3 ±0.7^{abde}	14.6 ±0.7^{abde}	10.7 ±0.4^{abd}
T.confusum	12.2 ±0.9^{abce}	4.5 ±0.8^{abce}	7.3 ±0.4^{abce}	4.7 ±0.3^{abce}
C. cephalonica E. kuehniella T. confusum	18.7 ±1.6^{abcd}	7.2 ±0.7^{abcd}	12.4 ±0.7^{abcd}	9.9 ±0.8^{abd}

a significant different for control at ($p < 0.05$); b significant different for *C. cephalonica* at ($p < 0.05$); c significant different for *E. kuehniella* at ($p<0.05$); d significant different for *T. confusum* at ($p < 0.05$); e significant different for three insect tested together at ($p < 0.05$)

Table 5: Total fungal counts in sieving and residual flour infested by *C. cephalonia, E. kuehniella* and *T. confusum* after 2 months of storage

Treatments	Log fungal counts	
	Sieving flour	Residual flour
Control	1.28 ^{bcde}	
C. cephalonica	4.45 ^{ace}	3.52^{acde}
E. kuehniella	3.39^{abd}	2.36^{abe}
T.confusum	4.34^{ace}	2.58^{abe}
C. cephalonica E. kuehniella T.confusum	3.30^{abd}	2.95^{abcd}

a significant different for control at ($p < 0.05$); b significant different for *C. cephalonica* at ($p < 0.05$); c significant different for *E. kuehniella* at ($p<0.05$); d significant different for *T. confusum* at ($p < 0.05$); e significant different for three insect tested together at ($p < 0.05$)

Meanwhile sieved and residual wheat flour infested with the three insect populations together had log total fungal counts of 3.3 and 2.95 cfu/gm respectively.

Fungal species and genera were isolated from control (without insect infestation) flour samples and from those infested with insects (Table 6). Seven mold species belonging to three genera were isolated from control wheat flour samples. They were *A. flavus, A. flavus* var. *columinaris, A. flavus* forming sclerotia, *A. oryzae, E. violaceae, P. chrysogenum* and *P. purpurogenum*. Seven fungal species belonging to four genera were isolated from wheat flour infested with *C. Cephalonia* namely *A. flavus, A. flavus var. columinaris, A. flavus forming* sclerotia, *A. niger, A. oryzae, Penicillium chrysogenum, Penicillium purpurogenum, Cladosporium sphaerospermum, Eurotium repens*.

In the case of wheat flour infested with *E. kuehniella*, 8 fungal species belonging to three genera were isolated. Meanwhile, 9 fungal species belonging to three genera were isolated from wheat flour samples infested with *T. confusum*. At the same time, 10 fungal species belonging to 4 genera were isolated from flour samples infested with the three insects under investigation together.

Cereals, flour and other crops are susceptible to attack by various species of fungi, many of which produce secondary toxic metabolites called mycotoxins. The presence of these mycotoxins in food commodities causes hazardous effects to humans and animals. Therefore, aflatoxins and ochratoxin A were detected in control flour samples and in insect-infested flour samples. Table (7) indicates that only aflatoxins (B_1, B_2) were detected in all tested flour samples, but at different levels. Very weak detection was observed in control flour samples and in flour samples infested with *E. kuehniella*, while moderate detection was observed in flour samples infested with *C. cephalonica* and *T. confusum*. Weak detection of AFB_1 and AFB_2 was observed in wheat flour samples infested with the three insects together.

Ochratoxin A was not detected in control flour samples and in flour samples infested with *C. cephalonica*, while ochratoxin A was detected in wheat flour infested with *E. kuehniella, T. confusum* and with the three insect populations altogether (Table 7).

Discussion

The apparent weight loss is defined as the difference in the weight of commodity before and after the practical infestation by insects. So, the total weight loss caused by infesting insects due to their contamination and the actual material they consume. The maximum residual flour was reported in our study in the sample infested by *C. cephalonia* and *E. kuehniella* because the larvae produce silken threads which develop into dense webbing. These silken threads may also form galleries (Ayyar, 1934). In heavy infestation, the product becomes tightly matted together with webbings (Kamel and Hassanein, 1967).

Results indicate that all flour samples suffered losses but the percentage of weight loss may be arranged in descending order as follows: *C. cephalonica* > three insects tested together > *E. kuehniella* > *T. confusum*. The effects of insect infestation on the weight loss of stored products draw the attention of many researchers. Moore *et al.* (1996) carried out a study to determine losses caused by

Table 6: Fungal species isolated from control and infested flour with insects by *C. cephalonia*, *E. kuehniella* and *T. confusum* after 2 months of storage

Treatments	Sieving flour	Residual flour
Control	*Aspergillums flavus*	
	A. flavus var. *columinaris*	
	A. flavus forming *sclerotia*	
	A. oryzae	
	Emercilla violaceae	
	Penicillium chrysogenum	
	P. purpurogenum	
C. cephalonica	*A. flavus*	*A. flavus*
	A. flavus var. *columinaris*	*A. flavus* var. *columinaris*
	A. flavus forming *sclerotia A. niger*	*A. oryzae*
	A. oryzae	*C. sphaerospermum*
	A. parasiticus	*E. repens*
	P. chrysogenum	*P. chrysogenum*
		P. purpurogenum
E. kuehniella	*A. flavus*	*A. flavus*
	A. flavus var. *columinaris*	*A. flavus* var. *columinaris*
	A. nidulance	*A. flavus* forming *sclerotia A. mellus*
	A. mellus	*A. niger*
	Cladosporium sphaerospermum	*A. oryzae*
	P. purpurogenum	*P. chrysogenum*
		P. purpurogenum
T. confusum	*A. flavus*	*A. flavus*
	A. flavus var. *columinaris*	*P. chrysogenum*
	A. flavus forming *sclerotia A. nidulance*	
	A. niger	
	A. oryzae	
	A. parasiticus	
	E. quadrilianiata	
	Eurotium chevalieri	
	P. purpurogenum	
C. cephalonica	*A. flavus*	*A. flavus*
E. kuehniella	*A. flavus* var. *columinaris*	*A. flavus* var. *columinaris*
T. confusum	*A. mellus*	*A. mellus*
	A. niger	*A. niger*
	A. oryzae	*A. oryzae*
	A. parasiticus	*A. parasiticus*
	E. quadrilianiata	*C. sphaerospermum*
	P. chrysogenum	*E. repens*
	P. purpurogenum	*E. quadrilianiata*
	E. quadrilianiata	*P. chrysogenum*
	P. chrysogenum	*P. purpurogenum*
	P. purpurogenum	*E. quadrilianiata*
		P. chrysogenum
		P. purpurogenum

Table 7: Detection of mycotoxins in control and infested flour by *C. cephalonia*, *E. kuehniella* and *T. confusum* after 2 months of storage

Treatments	Aflatoxins (B_1, B_2)		Ochratoxin A	
	Sieving flour	Residual flour	Sieving flour	Residual flour
Control	+	+	-	-
C. cephalonica	+++	+	-	-
E. kuehniella	+	+	+	-
T.confusum	+++	+	+++	+
C. cephalonica E. kuehniella T. confusum	++	+	++	-

+ weak; ++ moderate; +++ strong; - No detection

Sitotroga cerealella in dent corn. A single moth consumed an average of 32.9 mg during its development in a kernel of dent corn, which amounts to 10.35% weight loss per kernel per insect. Campbell and Sinha (1976) determined the damage done to stored wheat caused by larval and adult feeding of *Cryptolestes ferrugineus*, *Rhyzopertha dominica* and *Sitophilus granaries* by feeding individual insects on single wheat kernels. *Sitophilus granaries* caused 60%, *Rhyzopertha dominica* 17% and *Cryptolestes ferrugineus* 4% weight loss in single kernels weighing a mean of 29.5 mg. Tadele *et al.* (2011) discovered a mean weight loss of 67.1 and 6.9% recorded after infestation by 50 insects 200 g

grain after 90 days for *Prostephanus truncates* and *Sitophilus zeamais*, respectively. Recently Baoua *et al.* (2015) reported that *C. cephalonica and Tribolum spp* induced 17.1% of weight loss after seven months storage period on mill.

There was a significant correlation between the amount of flour consumed and insect population. Flour consumption by insects may be arranged in descending order as follows: three insects tested together > *C. cephalonica* > *T. confusum* > *E. kuehniella*, on the other hand insect population may be arranged as: *T. confusum* > three insects tested together > *C. cephalonica* > *E. kuehniella*. The exception was found in the case of *T. confusum* in spite of the fact that the population count was greater than other insects but it occupied the third rank in flour consumption.

Authors attributed this result to the size of *T. confusum* larvae and adults in comparison with the larvae of *C. cephalonica* and *E. kuehniella.* Arthur *et al.* (2012) studied the impact of *Rhyzopertha dominica* on milling rice after seven weeks of storage. The number of progeny produced by the parental adults positively correlated with feeding damage.

Infestation of flour by the *C. cephalonica, E. kuehniella and T. confusum* or a mixed population not only affected the quantity of flour but also influenced the quality parameters. The principle causes of loss in quantity and quality of cereals and products are the stored grain insects. According to Metcalf and Flint (1962) the pests of stored products are the most expensive of all insects, because they feed products that have been grown, harvested, manufactured and stored. There was a significant increase in the total protein contents in the all sieved and residual flour samples after infestation. Some literature showed an increase in protein contents, whereas others showed a reduction in the protein contents. For example, (Jood and Kapoor, 1992a, b; 1993) reported that *T. granarium* and *R. dominica* or a mixed population of both insect species reduced the protein content of wheat, maize and sorghum grains. On the other hand Samuels and Modgil (2003) stated that an increase in insect infestation and storage period significantly increase proteins content. Also, Ahmedani *et al.* (2009) reported an increase in the total protein of wheat varieties after six months from infestation by *Trogoderma granarium*.

On total carbohydrate determination, our results reported a significant decrease in the monosaccharaides and disaccharides in the all sieved and residual flour sample tested. These results are in agreement with the findings of Singh *et al.* (2013) who studied insect infestation by *Rhizopertha dominica* which induced a 13.5% decrease of total carbohydrate content. Also, Jood *et al.* (1993) found a significant reduction in carbohydrate contents of wheat, maize and sorghum when artificially infested with *T. granarium* and *R. dominica*. The results are also in line with the findings of previous researchers such as Hameed *et al.* (1984) who observed a significant decrease in carbohydrate contents of wheat grains due to the attack of *T. granarium* larvae. These results were confirmed by Daniel *et al.* (1977), Jood *et al.* (1996b), Prabhakumary and Sini (2008) and Ahmedani *et al.* (2009).

Results in Table 5, revealed a very low fungal count in control flour samples. This might be due to the effect of gamma irradiation (3.0 kGy), which was used for insect disinfestation of flour before artificial infestation, on destruction of fungal cells. Many investigators reported that irradiation in the range of 2–5 kGy greatly reduced total bacterial and fungal counts of different food commodities including grain and flour (Hammad *et al.*, 1987; Supriya *et al.*, 2014; Lung *et al.*, 2015).

The infestation of flour with these insects greatly increased total mold population , could be attributed to the generation of metabolic heat and water by insects in stored flour which increase the water activity (a_w) and temperature of flour to a level suitable for fungal growth and multiplication as reported by Mills (1986). Additionally, these insects carried fungus spores in their body (which act as vectors of fungi) that increased fungal counts. This increase in mold counts indicates possible synergistic interaction between insects and fungi. The high level of molds and insect infestation cause deterioration of flour quality at industrial processes and some of these molds produce mycotoxins, which pose a risk to the humans who consumed it. Generally, it is obvious from the results in Table (5) that the total fungal counts of all insects infested with flour samples were much higher than those of control flour samples and the extent of the increase was dependent on the type of the insect. This indicates a positive relationship between insect infestation and fungal counts. It is also clear from the results that total fungal counts of flour infested by a mixed population of the three insects under investigation were lower than that of flour infested with individual insects might be due to possible antagonistic interaction between the mixed population of insects and fungi.

Many fungal species belonging to many genera were isolated from wheat flour samples (Table 6). It is clear that *A. flavus* was more commonly found in control flour samples, as three different species of *A. flavus* were isolated from it. Many other investigators have isolated similar species of fungi from wheat flour. Doolotkeldieva (2010) and Al-Dfiery and Merjan (2015) found that the major genera of molds isolated from wheat flour and stored wheat flour according to decreasing frequency were *Aspergillus, Penicillium, Fusarium, Cladosporium, Alternaria, Mucor, Rhizobacteria, Trichoderma, Rhizopus, Nigrospora, Bipolaris* and *Macrophomina.*

It is worthy to mention that *A. niger, A. mellus, A. nidulance, Cladosporium sphaerospermum, Emercilla quadrilianiata* and *Eurotium repens* were only isolated from infested flour samples with insects. These fungi might be introduced through insects i.e., the insects act

as vectors of these fungi. The major fungi species found in flour (either control or infested) samples belonged to the genera *Aspergillus* and *Penicillium*. Also, it was reported that the major fungal species which were found in wheat grain and flour belonged to *Aspergillus* and *Penicillum* (Berghofer *et al.,* 2003; Mashinini and Dutton, 2006).

The detection of aflatoxins B_1, B_2 and ochratoxins A in insect infested flour samples suggests a relation and synergistic interaction between fungal species, mycotoxins contamination and insect infestation.

Food and Agricultural Organization (FAO) estimated that as much as 25% of foodstuffs worldwide are contaminated with mycotoxins leading to significant losses (Kabak *et al.,* 2006). Sweeney and Dobson (1998) reported that aflatoxins are a major class of mycotoxins produced mainly by two species of *Aspergillus*: *A flavus and A. paraciticus*. The obtained results about isolated fungi, i.e. the isolation of *A. flavus* from all flour samples under investigation confirm the detection of aflatoxins B_1 and B_2 in flour.

Many mold species can grow in grains and flour and produce ochratoxin A. Among them there are *A. ochreaceus, P. virdicatum, P. cyclopium, P. verricossium, A. niger, A. mellus* and *A. carbonarius* (Abarca *et al.,* 1994; Heenan *et al*., 1998; CAST, 2003). It is obvious that none of the mentioned fungi are known to produce ochratoxin A, which was isolated from flour samples either controls or insect infested. Thus the detection of ochratoxin A in some flour samples could be attributed to the fact that the mycotoxins may be found in the product without presence of mycotoxigenic fungi, as the mycotoxins may persist long after vegetative growth of fungi has occurred and the molds have died.

In conclusion, such results indicate the ultimate need for suitable methods that are effective in the disinfestation of insects and in the elimination of fungal contamination of wheat flour. The effectiveness of irradiation as a physical-cold process is well recognized to achieve this purpose but the establishment of suitable doses of irradiation for insect and microbial control with standards for food safety required mutual cooperation between different specialties such as entomology, microbiology and food irradiation departments.

Thus, we recommend the use of irradiation technology as a phytosanitary treatment for insects and as a microbial decontamination to overcome quarantine barrier and to improve the quality of wheat flour.

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