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EFFECT OF SILICA NANO-PARTICLES IN CONTROL OF MITE, *Tetranychus cucurbitacearum* (Sayed) AND AGRONOMIC TRAITS OF SOYBEAN PLANTS AND QUALITATIVE ASSESSMENT OF ITS GENOTOXICITY USING TOTAL PROTEIN AND RAPD ANALYSIS

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ABSTRACT

Control of the two spotted spider mite, *Tetranychus cucurbitacearum* (Sayed) using conventional acaricides resulted in many problems. Thus, this study aimed to evaluate the effect of silica nano-particles (SiNPs) as a new approach to control spider mite on soybean plants. Three concentrations of SiNPs (250, 350 and 450 ppm) were sprayed on two soybean varieties, Giza 35 and Giza 111 during 2014 season. The reduction percentage in the population of the mite was recorded under field conditions. The obtained results indicated that the mean reduction percentage in mite population appeared a concentration response. The concentration of 450 ppm of SiNPs induced the highest reduction of mite on both soybean varieties Giza 111 and Giza 35, with values of 78.91% and 62.34%, respectively, followed by concentrations of 250 (71.24% and 38%) and 350 ppm (62.16% and 41.92%),respectively throughout the experimental period. The motile stages of mite *T. cucurbitacearum* on soybean variety Giza 111 was more susceptible to SiNPs than variety Giza 35. On the other hand, the highest values of seed yield/fed were recorded for the two varieties grown at 450 ppm in contrast with the control and250 ppm which produced lower seed yield/fed. High differences in SDS-Polyacrylamide gel electrophoresis protein profiles were found between two soybean genotypes treated with different concentrations of SiNPs. Increasing SiNPs concentration, increased changes in protein profile in both soybean genotypes. Six RAPD primers revealed low to moderate polymorphism and genetic variations among the different concentrations of SiNPs and between both soybean genotypes. The changes in DNA profiles included variation in band intensity, presence or absence of certain DNA bands and even appearance of new bands. Genomic template stability test was performed for the qualitative measurement of changes in randomly amplified polymorphic DNA profiles. This study concluded that DNA stability was affected by SiNPs concentrations of 350 and 450 ppm for both soybean varieties as identified by RAPD markers.

Keywords: SiNPs, Spider mite,*Tetranychus cucurbitacearum,* Soybean,Total soluble protein,RAPD analysis and Genomic template stability.

INTRODUCTION

Soybean, *Glycine max* (L.) is one of the most important legume crops all over the world, as it shares with about 30% of the total world production of edible oil and more than 60% of the world production of high protein meal (chromosome number 2n = 40). A great number of phytophagous and predatory mite species were found inhabiting soybean plants during the crop growing (Abraham and Kuroli, 2003). The phytophagous mites are the main pests infesting soybean plants causing a great damage and loss in yield (Taha et al., 1995). The two spotted spider mite, *Tetranychus cucurbitacearum* (Sayed) is one of the major pests attacking different field crops, vegetables, fruits and ornamental plants (Edge and James, 1986). The continuous use of the conventional acaricides to control this pest has caused pest resurgence outbreaks of secondary pests through decimation of natural enemies and environmental pollution (John *et al*., 1986), as well as resistance developed in mite to many of these compounds (Huffaker *et al*., 1970). Therefore, it has become necessary to search safe compounds for pest control to minimize the use of the conventional synthetic pesticides. The genotoxic effect of SiNPs has been evaluated in a variety of biological systems (Park and Park, 2009; Galal and El-Samahy, 2012 and Barnes *et al*., 2008). The use of amorphous nanosilica as biopesticide has been reported (Barik *et al*., 2008).

Electrophoretic techniques of protein have been used as a successful tool to estimate the possible mutagenic potentialities produced due to continuous and accumulative pollution by chemicals and pesticides which correlated with the produced variation due to chromosomal aberrations resulted by environmental pollutants (George and Ghareeb, 2001 and Haiba *et al*., 2011).

Advances in molecular biology have led to the development of a number of selective and sensitive fingerprinting techniques for DNA analysis in the field of genotoxicology (Theodorakis and Bickham, 2004). Random amplified polymorphic DNA (RAPD), one of DNA fingerprinting methods, is generally used to effectively indicate genetic relationships by phylogenetic tree reconstruction (Jain *et al*., 2007; Ram *et al*., 2008; Subramanyam *et al*., 2009; Ince *et al*., 2010 and Marouelli *et al*., 2010).

Random amplified polymorphic DNA is a PCR-based technique and extremely efficient for DNA analysis in complex genomes as it is relatively inexpensive and yields information on a large number of loci without having to obtain sequence data for primer design (DeWolf *et al*., 2004 and Atienzar *et al*., 1999). RAPD profiles are achieved by PCR with single short primers of arbitrary nucleotide sequence under low annealing conditions. Fragments generated by RAPD are visualized after agarose gel electrophoresis and ethidium bromide staining. The resulting DNA profiles may differ due to band shifts, missing bands or the appearance of new bands. These bands can be scored to evaluate genetic similarities or dissimilarities. Furthermore, RAPD bands can be scored for geno mic template stability (GTS) evaluation to detect various types of DNA damage and mutations (rearrangement, point

mutations, small insertions or deletions of DNA and polyploidy changes) which suggests that RAPD bands may potentially form the basis of novel biomarkers assays for detection of DNA damage and mutations in the cells of bacteria, plants and animals (Savva, 1998 and Atienzar *et al*., 1999).

This study aimed to investigate the effect of silica nano particles sprayed on soybean (*Glycin max*) plants on the population of spider mite *Tetranychus cucurbitacearum* (Sayed). Also, the effect of SiNPs on soybean plants was studied. On the other hand, total soluble protein was evaluated to determine the effect of SiNPs on protein profile to describe genotoxic effects of SiNPs induced-DNA damage by RAPD analyses and also to compare GTS values calculated from the changes in RAPD profiles.

MATERIALS AND METHODS

This study was conducted at the Experimental Farm of Sakha Agricultural Station during 2014 soybean season. The laboratory experiment was applied at Genetic Department, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Experimental design

Complete randomized block design was adopted using two soybean cultivars; Giza 111 and Giza 35. Three silica nano-particle concentrations; 250, 350 and 450 ppm were used in addition to control (without treatments). The plot area measured 42 m^2 in three replicates (ten ridges, each of six meter long, and 70 cm between ridges).

Sampling

From each plot, samples of 10 soybean leaflets were picked up at random just before treatments, and 3, 6 and 9 days post-treatments. The leaflets were examined under binocular microscope to count and record the number of motile mites. For studying the agronomic traits, 10 guarded soybean plants were randomly chosen to record the following traits: number of branches, number of buds/plant, number of seeds/plant and seed yield.

Percentages of reduction in mite populations were counted using Henderson and Tilton equation (1955).where the equation used was as follows:

$$\text{Reduction } \% = 100 \times \left[1 - \left(\frac{n \text{ in Co before spraying}}{n \text{ in Co after spraying}} \times \frac{n \text{ in T after spraying}}{n \text{ in T before spraying}}\right)\right]$$

Where , n =insect population , T=treated and Co = control

Tested compound

The chemical compound: silicon dioxide nano-particles (SiO_2NP_s) purity 99.9% and its size is about < 15 n m. was supplied by Nanotech Egypt for Photo Electronics.

SDS-Polyacrylamide gel electrophoresis protein analysis

Fresh leaves were taken from soybean M_2 plants whose parents were previously treated with different concentrations of SiNPs and then milled to fine powder. Soluble water proteins were extracted using sucrose 20% according to Laemmli (1970). Centrifugation was performed at 10,000 rpm for

10 min and 40 µl supernatant of soluble proteins were loaded on SDS-slab gel of 12% acrylamide containing 10% SDS. Gel was run at a current of 15 mA for 1 h followed by 25 mA for 4-5 h. Following electrophoresis, the gel of total protein was stained for 2 h. with Coomassie Brilliant Blue G-250. Molecular weights of the different bands were calibrated using the wide range protein marker (PiNK prestained protein ladder) ranged from 15-175 kDa.

Genomic DNA extraction and DNA fingerprinting by RAPD markers

Total genomic DNA was extracted from M_2 fresh leaves using the i-genomic plant DNA Extraction Mini Kit (iNtRON Biotechnology, Inc. Cat.No. 17371, 50 columns). Extracted DNA was examined by subjecting it to 0.8% agarose gel electrophoresis stained with ethidium bromide. The quality and quantity of DNA were determined by a gel documenting instrument. Then, DNA samples were diluted to a final concentration of 40 ng/µl, and these dilutions were used as DNA template in the PCR reaction.

Polymerase chain reaction (PCR) amplification was carried out with six 10-base pair random primers (Bioneer-company) and genomic DNA as the template (Table 1). Amplification was performed in a reaction mixture of 25 µl containing 2xTaq PCR MasterMix (TIANGEN), approximately 40 ng of the genomic DNA dissolving in sterile Tris-EDTA (TE) and 2 µl of 10 µM primer.

Table 1. Primers used for random amplified polymorphic DNA analysis.

Code	Sequence (5′ to 3′)
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPB-12	CCTTGACGCA
OPB-14	TCCGCTCTGG

Amplifications were performed in a DNA thermocycler (BioRad., U.S.A) programmed for 3 min at 94°C (initial denaturing step), 35 consecutive cycles each consisting of 30 sec at 94°C (denaturing), 30 sec at 30°C (annealing), 1 min at 72°C (extension), and followed by 1 cycle for 5 min at 72°C (final extension step). Amplification products were detected by 1.5% agarose gel electrophoresis in Tris-Borate- EDTA (TBE) buffer and 1 Kb plus DNA bands were stained with ethidium bromide, visualized and photographed under UV light using gel documentation system (Syngene). The size and intensity of each amplification product were automatically estimated using the UV-Transilluminator image analyzer system. Genomic template stability (%) was calculated as following: GTS (%) = (1 - a/n) x100

Where a is the average number of polymorphic bands detected in each treated sample and n the number of total bands in the control. Polymorphism in RAPD profiles included disappearance of a normal band and appearance of a new band in comparison to control (Atienzar *et al.,* 1999). The average was calculated for each experimental group exposed to different concentrations of SiNPs.

Statistical analysis procedures

Data were subjected to analysis of variance, and significantly different means were compared using L.S.D or Duncan′s multiple range test (1955).

RESULTS AND DISCUSSION

Effect of silica nano-particals on population density of motile stages of *Tetranychus cucurbitacearum* on two soybean variteis

Data presented in Table (2) show the effect of spraying plants of Giza 111 soybean cultivar with nanosilica particles on the population density of motile mite, *Tetranychus cucurbitacearum*. The results revealed that the most effective concentration of SiNPs against the mite was 450 ppm, as it induced average reductin of 78.91% throughout the experimental period, followed by 250 ppm (71.24% reduction) and 350 ppm (62.16% mite population reduction). However, three days post-treatments, the highest mite reduction (81.20%) resulted from 250 ppm SiNPs, followed by 350 ppm (76.11%), while the least reduction was obtained from 450 ppm nano-silica particles. In general, the highest mite reduction was observed after six days post-treatment (76.33 – 88.23%) for all concentrations, while the least reductions were observed after nine days post-treatment (34.05 – 77.44%).

Table 2. Effect of silica nano-particles on population density of motile stages of *Tetranychus cucurbitacearum* on soybean Giza111 under field conditions.

Treatments	No. of motile stage before spraying	No. of motile stages per 10 leaflets after spraying and reduction percentage at						
		3 days		6 days		9 days		Mean Reduction
		No.	Red.	No.	Red.	No.	Red.	
250 ppm	200.00	76.67	81.29 a	20.00	84.10 ab	33.33	48.34 b	71.24
350 ppm	156.67	76.67	76.11 ab	23.33	76.33 b	33.33	34.05c	62.16
450 ppm	180.00	106.67	71.07 b	13.33	88.23 a	13.33	77.04 a	78.91
Control	206.67	423.33	-	130	-	66.67	-	-

Data presented in Table (3) show the effect of spraying nano-silica on soybean cultivar Giza 35 on population density of *T. cucurbitacearum*. Overall mean reduction of mite population resulted from 450 ppm nano-silica (62.34%). Lower reductions in mite population were obtained from 250 ppm (38 %) and 350 ppm (41.92%). The results revealed that lower reduction was obtained at six days post-treatments, while the reductions were highest at nine days post treatment (70.14-93.20%). Overall mean reduction showed a concentration-response.

Table (3):Effect of silica nano-particles on population density of motile stages of *Tetranychus cucurbitacearum* on soybean Giza35 under field conditions.

Treatments	No. of motile stage before spraying	No. of motile stages per 10 leaflets after spraying and reduction percentage at						
		3 days		6 days		9 days		Mean Reduction
		No.	Red.	No.	Red.	No.	Red.	
250 ppm	606.67	313.33	37.12 b	270.00	6.75 c	10.00	70.14 b	38
350 ppm	660.00	453.33	16.38 c	256.67	18.52 b	3.33	90.86 a	41.92
450 ppm	886.67	280.00	61.56 a	286.67	32.26 a	3.33	93.20 a	62.34
Control	1026.67	843.33	-	490.00	-	56.67	-	-

The results indicated that the motile stages of *T. cucurbitacearum* on soybean variety Giza 111 was more susceptible to the tested compound (SiNPs) than soybean Giza 35. Also, the concentration 450 ppm was more effective to the motile stages *T. cucurbitacearum* on two soybean varieties than other concentrations. These results are in agreement with those of Thabet (2015), who found that SiNPs reduced almost *Aphid craccivora* individuals with the highest concentration, 450 ppm, after just one day of treatment and reached 100% at 250, 300 and 375 ppm after 5 days of treatment.

Effect of SiNPs on morphological traits of soybean plants

As shown from the results presented in Table (4) number of branches/plant recorded the highest values at 350 ppm (4.50 and 3.70) among both varieties, Giza 111 and Giza 35, respectively. These results are in harmony with those obtained by Mohamed (1994), El-Desoky and El-Far (1996) and Radi (1999), they found that Giza 111 and Giza 35 plants gave significant values for plant height and number of branches/plant.

Number of pods/plant recorded the highest values at 450 ppm among both varieties, while, the lowest ones were recorded for the control followed by 250 ppm among both varieties. Generally, the higher mean number of pods /plant was recorded in Giza 111 followed by Giza 35, respectively.

Number of seeds per plant was highest at 450 ppm among both varieties (214 and 200, respectively), while control and 250 ppm produced the lowest number of seeds per plant. On the other hand, Giza 111 gave higher number of seeds per plant than Giza 35. These results are in general agreed with those obtained by Radi (1999), Hassan *et al.* (2002) and Mohamed and Morsi (2005). Hassan *et al*. (2002) evaluated some soybean cultivars, results showed that Giza 111 surpassed all tested cultivars in pod numbers followed by Giza 35 cultivars.

In general, 350 ppm induced significant increase in 100 seeds weight (16.5 and 15.5 g) over the two varieties. Giza 111 had heavier 100-seed weight (16.3 g) than Giza 35 (15.3 g). Significant differences in 100-seed weight among soybean genotypes under different environmental conditions were reported by Radi (1999) and Hassan *et al*. (2002).

The highest seed yield per plant was recorded at 450 ppm (35 and 31 g), compared to 350 ppm treatment (33 and 30 g), over the two varieties,

respectively. Seed yield per plant was varied among the different soybean varieties at all treatments. The results indicated that Giza 111 produced the greatest seed yield per plant (30.75 g) followed by Giza 35 (28 g).

On the other hand, the highest values of seed yield/ fed. were recorded at 450 ppm, while, the control and 250 ppm produced lower seed yield/fed among both varieties. These results are in the same trend with those obtained by El-Borai *et al*. (1993), Hassan *et al*. (2002), Hossain *et al*. (2003) and Ray *et al.* (2008), they found that, Giza 111 cultivar showed the highest seed yield/plant and feddan, followed by Giza 35 cultivar. The superiority of Giza 111 cultivar might be due to its vigorous and taller plants that produced more seeds/plant and feddan, with highest seed weight, which improved its seed yield per plant and per feddan.

Table 4. Mean performance of different traits, as affected by SiNPs.

Varieties	Treatments	No. of branches	No.of pods/ plant	No.of seeds /plant	100-seed weight(g)	Seed yield /plant(g)	Seed yield /fed(kg)
Giza111	250 ppm	4.20	74	172	16.2	28	1740
	350 ppm	4.50	81	200	16.5	33	2350
	450 ppm	4.40	92	214	16.3	35	2460
	Control	3.90	70	167	16.1	27	1620
Mean		4.25	79.25	188.30	16.30	30.75	2042.5
Giza35	250 ppm	3.40	65	172	15.1	26	1660
	350 ppm	3.70	70	196	15.5	30	2240
	450 ppm	3.60	77	200	15.3	31	2300
	control	3.50	58	164	15.2	25	1540
Mean		3.55	67.50	183	15.30	28.00	1935
$L.S.D_{0.05}$		0.14	3.40	9.40	0.09	2.71	13.40

Effect of SiNPs on the SDS-PAGE protein pattern

SDS-PAGE analysis was carried out on M_2 soybean leaf proteins whose parents were previously treated with different concentrations of SiNPs. Soybean plants were subjected to three different concentrations of SiNPs with an objective to find variation in protein banding pattern. There were high differences in protein banding pattern between the two tested genotypes and among the different concentrations of SiNPs. Most main proteins were similarly expressed at the three different concentrations in Giza 35.

Protein banding patterns illustrated in Fig. (1) exhibited the mutagenic effect of different SiNPs concentrations on soybean plants. SDS-PAGE protein pattern revealed that number of bands in Giza 35 ranged from 8 to 12, while in Giza 111 it was ranged from 4 to 10. Giza 35 and Giza 111 had two strong bands with a molecular weight in the range of 10 and 50 kDa as found in control and three SiNPs concentrations in both genotypes, but the two bands were differed in their intensity.

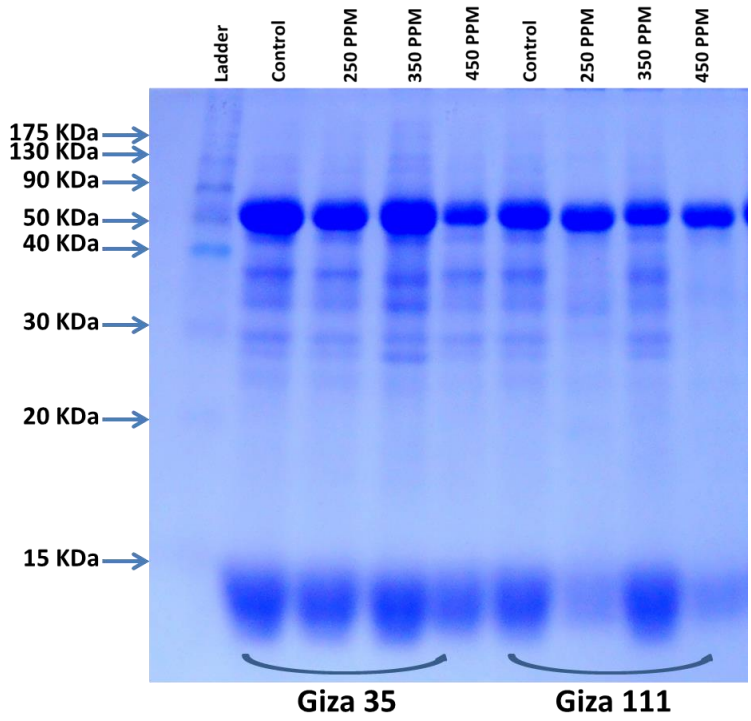

Figure 1. SDS-PAGE banding patterns of total soluble proteins for M_2 soybean plants at three concentrations of SiNPs in addition to control.

For Giza 35, 350 ppm SiNPs concentration caused appearance of two bands with molecular weights of 90 and 130 KDa, but 450 ppm SiNPs concentration caused disappearance of two bands with molecular weights of 25 and 75 KDa. Meanwhile, concentration of 350 ppm of SiNPs caused appearance of a new band in Giza 111 with molecular weight of 37.5 KDa. The disappearance of some bands may be resulted from inherited effects of SiNPs which explained the basis of mutational event on the regulatory genes that prevent or attenuate transcription (Muller and Gottschelk, 1973).

RAPD Based DNA Fingerprinting

Random Amplified Polymorphic DNA (RAPD) technique was used to detect variations among DNAs extracted from soybean plants treated with SiNPs and the control. RAPD profiles of control and treated plants and the data obtained from the amplification of six primers are shown in Fig. (2) and Table (5), respectively. Six oligonucleotide primers having 60-70 % GC content were used for screening fodder soybean genome for alterations, which generated specific and stable results with the total number of 16 and 18 bands for Giza 35 and Giza 111, respectively. Molecular size of RAPD

products ranged between 300 and 2000 bp. The RAPD profiles of treated and untreated groups showed low to moderate differences in banding patterns. If compared control and treatments, the differences observed in all RAPD profiles are clearly exhibited by the changes (appearance and/or disappearance of some bands) in the number, size and intensity of amplified DNA fragments for different primers.

The total number of bands scored for the six primers was 16 and 18 bands for Giza 35 and Giza 111, respectively. Out of them, five and eight bands were polymorphic and the percentages of polymorphism were 31.25 and 44.44% for Giza35 and Giza111, respectively. For Giza 35, only three primers showed polymorphism in a range of 33.33 to 66.67% between control and plants treated with SiNPs. Meanwhile, for Giza 111, five of six primers showed polymorphism which ranged between 33.33 and 75.0%.

Changes observed in the DNA profiles such as modifications in band intensity and loss of some bands may be due to the changes in oligonucleotide priming sites leading to genomic rearrangements, and less likely to point mutations or DNA damage in the primer binding sites which can block or reduce the efficiency of DNA polymerization in the PCR reaction (Atienzar *et al*., 2002; Liu *et al*., 2005; Gupta and Sarin, 2009 and Gao *et al*., 2010). Appearance of new PCR products occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers after structural change or because some changes in DNA sequence have occurred due to mutations (resulting in new annealing events), and/or large deletions (bringing two pre-existing annealing sites closer), and/or homologous recombination (juxtaposing two sequences that match the sequence of the primer) (Atienzar *et al*., 1999 and Liu *et al*., 2005).

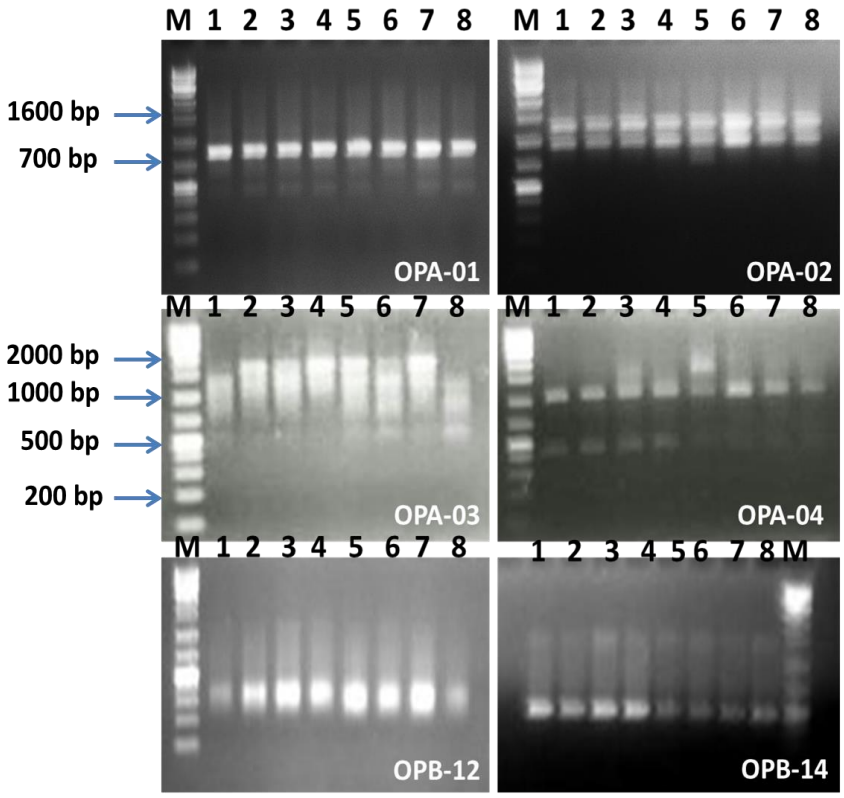

Figure 2. Random amplified polymorphic DNA pattern obtained by amplification of DNA from soybean plants.
Notes:
M = ladder DNA
Lane 1,2,3 and 4 (Giza 35) = control, 250 ppm, 350 ppm and 450 ppm, respectively.
Lane 5,6,7 and 8 (Giza 111) = control, 250 ppm, 350 ppm and 450 ppm, respectively.

Genomic template stability (GTS %)

DNA variation, induced in soybean cells treated with different concentrations of SiNPs, is shown in Table (6). The six tested primers gave specific and stable results, with apparent changes in the number and the intensity of amplified DNA bands. The different concentrations of SiNPs gave variable bands; compared to control, as reflected by changes in band intensity (increase/decrease), disappearance of bands, and appearance of new bands.

All SiNPs concentrations showed low GTS if compared to control. The GTS% decreased as SiNPs concentration increased. The maximum change in RAPD profiles (disappearance b and/or appearance a) was obtained in concentrations 350 and 450 ppm for Giza 35 and Giza 111, respectively if compared with control plants. Also, maximum change in band intensity (decrease d or increase c) was obtained in concentration of 450 ppm for Giza111 as compared to control plants. In this experiment, change in band intensity (decrease or increase) greatly increased at high SiNPs concentrations. Data showed a direct relationship between SiNPs concentration and band changes, thus suggesting nonrandom interaction between DNA and SiNPs.

In this study, DNA damage was shown by RAPD profiles via disappearance or appearance and alteration in intensity (decrease or increase) of bands. Bands intensity in some primers under different concentrations of SiNPs showed some changes (increase or decrease). Changes observed among RAPD profiles obtained from control and SiNPs treatments may be induced by direct and/or indirect interact with genomic DNA. This damage includes loss of one or more nucleotids which can lead to alterations of DNA sequence. The results indicated that SiNPs may interact with DNA causing genotoxic effect. This agreed with Lankoff *et al*. (2013), who determined the effect of different doses of unmodified and surface modified SiNPs (10, 25, 50 and 100 ug/ml) on DNA damage in human peripheral blood lymphocytes after 2 and 24 h. Their results revealed that unmodified SiNPs exhibited genotoxic properties at high doses. Also, Thabet (2015) studied the effect of different concentrations of SiNPs (75, 150, 225, 300, 375 and 425 ppm) on genotoxicity in faba bean plants which revealed that all concentrations showed decrease in GTS % if compared with control.

The genomic template stability, a qualitative measure reflecting changes in RAPD patterns, was used to compare the modifications in RAPD profiles with reductions in spider mite *Tetranychus cucurbilacearum* and soluble protein content of leaves in soybean plants. Following exposure to increasing SiNPs, spider mite *Tetranychus cucurbitacearum* and soluble protein content in soybean plants decreased gradually. Also, the genomic template stability decreased after exposure to SiNPs.

5-6

In conclusion, RAPD-PCR is a sensitive technique for detecting genetic alteration and it can be used as an investigational tool to detect the genotoxic effects of SiNPs on soybean. The higher doses of SiNPs caused toxic effects on DNA. The mean reduction percentage in the population of mite showed a concentration response. The higher concentration (450 ppm) of SiNPs induced highest reduction of mite on both varieties as well as, the former concentration recorded the greatest seed yield/plant among the two varieties studied herein.

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تأثير النانوسيليكا فى مكافحة العنكبوت الأحمر*Tetranychus cucurbitacearum* والصفات المحصولية لنباتات فول الصويا والتقدير الكمى لسميتها الوراثية بإستخدام تحليل البروتين الكلى و الـ RAPD.

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أدى اسـتخدام المبيـدات الأكاروسـية فـى مكافحـة العنكبـوت الأحمـر *Tetranychus cucurbitacearum* إلى حدوث كثير من المشاكل ، لذلك تم إجراء هذا البحث بهدف تقييم تأثير مركب النانوسيليكا كطريقة جديدة لمكافحة العنكبوت الأحمر على نبات فول الصويا. فى هذه الدراسة تم إستخدام ثلاثة تركيزات من النانوسيليكا هى 250 ، 350 ، 450 جزء فى المليون رشا على صنفين من نبات فول الصويا هما جيزة 35 و جيزة 111 خلال موسم 2014. سجلت النسبة المئوية للإنخفاض فى تعداد العنكبوت تحت الظروف الحقلية. أوضحت نتائج هذه الدراسة أن متوسط النسبة المئوية للإنخفاض فى تعداد العنكبوت زادت بزيادة تركيز مادة النانوسيليكا ، وكانت أعلى نسبة للإنخفاض فى تعداد العنكبوت للتركيز 450 جزء فى المليون على كل من الصنفين المستخدمين جيزة 111 ، جيزة 35 بنسبة 78.91٪ ، 62.34٪ على الترتيب يليه التركيزان 250 ، 350 جزء فى المليون بنسبة 67.32٪ ، 32٪ لصنف جيزة 111 ، 62.16٪ ، 41.92٪ للصنف جيزة 35 على الترتيب. أوضحت النتائج أيضاً أن الأطوار المتحركة للعنكبوت الأحمر على صنف جيزة 111 كانت أكثر حساسية لمركب النانوسيليكا من صنف جيزة 35. من ناحية أخرى أوضحت نتائج الدراسة أن التركيز 450 جزء فى المليون سجل أعلى معدل فى إنتاج الحبوب بالنسبة للفدان وذلك فى كل من الصنفين المذكورين بينما كان الكنترول والتركيز 250 جزء فى المليون أقل فى معدل إنتاج الحبوب بالنسبة للفدان. أوضحت الدراسة وجود اختلافات عالية في بروفيل البروتين بين الصنفين المختبرين حيث وجد أنه مع زيادة تركيز مادة النانوسيليكا يزداد التغير فى بروفيل البروتين فى صنفى فول الصويا. نتج عن استخدام ستة بادئات عشوائية أشكالا مظهرية منخفضة إلى متوسطة كما أعطت تغيرات وراثية بين تركيزات مادة النانوسيليكا وبين التراكيب الوراثية المدروسة. أشتملت التغيرات فى بروفيل الـ DNA على تغيرات فى كثافة الحزم، وظهور وغياب الحزم الأصلية وأيضاً ظهور حزم جديدة. أشارت النتائج أيضاً إلى أن ثبات جينوم الـ DNA تأثر بتركيزى 350 ، 450 جزء فى المليون بالنسبة للصنفين جيزة 35 ، جيزة 111 والتى تـــــــــــم التعـــــــــــرف عليهـــــــــــا بتحليـــــــــــل الـــــــــــ RAPD .

Table 5. Total number of bands, polymorphic bands and percentage of polymorphism in DNA-RAPD profiles of soybean plants treated with different concentrations of SiNPs.

Primers code	Genotype														
	Giza 35							Giza 111						
	Concentration (ppm)				Total bands	Polymorphic bands	Polymorphism (%)	Concentration (ppm)				Total bands	Polymorphic bands	Polymorphism (%)
	control	250	350	450				control	250	350	450			
OPA-01	3	3	3	3	3	0	0.00	3	2	3	3	3	1	33.33
OPA-02	2	2	2	2	2	0	0.00	3	2	2	2	3	1	33.33
OPA-03	3	3	3	2	3	2	66.67	4	4	3	3	4	3	75.00
OPA-04	2	2	3	2	3	1	33.33	3	2	2	2	3	1	33.33
OPB-12	1	1	3	3	3	2	66.67	3	3	3	1	3	2	66.67
OPB-14	2	2	2	2	2	0	0.00	2	2	2	2	2	0	0.00
Total	13	13	16	14	16	5	31.25	18	15	15	13	18	8	44.44

Table 6. Changes in DNA-RAPD profile of soybean plants treated with different concentrations of SiNPs.

Primer code	Genotypes																							
	Giza 35													Giza 111										
	No. of bands in control	Concentration (ppm)												No. of bands in control	Concentration (ppm)										
		250				350				450					250				350				450			
		A	b	c	d	a	b	c	D	a	b	c	d		A	b	c	d	a	b	c	d	a	b	c	d
OPA-01	3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	1	0	0	0	0	0	0
OPA-02	2	0	0	0	1	0	0	1	1	0	0	1	1	3	0	1	2	0	0	1	2	0	0	1	2	0
OPA-03	3	1	1	1	0	1	1	1	0	1	2	1	0	4	0	0	0	1	0	1	2	0	0	1	2	1
OPA-04	2	0	0	0	0	1	0	0	0	0	0	0	0	3	0	1	1	0	0	1	1	0	0	1	1	0
OPB-12	1	0	0	1	0	2	1	1	0	2	0	1	0	3	0	0	0	0	0	0	0	0	0	2	0	1
OPB-14	2	0	0	0	0	0	0	1	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	1	0
Total	13	1	1	2	1	4	2	4	1	3	2	4	1	18	0	3	3	1	0	4	5	0	0	5	6	2
a+b		2				6				5				a+b	3				4				5			
a+b+c+d		5				11				10				a+b+c+d	7				9				13			
GTS (%)		84.62				53.85				61.54				GTS (%)	83.33				77.78				72.22			

a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d:decrease in band intensity, a+b: polymorphic bands, GTS: genomic template stability.