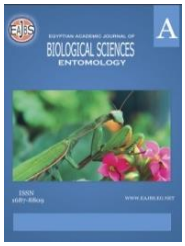
Egypt. Acad. J. Biolog. Sci., 13(2):195-204 (2020)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Does Flour Infested with Stored Grain Insects Causes Cytotoxicity of Human Skin and Lungs Cells?

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ARTICLE INFO
Article History
Received:1/4/2020
Accepted:15/5/2020

Keywords:
Cyotoxicity- stored grain -insects- food contamination- human cell.

ABSTRACT

In the present study two different cell cultures, derived from human skin melanocyte (HFB4) and Human Lung Fibroblast (ATCC) (WI-38) were used to examine. Three insects from two orders; Lepidoptera: *Ephestia kueniella* and Coleoptera: *Trogoderma granarium* & *Tribolium castaneum* were reared to study. Lung cells were the most sensitive than melanocyte. *T. castaneum* was recorded the most cytotoxicity than other insects in general. *T. castaneum* was the higher toxic in melano cells while *T. granarium* was the higher toxic in lung cells. However, morphological changes in treated cells were observed compared to non treated cells.

Our results indicate that grains and its products infested with stored grain insects caused toxic and death to cells which it exposure.

INTRODUCTION

The flour is subject to attack by several flour beetles, including the red flour beetle (*Tribolium castaneum*), is a polyphagous, cosmopolitan pest, feeding mostly on stored flour and other milled cereal products, broken wheat and farm stored products. In severe infestation, the flour turns grayish and mouldy and has pungent, disagreeable odor making it unfit for human consumption, Suresh *et al*, 2001.

Khapra beetle, *Trogoderma granarium* (Everts) is one of the world's most destructive stored-product pests. In fact, it has been recognized as an A2 quarantine organism for EPPO (1981) and ranked as one of the 100 worst invasive species worldwide, Lowe (2005). Losses caused by *T. granarium* (Everts) have been reported to range from 0.2 to 2.9 % over a period of 1 to 10.5 months (Irshad *et al*, 1988).

Mediterranean flour moth, *Ephestia kueniella* is a common pest of cereal grains, especially flour. It is frequently found in warm places with stored grain products, such as flour mills and bakeries, where it can breed year-round. Flour mills have a particular problem with the Mediterranean flour moth because the caterpillars spin silk that clogs machinery. The most effective pest control strategy for this moth is the sanitation of facilities and sealing grain containers to prevent infestation, but some pesticides may also be used, Jacobs and Calvin (1988).

Traditionally, animal models have been used to examine the toxic potential of pesticides. With the discovery of a greater number of new pesticides, the feasibility of using in vivo models to screen for toxicity becomes impractical with respect to time, ethical, and

cost considerations. In vitro cell, culture systems have proved to be useful for toxicity prediction on target organs by chemicals or drug exposure, Davila *et al.* (1998). Cell-based systems have become popular for studying interactions of chemicals with intact cells because it offers high-level integration. Various in vitro cell systems have been developed for studies of toxicity; Davila *et al.* (1998), Li *et al.* (2003) & Jana *et al*. (2012).

A complementary approach to conventional methodologies includes *in vitro* systems that assess the toxicity of chemical mixtures and identify components that may adversely impact biological processes. Compared to animal models, *in vitro* assays are inexpensive, rapid, and reduce and refine related animal testing, Bandele *et al.* (2012).

Cytotoxicity is one of the most important methods for biological evaluation as it has a series of advantages, along with the preferred and mandatory items. Given this information, the ability to accurately measure cytotoxicity can prove to be a very valuable tool in identifying compounds that might pose certain health risks in humans, (Li *et al*, 2015). By using a cytotoxic compound, healthy living cells can either be induced to undergo necrosis (accidental cell death) or apoptosis (programmed cell death). Whereas apoptotic cell death is slower, more orderly, and is genetically controlled, the cells may undergo necrosis, in which they rapidly lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), Celik, 2018.

MATERIALS AND METHODS

Insects and Culture:

Random samples were taken from wheat and flour infected with different insects from grain and stored materials. Each sample was infected with one type of insect that was infected in the laboratory. Random samples were taken from wheat and flour infected with different insects from grain and stored materials. Each sample was infected with one type of insect that was infected in the laboratory. Three insects used in the research, Mediterranean flour moth, *Ephestia kueniella*; Khapra beetle, *Trogoderma granarium* and red flour beetle, *Tribolium castaneum* were reared in the laboratory of Department of Stored Products and Grains Pests. Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. The insects reared for several generations on wheat and wheat flour media. The wheat grains were sterilized at a temperature of 55ºc for 6 hrs. in order to eliminate any hidden infestation before using. A control sample of flour used for comparison.

Cell Line Sources:

Human normal melanocyte (HFB4) and Human Lung Fibroblast (ATCC) (WI-38) cell lines were cultured in 75-cm2 cell culture flasks using RPMI medium with 10 % fetal bovine serum (FBS) as a culture medium in Tissue Culture Unit, VACSERA.

Cytotoxicity Test:

Cytotoxicity assay was determined by MTT stain (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) as a water-soluble tetrazolium salt, which is converted to an insoluble purple MTT - formazan Complex by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. The formazan product is impermeable to the cell membranes and therefore it accumulates in viable cells. Cell lines were cultured in 75-cm2 cell culture flasks using RPMI medium with 10% fetal bovine serum (FBS) as a culture medium for Human normal melanocyte (HFB4) and Human Lung Fibroblast (ATCC) (WI-38). The cells (104 – 106 cells /well) were cultured in the plate 96 well-flat shapes. Incubate the plates 24 hrs. until cell attachment complete. The cells were treated with the flour infested by different insects and incubated for 48 hrs. Media present in cell culture plates were discarded. The plates were washed with sterile PBS three times. MTT was dissolved in PBS as (5 mg/ ml) and filtered through 0.22 µm syringe filters. Plates were inoculated with (20

µl/well) of the previously prepared MTT solution. Plates were incubated at 37°C for 4 hrs. MTT solution was discarded and plates were washed with PBS three times. At the end of the incubation period, the produced formazan will appear as dark crystals in the bottom of the wells. DMSO (50 µl/well) was added. Plates were shacked on a plate shaker for 30 minutes in order to dissolve the intracellular blue formazan complex. Immediately measure the absorbance at 570 nm using an ELISA plate reader, According to MTT Cell Proliferation Assay Instruction Guide (2011).

Viability calculated as the following equation:

Viability % = mean values of Eliza of untreated cells/mean values of Eliza of treated cells*100.

So, the toxic or dead cells = 100 - viable cells.

Cell Morphology Analysis:

Morphological alterations and cell damage were qualitatively determined on the basis of microscopic signs of cellular damage (granulation and vacuolization of cytoplasm, rounding off and detachment of cells from the bottom of the cultivation vessel, rupture of cells), were evaluated by using an inverted microscope (Carl Zeiss, Germany) at a magnification of 400× after 48-hour exposure to treated materials with LC50.

Statistical Analysis:

LC50%, the half-maximal of lethal concentration values were calculated using (Sigma Plot software).

RESULTS AND DISCUSSION

Table (1) show the effect of flour and wheat infected with different types of stored grain insects, namely, *Ephestia kueniella, Trogoderma granarium,* and *Tribolium castaneum* on human skin cells. Different concentrations used from each infested flour with one type of insect; 0, 6.25, 12.5, 25, 50, and 100 micrograms per ml of the solvent containing cells under study. The results indicated that as the concentration of the material treated with cells increased, the toxic or death effect of cells increased. The higher percentage of the toxic cell was 86.25 % (13.75 % viable) and recorded in skin cells treated with flour infested by *T. castaneum* at conc. 100 % of treated material followed by toxic cell 67.59 % (32.41 % viable) for *T. granarium* and then for *E. kueniella* 65.75 % cytotoxicity (34.25 % viable) compared by flour control of material (non-treated flour) 61.13 % (38.87 % viable) at the same concentration and control of cell (conc.0) 0% (100 % viable).

Table 1: Cytotoxicity of human melano cells (skin) HFB-4 treated with flour infested by stored grain insects

Conc. Ug/ml	ELISA results	Viability %	ELISA results	Viability %	ELISA results	Viability %	ELISA results	Viability %
0	1.408 ±0.30	100	1.932±0.19	100	2.009 ±0.062	100	1.714 ±0.23	100
6.25	1.215 ±0.10	86.27	1.533±0.49	79. 38	1.902 ±0.12	94.67	1.661 ±0.22	96.94
12.5	1.184 ±0.27	84.09	1.445±0.27	74.82	1.883 ±0.06	93.71	1.395±0.14	81.4
25	1.131 ±0.03	80.33	1.391±0.33	72	1.559 ±0.30	77.6	1.374±0.76	80.19
50	1.056 ±0.04	75	1.268±0.21	65.62	1.534 ±0.13	76.34	0.899±0.20	52.48
100	0.547 ±0.12	38.87	0.662±0.06	34.25	0.651 ±0.33	32.41	0.236±0.04	13.75

Table (2) shows the effect of flour and wheat infected with the same obvious different types of stored grain insects on human lung cells. Different concentrations used from each infected material with one type of insect; 0, 6.25, 12.5, 25, 50, and 100 micrograms per ml of cells under study. The results indicated that as the concentration of the material on cells increased, the toxic or death effect of cells increased. The higher percentage of cytotoxicity was 81.62 (18.38 % viable) and recorded in lung cells treated with flour infested by *T.*

castaneum at conc. 100 followed by 73.15 (26.85 % viable) for *T. granarium* and then control treatment where recorded lower percentage 71.65 % (28.35 % viable) than *E. kueniella* 70.77 % (29.23 % viable) compared by control of material (non-treated flour) (38.87 viable) at the same concentration and control of cell (conc.0) 0% (100 % viable).

Table 2: Cytotoxicity of human lung cells (WI-38) treated with flour infested by stored grain insects.

Conc. Ug/ml	ELISA results	Viability %	ELISA results	Viability %	ELISA results	Viability %	ELISA results	Viability %
0	2.241±0.04	100	2.063±0.1	100	1.821±0.1	100	1.928±0.15	100
6.25	1.77±0.16	78.97	1.909±0.1	92.49	1.694±0.22	93.01	1.687±0.18	87.48
12.5	1.675±0.07	74.73	1.78±0.2	86.25	1.566±0.27	85.96	1.645±0.23	85.30
25	1.629±0.17	72.69	1.728±0.07	83.73	1.394±0.28	76.55	1.540±0.36	79.89
50	1.609±0.13	71.79	1.178±0.3	57.08	0.753±0.28	41.34	0.678±0.12	35.18
100	0.635±0.11	28.35	0.603±0.1	29.23	0.489±0.06	26.85	0.354±0.1	18.38

The LC50 for each treated material calculated as shown in table (3). The data represented that as LC50 value was high, this means that the treated material was less in cytotoxicity and safer from other material or by another meaning the insects were less dangerous than others. On the other hand, flour infested by *T. castanium* was less value of LC50, so this insect is the most dangerous insect, affecting the toxicity of cells from other insects in melano cells and recorded 47.56 µg/ml followed by LC50 of *E. kueniella* which recorded 68.61 µg/ml then LC50 of *T. granarium* (74.1 µg/ml) compared with control (87.53 µg/ml). While in lung cells, flour infested by *T. granarium* was the less value of LC50, so this insect is the most dangerous insect, affecting the toxicity of cells from other insects in lung cells and recorded 37.83 µg/ml followed by LC50 of *T. castanium* which recorded 40.91 µg/ml then LC50 of *E. kueniella* (59.38 µg/ml) compared with control (66.1 µg/ml). The cell lines used in this study have also been used as model systems for testing skin for touch, lung to breath, and inhalation during storage.

Table 3: Lethal concentration of treated material which caused 50 % cyotoxicity (LC_{50}) of human skin and lung cells.

Treatment	Lethal concentration (50%) (LC_{50}) µg/ml	
	Melano cells	WI-38 cells
Control	87.53	66.1
E. kueniella	68.61	59.38
T. granarium	74.1	37.83
T. castaneum	47.56	40.91

Morphology of treated cells (Figs. 1&2) appeared that there are various morphological abnormalities were recorded. deterioration of cells and cell walls has also reduced, some areas devoid of cells were also noticed in the same culture (Figure 1- e & 2- d)., in addition to the infiltration and bleeding of cells due to the rupture of the cell walls and so failure to do their functions, Furthermore, cell shrinkage and the formation of blebs on cell surface finally resulted in the generation of apoptotic bodies which indicated to cell death (Figs. 1- d & 2 - e) compared with normal cell (Figs. 1&2 (a)).

These results indicated that lung more sensitive to toxic materials from skin cells and flour infested by *T. castaneum* and *T. granarium* insects were more caused to toxic and death of human cells. These results go in line with Yun *et al.* (2017) who reported that cytotoxicity varied depending on the pesticides, concentrations, and cell type. Cytotoxicity is a biomarker for pesticide exposure and risk assessment. Meanwhile, the high rate of cell toxicity due to

the flour infested with *T. castaneum* insect may be contributed to benzoquinone secretion that the insect secretes through glands on its body to defend itself. This substance is known to be dangerous and to causes some signs of kidney and liver failure. Flour beetles have glands that produce defensive secretions with repellent and irritant properties against predators (Ruther *et al*., 2001). Methyl-1, 4-benzoquinone (MBQ), and ethyl-1, 4-benzoquinone (EBQ) are the major components of these defensive secretions (Eisner *et al*., 1998). Many studies indicated that benzoquinones secreted by flour beetles may have toxic and carcinogenic effects on humans and animals, (El-Mofty *et al*., 1992 and Elhassanen and El-Mofty 2003). The results of the reviewed studies indicate that benzoquinones secreted by flour beetles infesting the flour or grains may have a toxic effect on humans and animals, Elbadawy *et al* (2015). This effect may be direct or indirect. Quinones can make infested flour unsuitable for human consumption (Phillips and Burkholder 1984). Quinones are both acutely toxic, allergenic, and even carcinogenic to human beings (Ladisch *et al*., 1967). In addition El-Mofty *et al*., (1992) the researchers of the study founded that, the baking temperature did not minimize the carcinogenic effect of biscuits made from flour infested with *T. castaneum* beetles and the mutagenic effects on mice were still evident after contaminated flour had been cooked and consumed, also, concluded that *Tribolium* spp. are the only storage pests producing carcinogenic and teratogenic contaminants. These compounds give an unpleasant smell to stored food and may be responsible for liver and spleen tumours in small vertebrates (El-Mofty *et al*., 1992). On the other hand, the high rate of toxicity of the lung cells due to the flour infected with the *T. granarium* insects may be due to the presence of dense capillaries or hairs on the insect's body, leading to the irritation and death of the cells. (CABI, 2019) reported that larvae of T. granarium insects have dense tufts of hastisetae inserted on the posterolateral parts of the abdominal and thoracic tergites; the tufts become larger and denser posteriorly. This is in addition to the presence of insect feces inside the flour and also the high moisture content of the food material resulting from an insect infestation, which in turn leads to the presence of fungi that produce aflatoxin, which may be a factor in cell death and intoxication. The infestation of grain and stored products by insects encourages the growth of fungi including those that produce mycotoxins (Aflatoxins) and results in contamination of commodities with insect bodies and waste products etc. Some of which are toxic, repulsive, or allergenic (Freeman, 1976). Insects also play a significant role in the dissemination and proliferation of microorganisms including mycotoxigenic fungi in food commodities (Weston and Rattlingourd, 2000). Aflatoxins (AFs) produced by some species of *Aspergillus* on legume, cereals, and feed. These toxins can cause several diseases to humans or animals like acute liver damage, cirrhosis, and tumor induction. Elbadawy *et al.* (2015) stated that aflatoxin levels increased with the storage period and insect density, insect infestation leads to increase in fungal invasion (including aflatoxigenic fungi) and this further enhances the levels of aflatoxins contamination (Reddy *et al*., 2005). The fungal contaminations not only a serious health risk to consumers but also diminishes the nutritional value and economic benefits of the food. These fungi contaminate seeds in the field, where they can grow as saprophytes in crop debris on the soil, and in warehouses. A source of primary contamination can be sclerotia formed in damaged grains and in healthy maize. During dry seasons the plants are more susceptible to insect invasions that carry spores, beginning the development of the fungi. Aflatoxins can remain a longer time after the producing fungi die, therefore, grains can have dangerous levels of AF although they have not a moldy appearance. Maize seeds can be invaded by fungi during plant formation, as well as in post-harvest, transportation, and storage periods. The fungi reduce viability, nutritional and sanitary qualities to seeds and grains, Magda and Pavel (2009). Physical factors include the environmental conditions conducive to fungal

colonization and mycotoxin production such as temperature, relative humidity, and insect infestation. Insect management: the level of insect damage influences the extent of mycotoxins contamination. Avantaggio *et al*. (2002) found that insect damage of maize is a good predictor of Fusarium mycotoxins contamination. Insects carry spores of mycotoxins producing fungi from plant surfaces to the interior of the stalk or kernels or create infection wounds through their feeding habits (Munkvold, 2003). Therefore, proper management of insect pests through any appropriate control strategy would reduce mycotoxins contamination problem. Mycotoxins usually enter the body via ingestion of contaminated foods, but inhalation of toxigenic spores and direct dermal contact are also important routes. Clinical symptoms preceding death included vomiting, diarrhea, hemorrhage, breathing difficulty, chest pain, blisters, headache, fatigue, and dizziness. In addition to nephritic congestion, autopsy findings included necrosis of the lining of the stomach and upper small intestine, lungs, and liver (Zain, 2011). To preserve quality in storage, it is necessary to prevent biological activity through adequate drying to less than 10% moisture, elimination of insect activity that can increase moisture content (Lanyasunya *et al*., 2005). From our results, indicated that the cells treated with the control materials (non-infested flour) also have highly cytotoxicity in both skin and lung cells this may be due to the fact that the flour is naturally stored until it is used and there is a study that showed that the untreated flour contains aflatoxin with storage and that this percentage of aflatoxin increases with the increase in the storage period, Elbadawy *et al.* (2015).

Conclusion:

Through our study in this research, we found that exposure of stored grains and products to insect infestation leads to the intoxication of cells, whether skin cells or lung cells of the living organism, which leads to their death or the appearance of some distortions in the cells and this can occur by touch, as what happened with human skin cells or by inhalation during handling, packaging, and storage of stored products. This indicates the seriousness of insects that infest stored grains and their products. Therefore, care should be taken with due regard to safe storage methods.

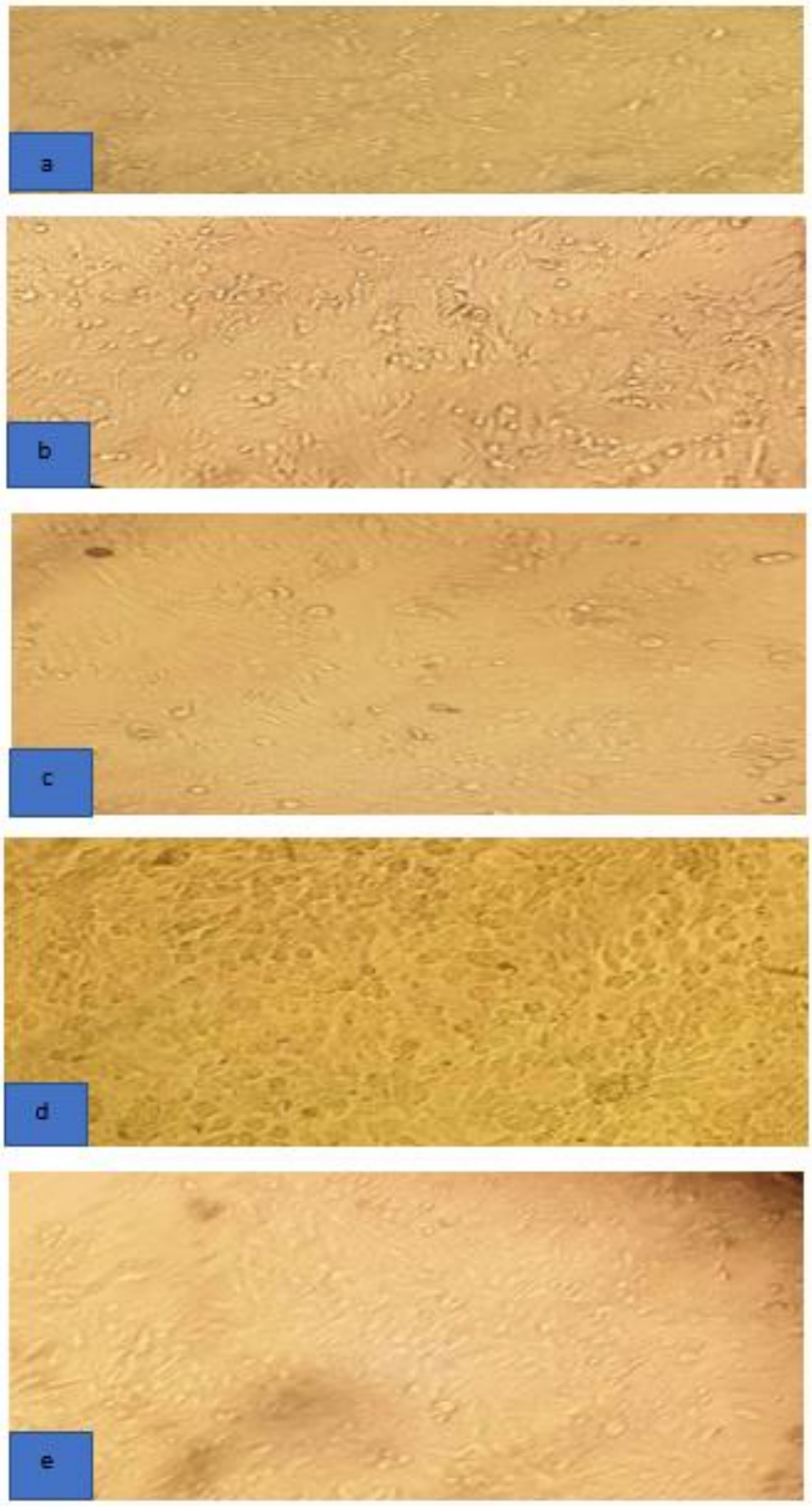

Fig. (1a-e): inverted microscope photos of changes in cell morphology of treated and non treated cells of human melano (skin) cells with flour infested by stored grain insects.
a- Normal of HFB-4 cell. **b**- Control (non-treated flour) on HFB-4 cell.
c- *E. kueniella* on HFB-4 cell. **d**- *T. granarium* on HFB-4 cell.
e- *T. castaneum* on HFB-4 cell

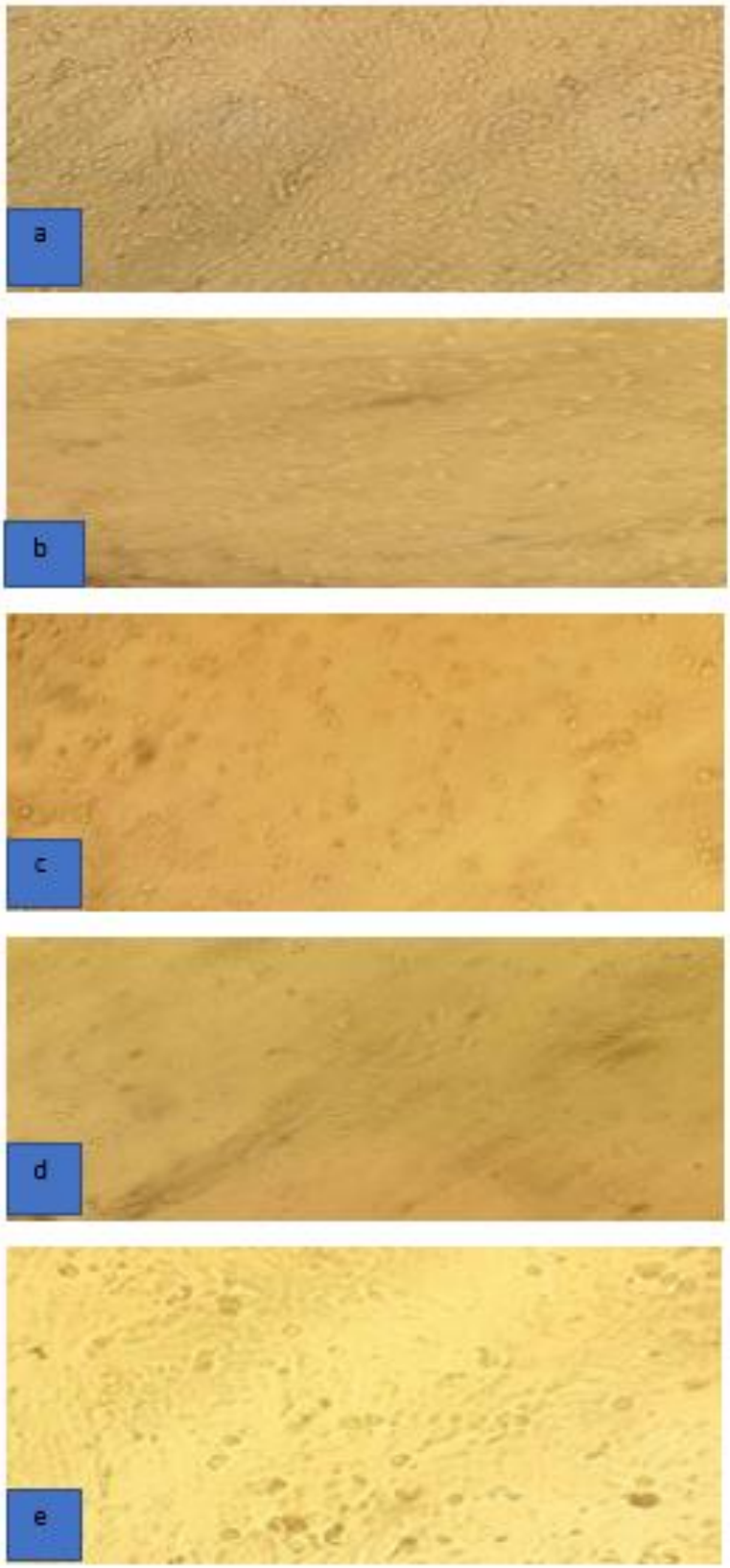

Fig. (2a-e): inverted microscope photos of changes in cell morphology of treated and non treated cells of human lung cells with flour infested by stored grain insects.

a- Normal of WI-38 cell. **b-** Control (non-treated flour) on WI-38 cell.
c- *E. kueniella* on WI-38 cell. **d-** *T. granarium* on WI-38 cell.
e- *T. castaneum* on WI-38 cell

REFERENCES

Avantaggio, G., Quaranta, F., Desidero, E., Visconti, A., 2002. Fumonisin contamination of maize hybrids visibly damaged by Sesamia. Journal of Science Food Agriculture, 83, 13–18.

CABI, current year. Fallopia japonica., 2019. In: Invasive Species Compendium. Wallingford, UK: CAB International. www.cabi.org/isc.

Davila, J. C., Rodriguez R. J., Melchert, R. B., Acostad., 1998. Predictive value of in vitro model systems in toxicology. Annals Review of Pharmacology and Toxicology, 38, 63.

Eisner, T., Eisner, M., Attygalle, B., Deyrup, M., Meinwald, J. 1998. Rendering the inedible edible: circumvention of a millipede's chemical defense by a predaceous beetle larva (Phengodidae). Proceeding of The National Academy of Sciences of United States of America, 95: 1108–1113.

Elhassaneen Y. A. and A. A. El-Mofty., 2003. Blood oxidant and antioxidant status in rats feeding with insect-infested wheat flour. Pakistan Journal of Biological Science, 6: 1354-1360.

El-Mofty, M. M., Khudoley, V. V., S. A. Sakr, S. A., Fathala, N. G., 1992. Flour infested with *Tribolium castaneum*, biscuits made of this flour, and 1, 4-benzoquinone induce neoplastic lesions in Swiss Albino mice. Nutrition and Cancer 17: 97-104.

Freeman, J. A., 1976. Problems of stored-products entomology in Britain arising out of the import of tropical products. Annals of Applied Biology 84, 120-124.

Irshad, M., Khan, A., Baloch, UK., 1988. Losses in wheat in public sector storage in Rawalpindi region during 1984-1985. Pakistan Journal of Agriculture Research, 9 (2) : 136-140.

Jana Polláková, Juraj Pistl, Natália Kovalkovičová, Tomáš Csank, Alica Kočišová, Jaroslav Legáth, 2012. Origin to Assess Cytotoxic Effects of the Pesticide Chlorpyrifos. Polish Journal of Environmental Studies. Vol. 21, No. 4 ,1001-1006.

Ladisch, R. K., Ladisch, S. K. and Howe, P. M., 1967. Quinoid secretions in grain and flour beetles. Nature, 215: 939-940.

Lanyasunya, T.P., Wamae, L.W., Musa, H.H., Olowofeso, O., Lokwaleput, I.K., 2005. The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. Pakistan Journal of Nutrition, 4 (3): 162–169.

Li W, Zhou J, Xu Y., 2015. Study of the *in vitro* cytotoxicity testing of medical devices (review). Biomedical Reports., 3:617-620.

Li, W., Choyd, F., Lam, M. S., Morgan, T., Sullivan, M. E., Post, J. M., 2003. Use of cultured cells of kidney origin to assess specific cytotoxic effects of nephrotoxins. Toxicology in Vitro, 17, (1), 107.

Lowe, S., Browne, M., Boudjelas, S., Depoorter, M., 2005. 100 of the World's Worst Invasive Alien Species: a selection from the global invasive species database. Invasive Species Specialist Group, World Conservation Union (IUCN). http://www.issg.org/booklet.pdf. Accessed 27 September 2005:2000.

Magda Carvajal and Pável Castillo, 2009. Tropical biology and conservation management. Vol.VII - Effects of Aflatoxins Contaminating Food on Human Health.

Mohamed, E. Zain, 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society, 15, 129–144.

MTT Cell Proliferation Assay Instruction Guide (2011):1 - 6.

Munkvold, G.P., 2003. Cultural and genetic approaches to managing mycotoxins in maize. Annal Review, Phytopathology, 41, 99–116.

OEPP/EPPO., 1981. Data sheets on quarantine organisms, *Trogoderma granarium*. Bulletin

121, 11 (1).

Omari, J. Bandele, Michael, F. Santillo, Martine, Ferguson, Paddy L.Wiesenfeld, 2012. Vitro toxicity screening of chemical mixtures using HepG2/C3A cells. Food and Chemical Toxicology; 50, (5): 1653-1659.

Phillips, J. K. and Burkholder, W. E., 1984. Health hazards of insects and mites in food. Insect Management for Food Storage and Processing, (F. J. Baur, (Ed), Amercian Association of Cereal Chemists, St. Paul, MN.,: 280-292.

Reddy, B. N., Brijitha, N., Raghavender, C. R., 2005. Aflatoxin contamination in insect damaged seeds of horsegram under storage. Mycotoxin Research, 21(3):187-91.

Ruther, J.; Reinecke, Tolasch, A. T., Hilker, M., 2001. Make love not war a common arthropod defence compound as sex pheromone in the forest cockchafer Melolontha hippocastani. Oecologia 128: 44–47.

Samy, S. Elbadawy, Hassan, B. H. Hussain, Tarik, El-desouky, Nilly, A. H. Abdelfattah, 2015. Influence of insect densities of *Tribolium castaneum* and *Oryzaephilus surinamensis* on the benzoquinone secretions and aflatoxins accumulation during wheat flour storage; Bulltin Entomological Socity Egypt, Economic Serial., 41: 111-125.

Steve, Jacobs and Dennis, Calvin, 1988. "Mediterranean Flour Moth". Penn State College of Agricultural Sciences.

Suresh S, White NDG, Jayas DS, Hulasare RB.,2001. Mortality resulting from interactions between the Red flour beetle and the rusty grain beetle. Proceedings of the Entomological Society of Manitoba, Volume 57: 11-18.

Tülay Aşkin Çelik, 2018. Introductory Chapter: Cytotoxicity. Chapter 1. :1-5.

Weston, P. A. and Rattlingourd, P. L. 2000. Progeny production by *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) on maize previously infested by *Sitotroga cerealla* (Lepidoptera: Gelechiidae). Journal of Economic Entomology, 93: 533-536.

XinmingYun, QingchunHuang, WenbingRao, CiyingXiao, TaoZhang, ZhifanMao, ZiyiWan, 2017. A comparative assessment of cytotoxicity of commonly used agricultural insecticides to human and insect cells. Ecotoxicology and Environmental Safety; 137: 179-185.