

# GENETICAL EFFECTS OF USING SILICA NANOPARTICLES AS BIOPESTICIDE ON *Drosophila melanogaster*

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Pesticides are used to kill organisms that are detrimental to agricultural production, including viruses, bacteria, fungi, parasites, weeds and insects. Modern pesticides place their own burdens on farming systems, for example accumulation in soils and ecosystems, which can have unexpected and often deleterious effects. The situation causes further concern when one considers that this affects both the environment and cost to the farmer (Mogul *et al.*, 1996). There is therefore an urgent need to (a) find alternatives to current pesticide deployment and (b) find ways of rapidly and locally detecting levels of the pesticide and either removing or degrading it. New technologies promise to deliver options for these challenges outlined. One of the most exciting new developments in pesticides is the prospect of nanopesticides and other nanomaterials.

Nanotechnology is being used to develop pesticides with new or enhanced activity, or more targeted application (such as through microencapsulation or affinity for specific target pests). Nanomaterials are defined by the US. National Nanotechnology Initiative as materials that are intentionally produced to

have particles whose size measures between one and one hundred nanometers in at least one dimension. Due to their extremely small size, nanomaterials can have different properties than their larger scale counterparts. Application of nanotechnology in agriculture is at a nascent stage. Nanotechnology has a huge potential to develop alternative pest control strategy and lower risk insecticidal molecules. There are a number of studies exploring the use of nanoparticles in the controlled release of bioactive substances in wood (Liu *et al.*, 2002 a, b, c) and the use of micro and nanoparticles as a tool for plant science (Taylor and Fauquet, 2002). While nanomaterials can have beneficial applications, laboratory studies have led to concerns that their small size and unique properties may pose new or increased risks to human health or the environment (Martin, 2007; EPA, 2011).

Silica is the common name for materials composed of silicon dioxide (SiO<sub>2</sub>) and occurs in crystalline and amorphous forms. As it is known to be biocompatible and biodegradable (Vallet-Regí and Balas, 2008), this has led a number of research groups to investigate its potential as a drug delivery vehicle for medical and veteri-

nary treatments, and more recently for pesticides (Li *et al.*, 2006; Liu *et al.*, 2006). As silica is rather inert, silica nanoparticles can be better alternative to the popular insecticides which are hazardous to human health and because of huge environmental concerns associated with them. In research experiments, nanosilica has been reported to provide insecticide activity on its own, through desiccation of insects' cuticles. It has also been successfully applied as a thin film to boost cereal germination and decrease fungal growth (<http://www.nanopool.eu/english/news.htm>). However, nanosilica is not a preferred auxiliary in crop protection products due to the adverse effect upon inhalation. Recently, the use of silica nanoparticles has been effective in the controlled release of substances into protoplasts within plant cells (Torney *et al.*, 2007). Although nanosilica could certainly provide benefits to society, its interaction with biological systems and potential genotoxic effects must be carefully addressed.

To date, nanotechnology is still in its early stage within agrochemical industry. Thus, information covering the potential toxicity of nanomaterials and their health and environmental impact are currently not being entirely explored. In response to the developing trend of nanosilica applications in agriculture sector, potential risks associated with its use must be transparent on biological systems. So, we aim to study the potential toxic effects of nanosilica particles on the fruit fly; *Drosophila melanogaster*, because of its distinct advantages for the study of

toxicology (Cummings and Kavlock, 2005; Petersen *et al.*, 2008). We employed the *Drosophila melanogaster* model to investigate the impacts of nanosilica exposure on phenotypic outcomes, chromosomal rearrangements and protein expression as well as its effect on DNA content. This may some how lead to prediction of a certain safe concentration of this nanoparticle that might be used in the various fields of nanotechnology including agricultural applications.

## MATERIALS AND METHODS

This study was carried out at the laboratory of Field Crop Pests Res. Dept., Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt and the laboratory of Genetics Dept., Fac. of Agric., Kafr El-Sheikh Univ., Kafr El-Sheikh, Egypt.

### *Silica nanoparticles*

Silica nanoparticles (SiNPs) with a narrow particle size distribution and controlled diameters of 10-20 nm were purchased from NanoTech Egypt Co., Dreamland, Wahat Road, 6<sup>th</sup> October, Egypt, and were used in the form of spherical (98% purity) without any further modification.

### *Drosophila population*

The flies used for these toxicity assays were the wild type of *D. melanogaster* flies founded from collections of natural populations from Egypt and incubated at  $25\pm 1^{\circ}\text{C}$  on standard *Drosophila* media.

### ***Toxicity assay and silica nanoparticles exposure***

To test the toxicity of SiNPs, *D. melanogaster* were reared on various amounts of it (0, 100, 250, 500 and 1000 ppm), each amount was applied in three replicates. A manageable number of newly emerged parental flies were placed in 250 ml culture bottles that contained standard medium. The eggs laid by these parents were allowed to develop into larvae. Then, one hundred first instar larvae were collected and transferred to another culture bottles containing 40 ml of treated food medium and maintained for one generation in standard conditions at  $25\pm 1^\circ\text{C}$ .

### ***Larval deformations, viability and body size assessment***

Given concerns about the toxicity of nanosilica in whole organisms, the effects on larvae and adults stages were investigated. During each larval developmental stage (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars); observation of overt phenotypes for ten larvae was documented. Different parts of larvae; i.e., mouth parts (pair hooks), midgut, hindgut and anus, were investigated and described under an ordinary microscope to examine symptoms resulting in SiNPs treatments. In addition, adults that had emerged from larvae exposed to SiNPs were assessed for viability which measured as the percentage emergence of adults relative to the initial number of larvae used (100 larvae). As indicators for adult body size, ten males and ten females of *D. melanogaster* flies emerged

from each replicate were sampled and measured for thorax and wing lengths using an ocular micrometer inserted into a 10x ocular lens in combination with a 3.2x objective (one micrometer unit equals 0.01 mm). The method of measuring live flies has been described in detail by Prevosti (1955).

### ***Salivary gland chromosomes and cytological procedures***

To test the effect of the SiNPs on the chromosomal rearrangements, newly emerged flies from different treatments were subsequently transferred to additional bottles with standard fresh medium and allowed to lay eggs for two days, then the parents were removed to obtain the F<sub>1</sub>'s. One hundred and fifty 3<sup>rd</sup> instar larvae from the progeny (50 larvae per replicate) reared in optimal condition of temperature (18°C) were sampled and scored for cytological analysis. Salivary glands were stained and squashed in 2% aceto-lactic-orcein solution to study the frequencies of chromosomal inversions. The chromosomal analysis was made for the second and third chromosomes and the observed inversions were identified by the help of salivary chromosome maps of Lindsley and Grell (1967).

### ***Protein and isozymes analysis***

Electrophoretic patterns of total protein as well as esterase and peroxidase isozymes were studied for both control and treated samples. Samples were prepared from whole body of adults by homogenate 100 mg flies of each treatment

in 1 ml of 20% sucrose according to El-Fadly *et al.* (1990).

Samples were submitted to polyacrylamide gel electrophoresis (PAGE) for determination of esterase and peroxidase isozymes, while total proteins were fractionated on SDS-PAGE (Laemmli, 1970). Following electrophoresis, the gel of total protein was stained overnight with Coomassie Brilliant Blue R-250. The gel was stained for esterase activity in a solution of  $\alpha$ - and  $\beta$ - naphthyl acetate and fast blue RR, while, peroxidase activity was determined using benzidine dihydrochloride according to Scandalios (1964).

#### ***Estimation of DNA content***

DNA was extracted from three to five adults of each treatment following Laayouni *et al.* (2000) and its concentration was determined spectrophotometrically at 260 nm.

#### ***Statistical analysis***

Results were subjected to one-way analysis of variance to compare the significance of differences between SiNPs concentrations. All levels of statistical significance were determined by LSD test at 95% confidence limit.

## **RESULTS AND DISCUSSION**

#### ***Effects of SiNPs on larval deform***

To determine whether any of the SiNPs concentrations causes symptoms or

enter the interior of the larvae, normal and treated larvae were examined under an optical microscope for deformations in larval mouth parts and body. As shown in Fig. (1), all SiNPs concentrations showed significant deformation in mouth or body parts, while the highest values were shown at the highest concentrations for any of three instars, compared with control.

During the 1<sup>st</sup> instar larvae, the mouth parts (pair hooks) are nearly straight in control treatment (diet free SiNPs) compared with larvae reared on diet containing SiNPs which showed in curvature pair hooks (Fig. 2). Therefore, the deformation in larva mouth parts (pair hooks) may lead to decrease larvae feeding and finally stop eating and die. On the other hand, the effect of SiNPs on the 2<sup>nd</sup> and 3<sup>rd</sup> instars appeared in deforming the mouth parts as well as blocked in hindgut and anus (Fig. 3-A), in addition to blocked in midgut (Fig. 3-B) specially at the diet containing 250, 500 and 1000 ppm of SiNPs. These symptoms appeared as lesion as compared to control (Fig. 3). We did not attempt to quantify uptake, but the optical microscope showed that the tested SiNPs became sequestered in larval tissue after exposure, which indicates transport across the gut lining. So, SiNPs in the food are uptaken into the larval gut (black areas compared to control) and larvae can't complete its life cycle because of SiNPs blocked the former places and therefore can't complete the molting process (El-Samahy, 2002).

### ***Effects of SiNPs on adult viability and body size***

From each replicate containing 100 larvae, the number of flies emerging was counted as a measure of SiNPs effects on larva-to-adult viability. The SiNPs did not alter total survivorship at a particular concentration as compared to control (Fig. 4), although it was somewhat higher for the control treatment (83.33%) with non detected significant differences ( $p < 0.05$ ). Therefore, the presence of SiNPs in the food media had no detectable effect on toxicity in this larva-to-adult assay. Petersen *et al.* (2008) reported that low toxicity may be due to that the nanomaterials entered the larval tissue but were purged within a few days and did not persist in tissues.

To determine whether SiNPs concentrations affect overall body size, newly emerged adults were sorted by sex and measured for thorax and wing lengths (Table 1). As it is normal for *Drosophila*, females size is larger than males in all treatments. The data showed that all the four concentrations of SiNPs significantly increased the thorax and wing lengths of *D. melanogaster* males and females as compared to control. The concentrations of SiNPs significantly increased the thorax lengths of *D. melanogaster* males from 27.43 (control) to 28.73 micrometer unit (250 ppm), then it was decreased again as concentrations increased (500 and 1000 ppm). The data of males also revealed that the concentration of 100 ppm gave the highest value in average (57.97 microme-

ter unit) for wing lengths as compared to control and the other concentrations. On the other hand, the four SiNPs concentrations did not differ significantly from each other for females for both traits, while it differed significantly comparing to the control.

There are few studies to which the present results can be directly compared. The study of Leeuw *et al.* (2007) showed no loss of viability or adult weight upon exposure of *Drosophila* larvae to 9 ppm Single-Walled Carbon Nanotubes dispersed in food paste. Blickley and McClellan-Green (2008) reported the existence of low toxicity of fullerene to embryo, larva and adult *Fundulus heteroclitus*. Velzeboer *et al.* (2008) found low toxicities of various nanomaterials including C60 and SWNT at concentrations up to 100  $\mu\text{g/L}$  in aquatic systems. On the other hand, Lin *et al.* (2006) reported that 15 and 46 nm amorphous silica nanoparticles produce both a dose- and time-dependent reduction in cell viability using cultured human alveolar epithelial cells (A549 cells). In other studies, silica did not reduce the viability of BEAS-2B human lung epithelial cells in a mitochondrial reductase assay (NTP, 2009).

### ***Effects of SiNPs on chromosomal rearrangements***

Different paracentric inversions have been detected in *D. melanogaster* flies which were treated with SiNPs. These chromosomal rearrangements were found only in heterozygous condition. The inversions were distributed on the second

and third chromosomes. These inversions were as follows: *(2L)Cy*, *(2R)Ns*, *(3L)P*, *(3L)M*, *(3R)Mo* and *(3R)C*. The results are presented in Table (2) and Fig. (5).

Data showed that when the two lowest concentrations of SiNPs (100 and 250 ppm) were applied, most of the inversions on the second and third chromosomes exhibited decline from the control, except for the third chromosomal inversion *(3L)P* which increased from 10.67% (control) to 17.33% (100 ppm). Thus, application of SiNPs caused a severe reduction in almost chromosomal inversions compared with control. This reduction in inversion frequency caused by SiNPs may be due to that the flies were in balance with their environment.

On the other hand, the data showed that the highest frequencies of the most observed inversions were detected when *Drosophila* flies were treated with SiNPs; either at 500 or 1000 ppm as compared to control and the lowest concentrations. The frequency of the second chromosomal inversion; *(2L)Cy* was 21.33% at 500 ppm (the same as a control) and *(2R)NS* increased from 6.67% (control) to 12.00% (1000 ppm). While the frequency of the third chromosomal inversion *(3R)C* increased from 23.33% (control) to 30.00 and 24.67% (500 and 1000 ppm, respectively). It was observed that inversions *(3L)M* and *(3R)Mo* on the third chromosome were the most affected ones when *Drosophila* flies were treated with 500 and 1000 ppm of SiNPs. The inversion *(3L)M* was only observed after the appli-

cation of the two highest treatments as compared to the control. This means that the highest concentrations of SiNPs increased the inversions in their frequencies. Because chromosomal inversions can have large fitness effects (Wright and Dobzhansky, 1946; Dobzhansky, 1947), they are extremely valuable in monitoring genetic variation for stress-response. The fly needs these inversions to keep certain genes intact together. Flies use these inversions to cope with the surrounding environment and utilize the available food components. On the other hand, the inversion *(3R)Mo* was not observed at all in these two concentrations as compared to control.

Several studies evaluated the cytotoxic potential of SiNPs. These studies showed that the cytotoxicity of nanosilica was cell-type specific (NTP, 2009). But the present study revealed that the two low concentrations of SiNPs (100 and 250 ppm) may have not the potentials with mutagenicity and damages of chromosomal levels in salivary gland chromosomes. From these results, we suggest that SiNPs; at the low concentrations, are not able to cause mutagenicity on chromosomal levels in *Drosophila* salivary chromosomes.

Despite these abnormalities, there is limited evidence to suggest that SiNPs are genotoxic and some recent studies utilizing the comet assay have demonstrated that SiNPs ranging in size from 20 to 400 nm do not exert significant genotoxicity (Jin *et al.*, 2007; Barnes *et al.*, 2008). In contrast, one investigation

based on the micronucleus assay found that these nanoparticles do indeed induce chromosomal damage (Wang *et al.*, 2007).

### ***Effects of SiNPs on protein expression***

Analysis of the proteins in *D. melanogaster* cells that were differentially expressed in response to SiNPs was conducted to understand the molecular mechanism of SiNPs-induced toxicity at the protein level. While study of individual proteins facilitates the investigation of the chemical nature and the physiological functions of each protein (Firling, 1977).

#### ***a. SDS-Protein analysis***

Figure (6) and Table (3) show the SDS-PAGE pattern from the total protein profile of *D. melanogaster* after one generation of exposure to different concentrations of SiNPs. A successive increase in the protein profiling pattern was observed which could be as a result of the application of high concentrations of SiNPs. The gel scan for the control treatment showed that the protein profile of *D. melanogaster* consisted of 22 bands. In comparison with the control (lane 1), it can be clearly observed that there are 24 bands (two new bands) for *Drosophila* samples treated with 100, 250 and 500 ppm (lanes 2, 3 and 4, respectively) and 27 bands (five new bands) for the highest concentration; 1000 ppm (lane 5), that could be resulted due to the stress created by the addition of SiNPs to the *Drosophila* medium. Six bands were categorized as new bands (comparing with control) which have the numbers 2, 5, 11, 16, 17 and 20; indicated by ar-

rows (Fig. 6). Band No. 17 was shown as a result of using the lowest concentration; 100 ppm, two bands (No. 2 & 16) were existed in the three concentrations; 250, 500 and 1000 ppm, two bands (No. 5 and 20) appeared by the highest concentration; 1000 ppm, and band No. 11 was found in all the four SiNPs concentrations. The rest bands were registered for both control and treated samples as common bands, except only for band No. 4 which totally eliminated from 250 and 500 treatments. Some bands became more intense after SiNPs exposure in comparison with control. Six bands (No. 6, 7, 8, 10, 12 and 13) showed increase in the levels of proteins intensity after 100 ppm treatment, while bands No. 1, 3 and 18 increased after 250 ppm treatment. Only band No. 18 increased in its intensity after 500 ppm treatment, while bands No. 3, 6, 10, 13 and 18 increased after 1000 ppm treatment. The results of protein analysis of samples exposed to 500 ppm of SiNPs exhibited low intensity for bands No. 1 and 6, while band No. 7 showed low intensity for the three highest concentrations; 250, 500 and 1000 ppm, in comparison to control. The other bands were represented by equal intensity as compared to control sample.

The alterations in protein expression in the presence of SiNPs provided further evidence of the toxicological effects of these nanoparticles, and provided valuable clues to elucidate the molecular mechanisms underlying the toxicological effects of SiNPs exposure. These protein profile alterations seem to be due to a direct effect of SiNPs, where SiNPs were detected in the organs of *D. melanogaster*

as reported previously in this investigation. This result was in agreement with those of Geiser *et al.* (2005), Park and Park (2009) and Taylor *et al.* (2010), they reported that nanoparticles can enter into the cytoplasm and cell organelles by endocytosis independent pathways due to their unique properties. The process includes: diffusion through membrane pores, adhesive interactions and so on. The overexpressions of some of the proteins observed in the present study could be due to the fact that the nanomaterial interacts with cellular proteins such as those involved in the cell division process (Brunner *et al.*, 2006). Cho *et al.* (2007) observed significantly increased levels of total protein in BALF up to one week after treatment with 50 mg/kg of ultra fine amorphous silica with a primary particle diameter of 14 nm. However, the weak intensity suggests the partial removal of proteins from the nanocomposites and retention of only those actually attached to the nanoparticle surfaces for capping (Singh *et al.*, 2008). Moreover, the binding of proteins to nanoparticles may also induce modifications of the proteins. Yang *et al.* (2010) showed that the levels of the differentially expressed proteins were associated with the particle size; the alterations of protein expression were more apparent in 15-nm SiO<sub>2</sub>-treated cells than that in 30-nm or micro-sized SiO<sub>2</sub>-treated cells.

### ***b. Isozymes analysis***

Figures (7 and 8) show the changes in the activities of esterase and peroxidase isozymes after the administration of the nanosilica in *D. melanogaster*.

Thirteen electrophoretic esterase bands were presented after treatment with SiNPs (Fig. 7), ten out of them were common for the control and the treated adults. The first common band (No. 1) had lower intensities for the two higher concentrations; 500 and 1000 ppm, while band No. 4 exhibited low intensity for only treatment of 500 ppm. On the other hand, band No. 13 had higher intensity for the lowest SiNPs treatment; 100 ppm, comparing with control and other treatments. The other common bands (No. 3, 5, 6, 7, 8, 10 and 12) had the same intensities for the control and all SiNPs treatments. Band No. 2 which was appeared in the control and treatments of 100 and 250 ppm was absent in the 500 and 1000 ppm treatments. At the same time, a new band (No. 9) was induced in flies treated with 500 and 1000 ppm and was lacking in those fed upon the control as well as 100 and 250 ppm SiNPs. This band was considered to be specific for both the highest SiNPs treatments, while it was increased in its intensity when the concentration increased. Band No. 11 was absent in the treatment with 250 ppm.

The results of peroxidase analysis revealed two common bands for the control and the SiNPs treated samples (Fig. 8). The intensity of both bands was increased at all doses of SiNPs compared to those of the corresponding control, indicating that the SiNPs induces stress.

It is evident from the data that there are specific changes at the levels of total protein and the two isozymes tested;

esterases and peroxidases. This indicates that severe side effects were presented due to the application of the SiNPs as pesticide to the insects and other organisms. In agreement with previous findings, Seth *et al.* (2007) showed the effect of nanosilica on *in vivo* level of esterases and peroxidases, they found that there were random changes in the level of esterases indicating that there was no possibility of the biotoxicity of these nanosilica inside chicken body, where the application of nanosilica increased the level of peroxidase significantly over control in liver and kidney tissues of chickens. Silica nanoparticles (10 nm and 80 nm) were also found to induce little effect on the activities of glutathione peroxidase in human lung epithelial cells; A549 cells (Akhtar *et al.*, 2010).

#### ***Effects of SiNPs on DNA content***

Figure (9) shows the changes in the level of DNA content in the control and SiNPs treatments in *D. melanogaster* adults. It is evident that SiNPs significantly reduced DNA contents in dose dependent manner, where the highest decline was observed at the highest concentration. This means that the SiNPs tested in this study (10-20 nm) have toxic effect on the level of nucleic acid DNA content.

Recently, Gerloff *et al.* (2009) investigated the DNA damaging properties of amorphous fumed nano-silica (14 nm) in the human colon epithelial cell-line, Caco-2, they reported that exposure to nano-silica for up to 24 h caused significant DNA damage. Sarih *et al.* (1993) found that silica-treated macrophages

underwent apoptosis. This was demonstrated by quantification of apoptotic cells by a flow cytometric analysis based on the reduction of cellular DNA content exhibited by apoptotic cells. Flow cytometric analysis showed that SiO<sub>2</sub> nanoparticles can cause G2/M phase arrest and apoptotic sub-G1 population increase in a dose-dependent manner. Indeed, it has been shown that nanoparticles of silica have an impact on nuclear integrity by entering the nucleus (Geiser *et al.*, 2005; Liu *et al.*, 2007) and forming intranuclear protein aggregates that can lead to inhibition of replication, transcription, and cell proliferation (Chen and Von, 2005). SiO<sub>2</sub> particles between 40 nm and 5 μm were applied to epithelial cells in culture and observed that particles of all tested sizes penetrated the cytoplasm; however, nuclear localization was observed exclusively in cells treated with SiO<sub>2</sub> nanoparticles between 40 and 70 nm (Chen and Von, 2005). In addition, they reported that silica nanoparticles >200 nm failed to penetrate the nucleus, do not alter nuclear structure and function, and also do not interfere with gene expression. Interestingly, Jin *et al.* (2007) reported that the luminescent amorphous SiNPs (50 nm) penetrated the rat alveolar macrophage cells and human lung epithelial cells (A549 cells) but were not detected in the nuclear region and did not cause significant toxic effects at the molecular and cellular levels below a concentration of 0.1 mg/ml.

Nanoparticles may induce genotoxicity by interacting directly with

DNA or through indirect means. It may pass through cellular membranes and gain access to the nucleus. If the nanomaterials were to locate within the nucleus, then direct interaction between them and the DNA molecule or DNA-related proteins may lead to physical damage to the genetic material. Additionally, if nanomaterials were able to accumulate within a cell but not necessarily gain access to the nucleus, they may still come into direct contact with DNA during mitosis when the nuclear membrane breaks down, providing ample opportunity for DNA aberrations to arise. SiNPs have been shown to enter the cell nucleus where they could potentially bind to the DNA phosphate backbone (Chen and Von, 2005). Alternatively, DNA damage may arise through indirect mechanisms where the nanomaterial does not physically interact with the DNA molecule, but with other cellular proteins such as those involved in the cell division process. Additionally, they may induce other cellular responses that in turn lead to genotoxicity, such as causing oxidative stress, inflammation and aberrant signaling responses (Brunner *et al.*, 2006). The generation of oxidative stress has been documented both for crystalline silica and for high doses of amorphous silica nanoparticles (Lin *et al.*, 2006; Wang *et al.*, 2009). Since the SiNPs can cause increased ROS levels and given that the hydroxyl radical close to the DNA could readily lead to the induction of DNA strand breaks and oxidised bases (Valko *et al.*, 2006) which could have important implications in the development of cancer. While, in a recent review, Gonzalez *et al.*

(2010) compared two genotoxicity tests; the alkaline comet assay and the micronucleus test, in terms of chemical composition and size of engineered nano-silica: engineered nano-silica did not seem to induce DNA strand breakage.

## SUMMARY

Employing nanomaterials and nanoparticles in the industrial and research area could reduce use of certain agrochemicals such as pesticides, and further provide a better ability to control the application and dosage of active substance to the target. Here, the use of silica nanoparticles (SiNPs) as biopesticide was applied in attempt to bring a number of benefits into potential applications of nanotechnology to pesticides; in addition, to provide a review to explain *in vivo* biological effects using *Drosophila melanogaster* fruit fly. In this study, SiNPs were used in the form of nanometer silicon dioxide (10-20 nm SiO<sub>2</sub>). The effects of exposure to SiNPs (100, 250, 500 and 1000 ppm) on larval deform, larva-to-adult viability, body size, chromosomal rearrangements, protein and isozymes expression as well as DNA content were evaluated in *Drosophila* flies by using morphological, cytological and biochemical analysis.

All SiNPs concentrations had no toxic effect on larva-to-adult viability or body size of *D. melanogaster*, although the ingested SiNPs concentrations showed significantly deformation in mouth and body parts and became incorporated into organs of *D. melanogaster* larvae in a dose-dependent manner; compared with

control. This suggests that SiNPs ingested by these insects have negligible physiological impact. On the other hand, we cannot exclude other genotoxic effects. SiNPs at the concentrations of 500 and 1000 ppm appeared to be more affective on salivary gland chromosomes than the other two concentrations. SiNPs induced specific changes in the number and intensity of total protein as well as the activity of esterase and peroxidase isozymes. Moreover, SiNPs significantly reduced DNA content in dose dependent manner. These toxic effects were closely related to the concentration used.

From all the above mentioned results, the level of SiNPs could now be determined to be introduced to control the insects based on the physiological level, in addition to maintain and protect other organisms at the genetically level of these nanoparticles.

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Table (1): Lengths of thorax and wing of adult *D. melanogaster* flies fed throughout the larval period on different concentrations of SiNPs. Measurements of length are in micrometer unities.

Treatment	Thorax length		Wing length	
	Male	Female	Male	Female
Control	27.43 ± 0.17 <sup>c</sup>	30.60 ± 0.18 <sup>b</sup>	54.50 ± 0.26 <sup>c</sup>	61.87 ± 0.37 <sup>b</sup>
100 ppm	28.17 ± 0.20 <sup>b</sup>	31.83 ± 0.25 <sup>a</sup>	57.97 ± 0.35 <sup>a</sup>	65.00 ± 0.43 <sup>a</sup>
250 ppm	28.73 ± 0.25 <sup>a</sup>	31.77 ± 0.22 <sup>a</sup>	57.57 ± 0.42 <sup>ab</sup>	64.70 ± 0.50 <sup>a</sup>
500 ppm	28.03 ± 0.16 <sup>b</sup>	31.43 ± 0.24 <sup>a</sup>	57.17 ± 0.31 <sup>ab</sup>	64.33 ± 0.45 <sup>a</sup>
1000 ppm	27.83 ± 0.21 <sup>bc</sup>	31.50 ± 0.21 <sup>a</sup>	56.80 ± 0.37 <sup>b</sup>	64.83 ± 0.37 <sup>a</sup>

Different superscript letters within each column indicate significant differences at the 5% level.

Table (2): Frequencies of chromosomal inversions recorded in *D. melanogaster* after treatment with SiNPs.

Inversion Treatment		Chromosome II		(a) Chromosome III			
		(2L)Cy	(2R)Ns	(3L)P	(3L)M	(3R)MO	(3R)C
Control	N	32	10	16	-	6	35
	%	21.33	6.67	10.67	-	4.00	23.33
100 ppm	N	9	4	26	-	4	13
	%	6.00	2.67	17.33	-	2.67	8.67
250 ppm	N	5	9	14	-	5	14
	%	3.33	6.00	9.33	-	3.33	9.33
500 ppm	N	32	9	9	4	-	45
	%	21.33	6.00	6.00	2.67	-	30.00
1000 ppm	N	15	18	22	3	-	37
	%	10.00	12.00	14.67	2.00	-	24.67

Total No. of chromosomes examined = 150 chromosomes

Table (3): SDS-PAGE gel scanning of protein bands of *D. melanogaster* treated with SiNPs.

No. of bands	Relative mobility (R <sub>f</sub> )	(Lane M) Marker (KDa)	(Lane 1) Control	(Lane 2) 100 ppm	(Lane 3) 250 ppm	(Lane 4) 500 ppm	(Lane 5) 1000 ppm	
1*	0.026	205.0	+++	+++	++++	++	+++	
2	0.054		-	-	++	+	++	
3*	0.111		+	+	++	+	++	
4	0.145		+	+	-	-	+	
5	0.175		-	-	-	-	+	
6*	0.232	116.0	+++	++++	+++	++	++++	
7*	0.275		+++	++++	++	++	++	
8*	0.313		+	+++	++	++	++	
9*	0.347		+	+	+	+	+	
10*	0.385		++	+++	++	++	+++	
11	0.402	97.4	-	++	+	+	++	
12*	0.453		++	+++	++	++	++	
13*	0.481		+++	++++	+++	+++	++++	
14*	0.517		++	++	++	++	++	
15*	0.555		++	++	++	++	++	
16	0.566	66.0	-	-	+	+	+	
17	0.598		-	+++	-	-	-	
18*	0.628		+	+	++	++	++	
19*	0.658		+	+	+	+	+	
20	0.677		-	-	-	-	++	
21*	0.696	45.0	+++	+++	+++	+++	+++	
22*	0.730		++	++	++	++	++	
23*	0.760		+	+	+	+	+	
24*	0.781		+	+	+	+	+	
25*	0.858		+	+	+	+	+	
26*	0.890	29.0	++	++	++	++	++	
27*	0.928		+	+	+	+	+	
28*	0.968		++	++	++	++	++	
Total				22	24	24	24	27

\* Common bands - : Absent + : Very faint ++ : Faint +++ : Dark ++++ : Very dark

Fig. (1): Deformed percentage of *D. melanogaster* larvae (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars) under different concentrations of SiNPs. Different letters indicate significant differences at the 5% level.

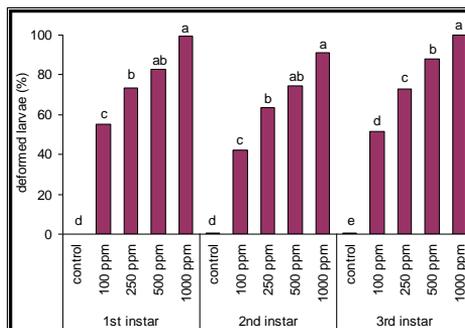


Fig. (2): Toxic effects of SiNPs on mouth parts (pair hooks) of *Drosophila* larvae.

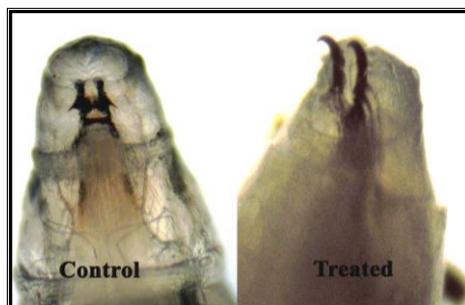
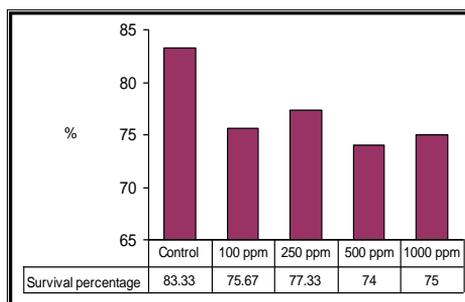


Fig. (3): Effect of SiNPs on *D. melanogaster* larvae corresponding to the control. (A) blocked in hindgut and anus, (B) blocked in midgut.



Fig. (4): Toxic effects of SiNPs on larva-to-adult viability of *D. melanogaster*.



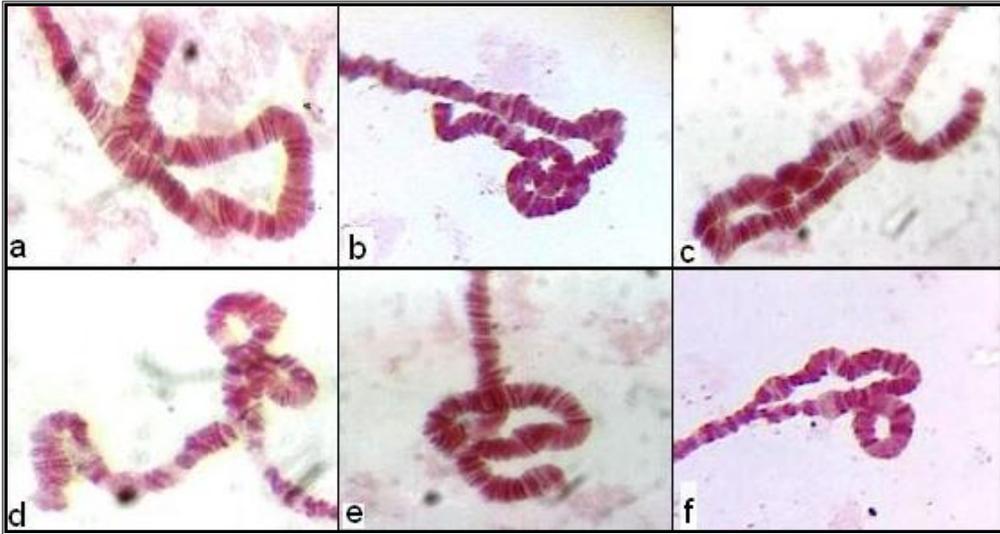


Fig. (5): Microphotographs of chromosomal inversions in *D. melanogaster* after treatment with SiNPs; a:  $(2L)Cy$ , b:  $(2R)Ns$ , c:  $(3L)P$ , d:  $(3L)M$ , e:  $(3R)Mo$  and f:  $(3R)C$ .

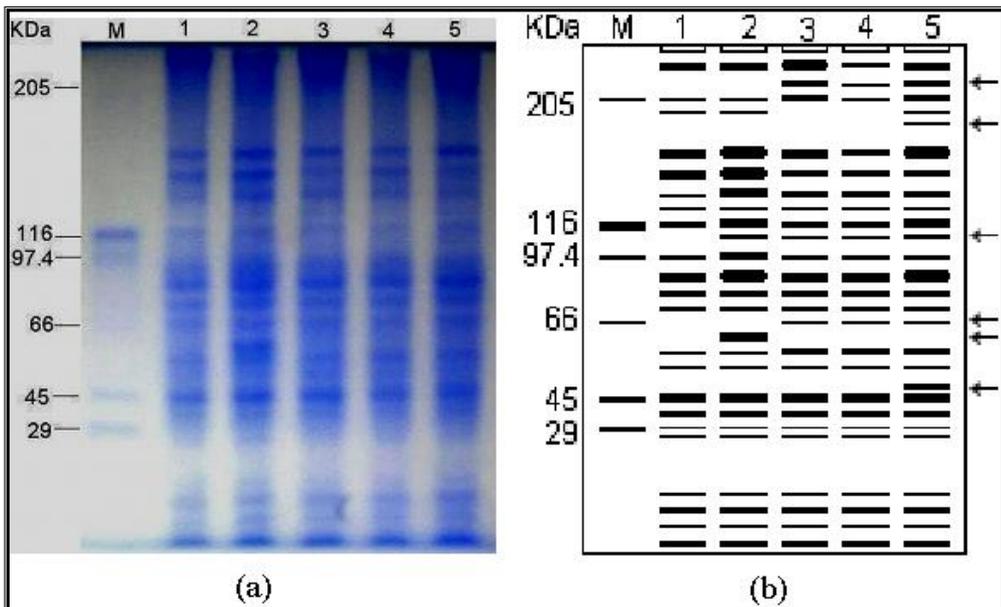


Fig. (6): Gel image (a) and its diagram (b) of *D. melanogaster* flies homogenate on SDS-PAGE gel. Lane M has molecular weight markers for 205, 116, 97.4, 66, 45 and 29 kDa. Lane 1 for control and lanes 2, 3, 4 and 5 for 100, 250, 500 and 1000 ppm SiNPs-exposed *Drosophila*, respectively.

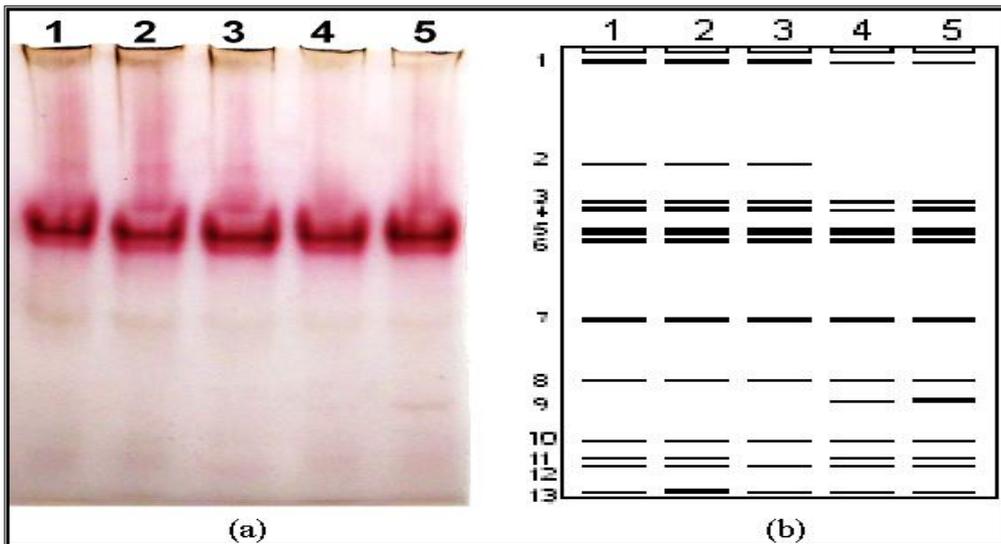


Fig. (7): Electrophoretic patterns (a) and their diagram (b) of esterase isozymes of *D. melanogaster* treated with SiNPs. Lane 1: control. Lanes 2, 3, 4 and 5: treatments of SiNPs; 100, 250, 500 and 1000 ppm, respectively.

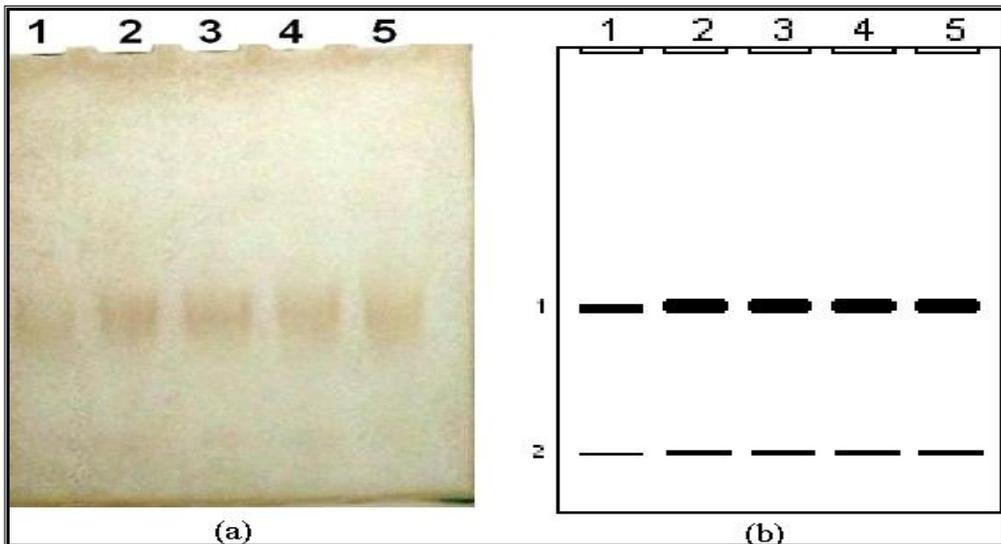


Fig. (8): Electrophoretic patterns (a) and their diagram (b) of peroxidase isozymes of *D. melanogaster* treated with SiNPs. Lane 1: control. Lanes 2, 3, 4 and 5: treatments of SiNPs; 100, 250, 500 and 1000 ppm, respectively.

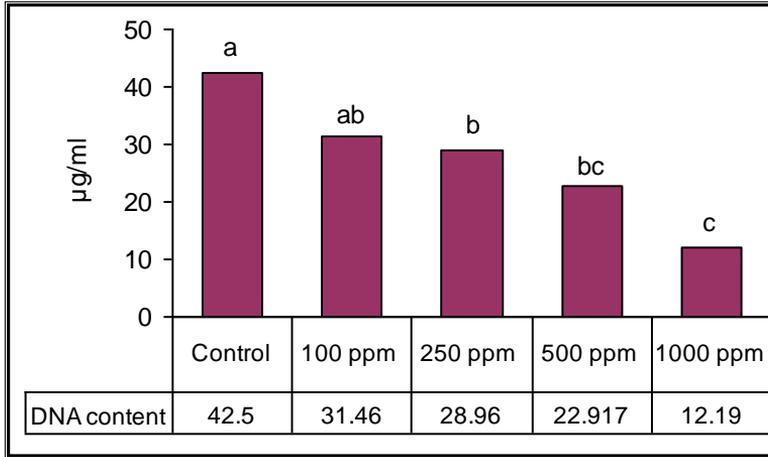


Fig. (9): Changes in the level of DNA content in *D. melanogaster* after treatment with SiNPs.