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Research Article

Cytotoxicity and Genotoxicity Evaluation of Some Stored Grain Insects and Their Infested Flour Using the BHK-21 Cell Line in an *In Vitro* Experimental Model

Nilly A. H. Abdelfattah,[1] **Enas A. Hasan**,[2] **and Sameer H. Qari**[3]

[1]*Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza 12619, Egypt*
[2]*The Holding Company of the Biological Products & Vaccines, 51 Wezaret Al Zeraa St., Agouza, Giza, Egypt*
[3]*Department of Biology, Genetics and Molecular Biology Central Laboratory, Aljumum University College, Umm Al-Qura University, Makkah, Saudi Arabia*

Correspondence should be addressed to Sameer H. Qari; shqari@uqu.edu.sa

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Globally, stored grain is vulnerable to pest infestation, resulting in significant economic losses for some crops. Wheat is one of the most important crops in the world. Many sucking, piercing insects infect wheat in the form of grains or flour and may produce toxic residues that are harmful to human health. The current study aimed to estimate the safe use of four stored grain insects by evaluating the potential genotoxic effects and cytotoxicity of crushed insects (*T. granarium*, *S. oryzae*, *R. dominica*, and *T. castaneum*) and their flour residues. MTT and comet assays were conducted to assess the effects of six concentrations of insect flour residues (0, 6.5, 12.5, 25, 50, and 100%) on the baby hamster kidney cell line (BHK-21). The lowest BHK-21 cell viability was noted against *T. granarium* (LC_{50}% 36.42 μg/ml) followed by *T. castaneum* flour (LC_{50}% 46.73 μg/ml) compared to the control (LC_{50}% 808.2 μg/ml). Significantly high DNA comet (%) was observed in the treatments of *T. castaneum* flour (18.8%), *S. oryzae* wheat (15.6%), *T. granarium* (15.4%), *T. castaneum* (13.6%), and *T. granarium* wheat (13.1%). FTIR spectra of stored grain insects and their flour residues identified various functional metabolite groups, including alkynes and phenols, which could enhance cell apoptosis and genotoxicity. *T. granarium*, *T. castaneum,* and their flour residues had the highest cytotoxic and genotoxic effects on the BHK-21 cell line. The current study concludes that insect residues in flour may have cytotoxic and genotoxic effects on living cells, potentially affecting public health, particularly after consuming *T. granarium* and *T. castaneum*-infested flour. Therefore, good storage of stored grains and their products is recommended.

1. Introduction

Globally, 25% to 33.33% of grain crops and approximately 50% to 60% of cereal crops are lost due to inefficient storage techniques [1]. Insects also severely damage the quality of stored grains. Stored grain pests preferentially consume grain embryos, which reduce the protein content, resulting in a lower germination percentage. Rust-red flour beetle (*Tribolium castaneum*), lesser grain borer (*Rhyzopertha dominica*), and rice weevil (*Sitophilus oryzae*) are important stored grain pests, which could limit grain exports by damaging grain quality [2]. The infestation of these insect pests increases the costs for grain growers either directly through farm pest control or indirectly through the control of weevils in bulk storage [3]. Grain insects are categorized into primary and secondary pests. Primary grain insects damage whole and unbroken grains, whereas secondary pests consume only milled products, dust, and damaged grains [3].

The application of pesticides for stored grain pest management poses an additional toxic threat to food commodities and the environment. Several animal models have been traditionally applied to assess pesticide toxicity. *In vivo* models are becoming impractical because of the latest

considerations regarding ethics, cost, and time [4]. The discovery and manufacturing of new pesticides have further reduced the applicability of *in vivo* models. Therefore, *in vitro* cell culture-based models have emerged as an efficient and swift technique for the assessment of pesticides, chemicals, and drug toxicity [5]. High-level integration of cell-based systems could effectively investigate chemical interactions with intact cells [6].

Multiple *in vitro* cell systems have been developed to investigate pesticide toxicities [6–8]. *In vitro* systems have emerged as a complementary approach for identifying the components of chemical mixtures and assessing their toxicity in biological processes. I*n vitro* assays are rapid and inexpensive compared to conventional animal testing [9]. Cytotoxicity is an important biological evaluation technique that offers a series of advantages. Accurate cytotoxicity measurement could efficiently identify health risks in humans [10]. Cytotoxic compounds could cause apoptosis (programmed cell death) and necrosis (accidental cell death) in healthy cells. Apoptotic cell death is slower and genetically controlled, whereas necrosis is characterized by rapid loss of cell membrane and cell lysis, leading to rapid cell death [11]. Ultimately, cell proliferation decreases, which causes lower cell viability [12].

The kidneys actively eliminate xenobiotics from the animal body [13]. Therefore, several studies have demonstrated the importance of kidney and liver cell cultures in toxicity screening before conducting *in vivo* trials on animals [6]. Apoptosis detection through acridine orange/ethidium bromide fluorescent double staining is considered an accurate and rapid technique [14]. Acridine orange could penetrate the plasma membrane of both viable and nonviable cells and bind with DNA to emit green fluorescence, whereas ethidium bromide could only penetrate nonviable cells with the ruptured cell membrane and bind with DNA to emit red fluorescence [15]. Metabolic processes and environmental factors cause cellular DNA damage at a rate of 1,000 to 1,000,000 molecular lesions/cell/day. Such lesions in approximately 6 billion bases (3 billion base pairs) of the human genome and critical genes could affect normal cellular functions and enhance the likelihood of cancer. The comet assay, or single-cell gel electrophoresis (SCGE) assay, is commonly applied to measure DNA damage in individual cells [16]. Damaged cellular DNA consisting of fragments and strand breaks is separated from intact DNA under an electrophoretic field that yields a classic "comet tail" shape under the microscope. The comet tail is measured to evaluate the extent of DNA damage either visually or by using image analysis software. It is a simple and efficient method to measure damaged and repaired DNA at the cell level. Negatively charged DNA fragments are pulled through agarose gel under an electric field, which appears like a comet [16]. The applicability of the comet assay has considerably increased during the last two decades for the assessment of DNA damage and repair [17]. The comet assay combines single-cell cytogenetic assays with biochemical detection of DNA single-strand breaks (strand breaks and incomplete excision repair sites), cross-linking, and alkali-labile sites (ALSs) [17, 18].

The infrared (IR) spectroscopy technique is widely used by chemists for determining and identifying compound structures [19]. The properties of chemical compounds differ due to various functional groups. IR spectroscopy is an economical, rapid, and nondestructive physical method that is universally applied for structural analysis. It could also be used as a source of physical parameters for determining the crystal lattice and eliciting empirical qualitative relationships between specimens [19–21].

Many populations are not inclined to eat flour infested with stored grain pests because they believe that it is inedible. Maybe this is logical if stored grain insects and their food contain toxic residues. The objective of the present study was to determine the safety of using four stored grain insects by assessing potential genotoxic effects and cytotoxicity of crushed insects (*T. granarium, S. oryzae, R. dominica, and T. castaneum*) and their flour residues. Their potential toxicity to the BHK-21 cell line and DNA was evaluated through MTT, comet assays, fluorescent microscopy, and FTIR.

2. Materials and Methods

2.1. Insects and Cultures. *Trogoderma granarium*, *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum* adults were reared in the Department of Stored Products Laboratory and Grains Pests, Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt. Multiple generations of insects were reared on wheat grains. The wheat grains were sterilized at 55°C for 6 h to eliminate hidden infestations before use. Insect cultures of the same age were initiated by placing the adults of each species separately in a jar (200 ml). The jars were covered with muslin cloths and fixed with rubber bands. For starting the experiment, 25 adults of each species were introduced separately into jars containing wheat or flour (500 gm) and placed in an incubator for three months (30 ± 2°C and 65 ± 5% R.H). Treatments and controls were prepared in triplicate under similar conditions [22]. After three months, insect samples and infested wheat and flour were collected for further analysis.

2.2. Cell Line Source. The baby hamster kidney cell line (BHK-21-ATCC® CCL-10™) was obtained from the Tissue Culture Unit, VACSERA, Giza, Egypt.

2.3. Cytotoxicity Assessment. The MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was conducted to estimate cell survivability according to Meerloo et al. [23]. MEM (H) medium was used to culture cells in 96-well plates and incubated at 37°C in a CO_2 incubator for 24 h. Then, the MEM (H) medium was supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 mg/ml), l-glutamine (3 mM), and 25 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), and the pH of the medium was adjusted to 7.2 by using bicarbonate solution. The cells were incubated at 37°C under humidified atmosphere (95% air and 5% CO_2) [24].

MTT (5 mg/mL stock in PBS) was added to each well, and the plates were further incubated for 4 h. Finally, supernatants were discarded, and DMSO (200 μL) was gently mixed into each well [25]. Different concentrations (0, 6.25, 12.5, 25, 50, and 100 μg/ml) were prepared from the cell containing solvent. The intensity of light absorbance was read by using an enzyme-linked immunosorbent assay (ELISA) plate reader BioTek (ELX-800) at 570 nm and calculated as follows: cell survival (%) = (sample × control)/control × 100.

2.4. Morphological Investigation under an Inverted Microscope. The cells treated at LC_{50} values were incubated for 48 hours and subjected to inverted microscopy (Carl Zeiss, Germany) at a magnification of 10x. The characteristic cellular damages such as cytoplasmic vacuolization and granulation, rounding off, rupturing of cells, and cell detachment from the plate bottom were observed to assess morphological alterations.

2.5. Apoptosis Assay with a Fluorescent Microscope. Cell apoptosis-related morphological changes were monitored under a fluorescent microscope (Leica DM LB2, Germany). Cells were treated with a dual stain of acridine-orange/ethidium bromide (AO/EB) and observed at 40x magnification [26].

2.6. Comet Assay. The single-cell DNA comet assay was carried out to study the DNA strand breakage in the BHK-21 cell line after treatment with *T. granarium, S. oryzae, R. dominica,* and *T. castaneum* flour residues. DNA damage was measured through single-cell gel electrophoresis by following the methodology of Singh et al. [27] with minor modifications.

2.7. Fourier-Transform Infrared (FTIR) Spectroscopy. The functional groups in *T. granarium, S. oryzae, R. dominica,* and *T. castaneum*-lyophilized aqueous extracts and infested flour were identified in comparison to control treatments by using Fourier-transform infrared (FT-IR) (FT/IR-6000 compact spectrometers, JASCO inc., Japan). A fine sample powder was mixed with potassium bromide (KBr) (Sigma-Aldrich, FT-IR grade) to prepare pellets. Spectra were recorded in a transmission mode from 4000 to 400 cm^{-1}. The FTIR spectroscopy interferogram was obtained, and functional groups were identified based on the wavenumber and absorption [28].

2.8. Statistical Analysis. The results are expressed as a mean ± SE, and significant differences between treatments and control conditions were compared using an ANOVA at the 5% probability level, and if so, Tukey's mean comparison test was used ($P \leq 0.05$). The data from time-mortality (survival) bioassays were subjected to a nonparametric survival analysis using Sigma Plot software (version 12.0) to obtain survival curves and estimates of the median survival time and calculate (50%) lethal concentrations (LC50 μg/ml). The tail moment (arbitrary unit) was counted as follows: length of DNA migration (pixel) × percentage (%) of migrated DNA.

3. Results

3.1. Population Dynamics. Table 1 presents the number of insects of each species used in the experiments after 3 months of rearing. Twenty-five insects of each species were separately released to 500 grams of the food environment, which included wheat for *T. granarium, S. oryzae,* and *R. dominica* and flour for *T. castaneum*. The highest numerical density (2613 insects) was noted for *T. granarium*, followed by *R. dominica* (765 insects) and *T. castaneum* (350 insects). The lowest numerical density (130 insects) was observed for *S. oryza*e.

3.2. Cytotoxicity Assessment. Table 2 depicts flour and wheat infested by *T. granarium*, *S. oryzae*, *R. dominica*, and *T. castaneum* and their impact on BHK-21 cell viability (%). Different concentrations of infested flour (0, 6.25, 12.5, 25, 50, and 100 μg/ml of the cells containing solvent) were used for each insect species. An overall decreased cell viability was noted after treatments. The highest cell viability of 77.03 ± 0.84 was observed at a concentration of 100% in *R. dominica*treatment, followed by 74.14 ± 4.61 cell viability in *S. oryzae* flour. The lowest cell viability of 31.69 ± 1.86 was observed at a concentration of 100% in *T. granarium*, followed by 32.56 ± 3.21 in *T. castaneum* treatment as compared to nontreated control cells (conc.0) (100% viable).

IC_{50} (%) values of each treatment were calculated for BHK-21 cells as shown in Table 2. The treatments (insects and their secretions) with higher IC_{50} (%) values were less cytotoxic and safer than other treatments. The lowest IC_{50} (%) value of *T. granarium* treatments against BHK-21 cells demonstrated it as the most damaging insect. It posed higher toxicity to cells (36.42 μg/ml), followed by IC_{50} (%) of *T. castaneum*-infested flour (46.73 μg/ml) and IC_{50} (%) of *T. castaneum* (65.04 μg/ml). *S. oryzae* and *R. dominica* (insects and infested flour) caused the lowest cytotoxicity that was noted as 410.30, 376.1, and 138.5 μg/ml, respectively, compared to positive control flour treatment (808.2 μg/ml).

3.3. Cell Morphology under an Inverted Microscope. Morphological investigations revealed various abnormalities in treated cells (Figures 1 and 2). The abnormalities mainly included the deterioration of cells, reduction of cell walls, and absence of cells in certain areas of the same culture in *S. oryzae* and *R. dominica* treatments. Furthermore, cells failed to perform their functions due to the infiltration and bleeding of cells after the rupturing of cell walls. Cell shrinkage and the formation of blebs on the cell surface were also noted, which finally led to the generation of apoptotic bodies. Also, the formation of these bodies in *T. granarium* and *T. castaneum* indicated cell death as compared to the normal cells.

Table 1: Population dynamics of adult insects after 3 months of rearing at 30°C and 65 ± 5 R.H.

Insect*	Control	*T. granarium*	*S. oryzae*	*R. dominica*	*T. castaneum*
Population dynamic (number)	0	2613a	765b	130d	350c

*Initial number of insects: 25/500 gm of the sample. The values with different letters are significantly different at $P < 0.05$.

3.4. Fluorescent Microscopy of Cell Apoptosis. Viable cells stained only by AO were bright green with an intact structure, whereas AO-stained early apoptotic cells depicted a bright green area in the nucleus in *T. castaneum* treatments (Figures 3 and 4). AO and EB-stained late apoptotic cells appeared red-orange with the condensation of visible chromatin as dense orange areas as in *T. granarium* treatments (Figure 4).

3.5. DNA Comet Assay. Table 3 demonstrates the amount of comet (%) in the kidney cells of mice exposed to stored grain insects and their infected flour. The largest cells were observed in rice weevil (*S. oryzae*)-infested flour treatment. The highest DNA damage was observed in *T. granarium* treatment, whereas *T. castaneum*-infested flour treatment caused the least comet detachment intensity (%), followed by *S. oryzae*. *T. granarium* treatment (insect and infested flour) and *T. castaneum* treatment exhibited the highest intensity of comet detachment from the cell nucleus, which is marked by white arrows (Figure 5). The moderate comet detachment intensity was observed in other treatments compared to control and normal cells, which are marked by blue arrows. The nuclei of these cells were attached, which are marked by green arrows.

Figures 5 and 6 demonstrate the comet assay-based DNA damage to BHK-21 kidney cells caused by stored grain insects. The degrees of damage in different treatments were estimated and labeled with arrows.

3.6. FTIR Analysis. FTIR analysis was carried out to characterize the infrared active functional groups in *T. granarium*, *S. oryzae*, *R. dominica,* and *T. castaneum*-lyophilized aqueous extracts and their respective infested flour. The uninfested flour served as a control. The results of the FTIR analysis are presented in Figures 7 and 8 and Table 4. The results indicated the presence of C-H (alkanes), O–H (alcohols and esters), N–H (amide), C≡N (nitrites), C☐O (amide and ketone), C–O (ether), C=O (anhydrides), NO_2 (nitro compounds), R-S-H (thiols), C–Cl (aryl chloride), C-Br (aryl bromide), and C–I (aryl iodide) in insects and their respective infested flour (Table 4). Figure 7 show that *T. granarium*, *S. oryzae*, *R. dominica*, and *T. castaneum* treatments presented varied peaks ranging from 620 cm^{-1} to 3276 cm^{-1}. The broad absorption peaks at 3331 cm^{-1} and 1630 cm^{-1} corresponded to N-H vibration bending amine and stretching C=C conjugated alkene, respectively [29]. A strong broad peak at 620 cm^{-1} referred to the C=C bending of alkene, whereas the peak at 3277 cm^{-1} represented the stretching of N-H amine and O-H groups in proteins [30]. The infrared active functional groups in insect-infested flour were more concentrated in the fingerprint region (<3250>900 cm^{-1}) than those in the uninfested control. The infrared active functional groups in insect-infested flour included –OH, (phenol and carboxylic acid), N–H (amide and amine), C–N (nitrile), C≡C (alkynes), H-C=O and C☐O (aldehydes, carboxylic acid, ester, amide, and ketone), C–F (aryl fluoride), C–O (ester, ether, and phenol), C–Cl (aryl chloride), and C–I (aryl iodide), as shown in (Figure 8 and Table 4). Nontreated grains (control) contained C-H (alkynes), O–H (phenol and carboxylic acid), N–H (amide and amine), C–O (amide and ketone), C–N (amide), C–F (aryl fluoride), C–O (phenol and ester), C-I (aryl iodide), C-Br (aryl bromide), and C–Cl (aryl chloride) functional groups.

4. Discussion

The study revealed the highest numerical density in *T. granarium* after 3 months of rearing under normal conditions than in other insects. These results are in line with the findings by a previous study [31]. They stated that the population growth of *T. granarium* (at 35°C) was 10–250 times higher than that of other stored grain insects. Therefore, it is considered a dangerous stored grain pest. Morrison et al. [32] have also reported *T. granarium* as an economically destructive pest of stored products that is quarantined in the USA.

The kidneys actively eliminate xenobiotics from the body. Therefore, the BHK-21 cell line (baby hamster kidney cells) was used in the present study to assess the toxic impacts of four important stored grain pests [13]. Multiple studies have established that the kidney and liver cell cultures could serve as efficient tools for toxicity screening before conducting animal-based*in vivo* investigations [6]. *T. granarium* treatments (insect or infested flour) presented the highest cytotoxicity values, followed by *T. castaneum*. Morphological examination and the apoptosis assay revealed cytotoxicity-related cell abnormalities, which included the improper shape of cells, formation of a bright green-colored nucleus, orange-colored cytoplasm of cells, and programmed cell death compared to normal cells and cells treated with uninfested flour (control). Cell wall rupture, change in cytoplasmic color, and cell separation were not observed in control cells [28].

T. granarium and *T. castaneum*-treated cells exhibited the largest comet appearance with clear DNA separation in the nucleus. The control cells were strictly attached to the nucleus without comet formation. The cytotoxicity in the treated cells could be attributed to the insect's gland secretions in food as a defense mechanism, which varies among insects. Several studies have established the presence of benzoquinone as a defense tool in the flour infested by *T. castaneum* [33, 34], *R. dominica* [35], and *S. oryzae* [36]. Abdelfattah and Hassan [37] have reported that *T. castaneum* and *T. granarium* infestations were more toxic to the human lungs and skin cells.

Table 2: Baby hamster kidney cell line (BHK-21) viability (%) at different concentrations of stored grain insects and infested flour as well as determination of IC_{50} %.

Conc. (μg/ml)	Control flour	Grinded insects				Flour			
		T. granarium	*S. oryzae*	*R. dominica*	*T. castaneum*	*T. granarium*	*S. oryzae*	*R. dominica*	*T. castaneum*
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
6.5	99.71 ± 3.75	95.69 ± 1.23*	95.64 ± 3.84*	95.93 ± 2.25*	96.51 ± 3.11*	99.13 ± 0.86	96.26 ± 3.75*	94.48 ± 1.08*	95.69 ± 5.1*
12.5	99.13 ± 4.28	91.38 ± 3.15*	85.47 ± 4.32*	95.35 ± 3.62*	83.14 ± 4.55*	96.51 ± 2.11*	92.82 ± 2.39*	89.83 ± 4.26*	91.38 ± 1.96*
25	98.26 ± 3.52	54.31 ± 4.21*	71.80 ± 5.37*	93.90 ± 2.22*	65.41 ± 3.48*	93.02 ± 3.88*	88.79 ± 0.99*	87.21 ± 0.87*	54.31 ± 2.78*
50	92.44 ± 2.56*	44.54 ± 2.05*	52.62 ± 3.33*	87.50 ± 3.48*	58.72 ± 4.01*	81.98 ± 2.65*	83.05 ± 3.85*	76.16 ± 2.44*	44.54 ± 3.46*
100	89.83 ± 4.21*	31.69 ± 1.86*	44.48 ± 2.69*	77.03 ± 0.84*	32.56 ± 3.21*	47.67 ± 3.48*	74.14 ± 4.61*	56.98 ± 4.10*	37.93 ± 1.32*
IC 50%	808.2	36.42	67.72	376.1	56.04	96.78	410.3	138.5	46.73

*Significant difference at $P = 0.05$ compared with the control; values represent the ratio of the mean of four replicates ± SE (standard error).

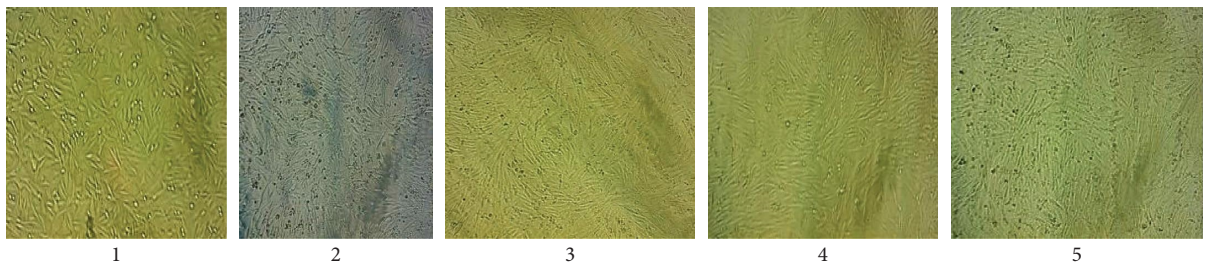

Figure 1: Morphology of baby hamster kidney cells (BHK-21cell line) under the inverted microscope after exposure to stored grains insects. (1) Normal cells (control), (2) *T. granarium*-treated cells, (3) *S. oryzae*-treated cells, (4) *R. dominica*-treated cells, and (5) *T. castaneum*-treated cells.

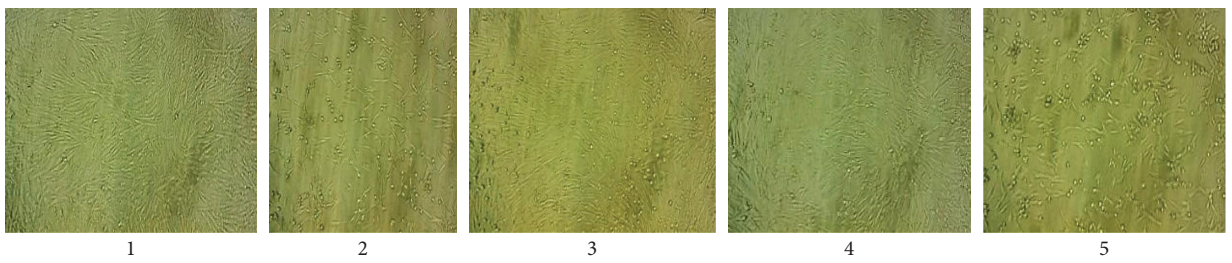

Figure 2: Morphology of baby hamster kidney cells (BHK-21 cell line) under the inverted microscope after exposure to insect-infested flour. (1) Cells treated with uninfested flour (control), (2) cells treated with *T. granarium*-infested flour, (3) cells treated with *S. oryzae*-infested flour, (4) cells treated with *R. dominica*-infested flour, and (5) cells treated with *T. castaneum*-infested flour.

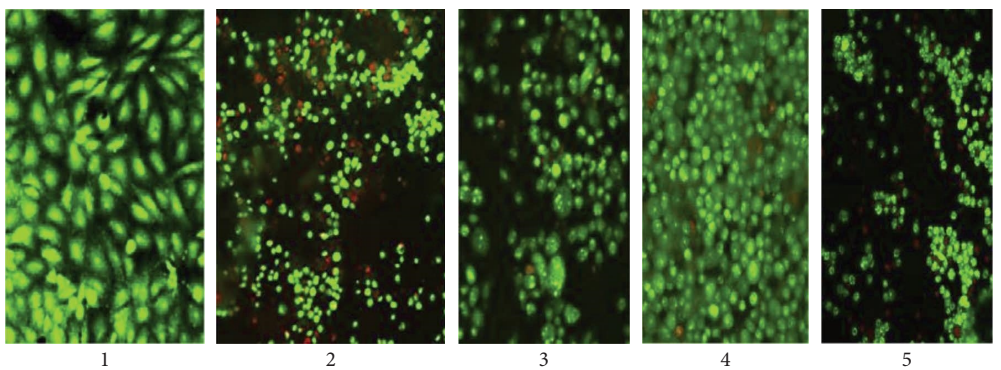

Figure 3: Apoptosis of baby hamster kidney cells (BHK-21 cell line) under the fluorescent microscope after exposure to stored grain insects. (1) Normal cells (control), (2) *T. granarium*-treated cells, (3) *S. oryzae*-treated cells, (4) *R. dominica*-treated cells, and (5) *T. castaneum*-treated cells.

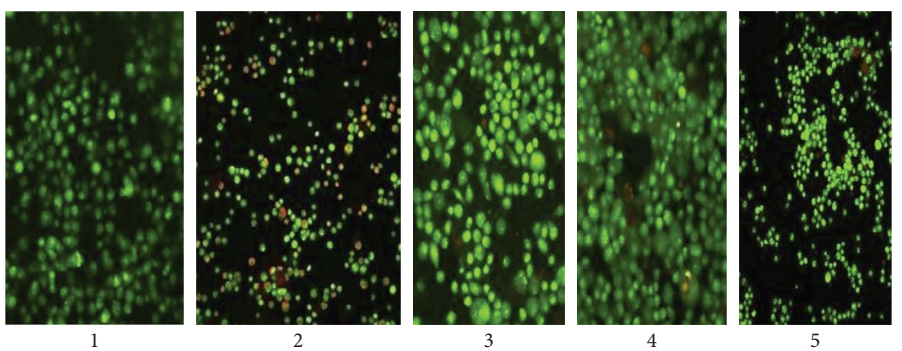

Figure 4: Apoptosis of baby hamster kidney cells (BHK-21 cell line) under the fluorescent microscope after exposure to insect-infested flour. (1) Cells treated with uninfested flour (control), (2) cells treated with *T. granarium*-infested flour, (3) cells treated with *S. oryzae*-infested flour, (4) cells treated with *R. dominica*-infested flour, and (5) cells treated with *T. castaneum*-infested flour.

Table 3: DNA comet assay of baby hamster kidney cells (BHK-21 cell line) after exposure to stored grain insects and their flour.

Treatments	Comet (%)	Tail length (px)	DNA in tail (%)	Tail moment	Olive moment
Control cell	10.4	6.77	15.76	1.13	1.18
T. granarium	15.4	7.85	26.45	3.02	2.36
S. oryzae	10.7	4.38	10.40	0.43	0.99
R. dominica	11.5	6.22	7.96	0.42	1.04
T. castaneum	13.6	3.63	7.00	0.26	0.75
Control flour	11.1	9.35	22.89	3.16	2.64
T. granarium wheat	13.1	6.25	10.89	0.65	1.26
S. oryzae wheat	15.6	5.35	8.82	0.51	0.91
R. dominica wheat	12.8	5.61	10.25	0.50	1.16
T. castaneum wheat	18.8	6.38	16.90	1.44	1.59

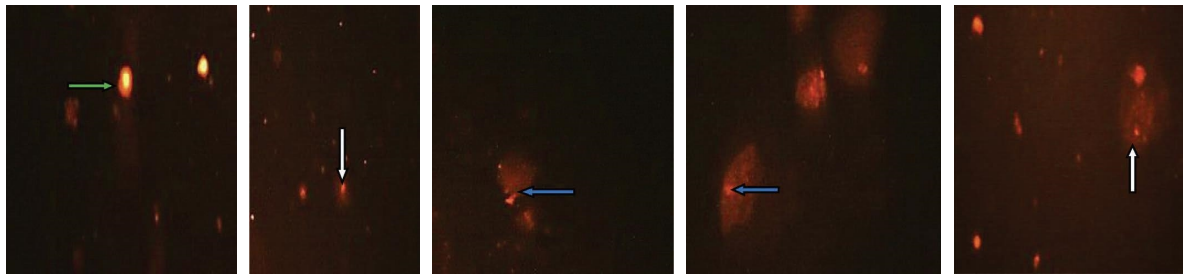

Figure 5: Comet assay images of baby hamster kidney cells (BHK-21 cell line) after exposure to stored grains insects. (1) Normal cells (control), (2) *T. granarium*-treated cells, (3) *S. oryzae*-treated cells, (4) *R. dominica*-treated cells, and (5) *T. castaneum*-treated cells. The green arrow represents intact nuclei, the white arrow represents the high degree of damage, and the blue arrow represents the low degree of damage.

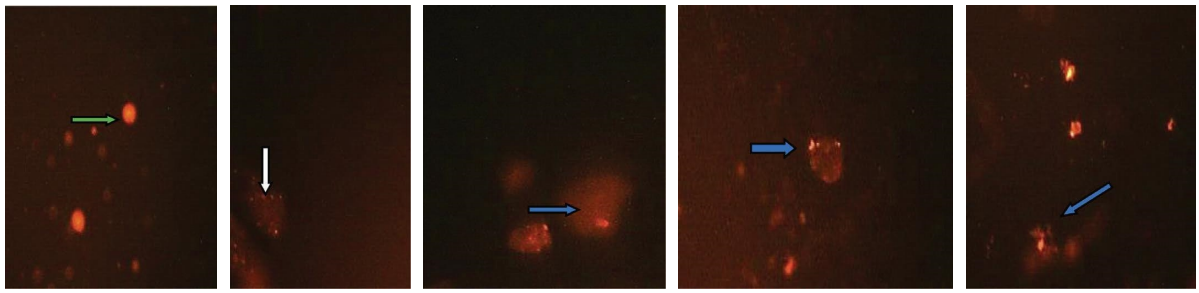

Figure 6: Comet assay images of baby hamster kidney cells (BHK-21 cell line) after exposure to insect-infested flour. (1) Cells treated with uninfested flour (control), (2) cells treated with *T. granarium*-infested flour, (3) cells treated with *S. oryzae*-infested flour, (4) cells treated with *R. dominica*-infested flour, and (5) cells treated with *T. castaneum*-infested flour. The green arrow represents intact nuclei, the white arrow represents the high degree of damage, and the blue arrow represents the low degree of damage.

Saquib et al. [38] have demonstrated cytotoxic and necrotic effects of phorate (organophosphate insecticide) exposure on human amniotic epithelial (WISH) cells. Similarly, insect infestations could exert cytotoxic and genetic effects on the target organism through smelling, touching, or eating. These findings have highlighted the necessity of safe grain storage to maintain quality and avoid pest infestations. In countries where *T. granarium* has been declared a quarantine pest, highly efficient monitoring tools are applied at seaports and international airports to counter the ongoing threat of *T. granarium* invasion [39].

A close comparison with nontreated grains (control) depicted that C–O (ether), C–Cl (aryl chloride), and C–I (aryl iodide) functional groups located in the fingerprint region were mainly responsible for the cytotoxicity of flour infested by four stored grain insects (Figure 8) (Table 4). Based on the chemical composition of active ingredients, Zacharia [40] has classified cell apoptosis into four main groups (organochlorines, phenols, alkaloids, and alcohol). Interestingly, the IR peaks at 3200, 995, and $<840\ cm^{-1}$ corresponded to the oxidative stress and base intercalations [28, 29, 41]. The functional groups identified by FTIR spectra in four stored grain insects could be

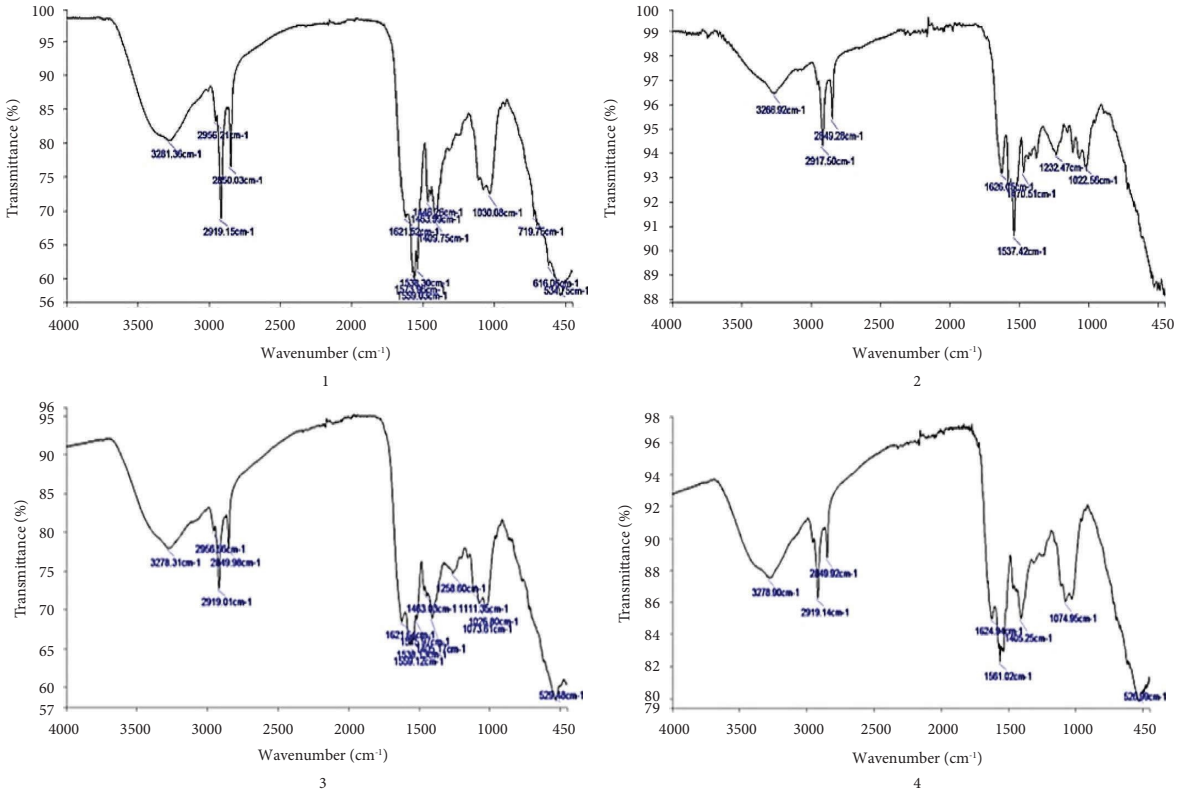

Figure 7: FTIR of stored grain insects. (1) *T. granarium*-treated cells, (2) *S. oryzae*-treated cells, (3) *R. dominica*-treated cells, and (4) *T. castaneum*-treated cells.

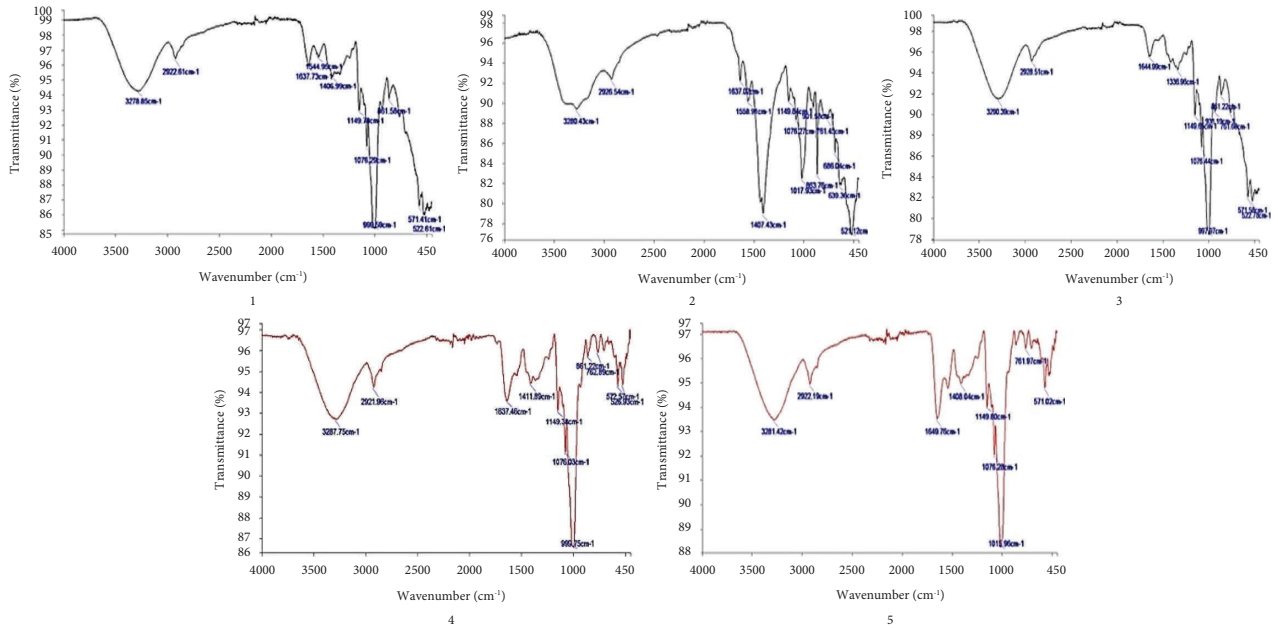

Figure 8: FTIR of insect-infested flour. (1) Cells treated with uninfested flour (control), (2) cells treated with *T. granarium*-infested flour, (3) cells treated with *S. oryzae*-infested flour, (4) cells treated with *R. dominica*-infested flour, and (5) cells treated with *T. castaneum*-infested flour.

Table 4: Characteristic FTIR absorption peaks of functional groups*.

Function groups	Position (cm^{-1})	Intensity*	Notes
Alkanes			
C-H stretch	2990–2850	m to s	
Alkenes			
=C-H stretch	3100–3000	m	
C=C stretch	1680–1620 (sat.)	w to m	
	1650–1600 (conj.)	w to m	
=C-H bend	995–685	S	See Table 2 for details
Alkynes			
≡C-H stretch	3310–3200	S	
C≡C stretch	2250–2100	m to w	
Aromatic compounds			
C-H stretch	3100–3000	m to w	
C=C stretch region	1625–1440	m to	Hidden in fingerprint
C-H bend	900–680	S	See Table 2 for details
Alcohols**			
O-H stretch	3550–3200	br, s	Hydrogen bonded (typical)
Amines			
N-H stretch secondary (one band)	3550–3250	br, m	Primary (two bands)
Nitriles			
C≡N stretch	2280–2200	S	
Aldehydes			
C-H stretch	2900–2800 and		
	2800–2700	S	
H-C=O fermi doublet C=O stretch	1740–1720 (sat.)	S	
	1715–1680 (conj.)	S	
Ketones			
C=O stretch	1750–1705 (sat.)		
	1700–1665 (conj.)	S	
Esters**			
C=O stretch	1765–1735 (sat.)	S	
	1730–1715 (conj.)	S	
Carboxylic acids**			
O-H stretch	3200–2500	br, m to w	
C=O stretch	1725–1700 (sat.)	S	
	1715–1680 (conj.)	S	
Amides			
N-H stretch	3500–3150	m	Primary (two bands) secondary (one band)
C=O stretch	1700–1630	S	
Anhydrides**			
C=O stretch	1850–1800	S	
	1790–1740	S	
Acid chlorides			
C=O stretch	1815–1770	S	
Nitro compounds			
NO_2 stretch	1570–1490 and	S	
	1390–1300	S	
Thiols†			
R-S-H stretch	2550–2600		
Alkyl and aryl halides†			
C-F stretch	1000–1400		Hidden in the fingerprint region
C-Cl stretch	<600–840		
C-Br stretch	<700		
C-I stretch	<600		

*s = strong; m = medium; w = weak; br = broad; sat. = saturated; conj. = conjugated; **alcohols, esters, carboxylic acids, and anhydrides also absorb in the fingerprint region due to the C-O stretch (1300–1000, s).

responsible for cytotoxic and genotoxic effects on hamster kidney cells.

Infrared spectroscopy employs infrared light to monitor the interaction among functional groups of chemical molecules. Infrared light generates predictable vibrations to express the "fingerprint" characteristic of chemical or biochemical substances in a sample. This technique is convenient, cost-effective, and quickly provides accurate and reliable results [19, 42, 43]. The use of infrared spectroscopy for the analysis of food and dairy products is significantly increasing, which demands miniaturization, better instrumentation, and on-site analysis capability for rapid results [28, 44]. The infrared spectroscopy of dairy samples mainly covers two areas: (1) quantification of concentrations and quality parameters and (2) testing of purity, adulteration, and identity. Several dairy researchers are working to expand the role of this technique at the industrial level. The main focus is to better understand the changes in dairy products, microbial populations, and ingredients during the processing and optimization of processes for safe and uniform product quality [21, 28].

5. Conclusion

The current study elaborates on the toxicity of four important stored grain insects and their flour residues through the MTT assay, comet assay, and FTIR spectra. The study further established the presence of metabolite functional groups, including alkenes and phenols, which can promote apoptosis and genotoxicity. Therefore, it can be concluded that insect residues in flour could have cytotoxic and genotoxic effects on living cells that might affect public health, especially after consuming *T. granarium* and *T. castaneum*-infested flour. Therefore, good storage of stored grains and their products is recommended. Future research is required to identify the primary and secondary chemical components responsible for cytotoxicity and genotoxicity in flour infested with these types of pests.

Data Availability

All the data are included in this article.

Ethical Approval

The study was conducted in accordance with the Declaration of the Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza 12619, Egypt Ethics Committee.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

SQ and NA were responsible for conceptualization; NA and EH were responsible for methodology; NA and EH were responsible for formal analysis; SQ, NA, and EH were responsible for investigation; NA was responsible for writing and preparing the manuscript; SQ was responsible for writing and reviewing and editing the manuscript; SQ and NA were responsible for supervision; SQ was responsible for project administration and funding acquisition. All authors read and agreed to the published version of the manuscript.

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