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Genotoxic Effects of Some Plant Oils and Abamectin in *Vigna unguiculata* L. Stored Seeds as Revealed by the iPBS-DNA Fingerprinting

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ESSENTIAL oils and the bio-insecticides have been utilized to protect the plants aginst different kind of insects. However, it is really improtent to study the side effects of these essential oils and insecticides on plants. The iPBS technique based on Long Terminal Repeat (LTR) retrotransposons was applied in this study to detect the DNA damage in cowpea seeds exposed to three plant oils of *Trigonella foenum-graecum*, *Panax ginseng*, *Origanum majorana* and the bio-insecticide abamectin. Different levels of polymorphism ranged from 37.50% polymorphism for primer 2394 to 82.35% polymorphism for primer 2270 with a total average of 52.00%. Also, decrease in genetic template stability (GTS%) values were recorded with all treatments as compared with the control. The value of Band Sharing Index (BSI) for each treatment was calculated and the results showed that mixture 2 (LC20 abamectin + 2LC95 ginseng) showed the lowest (BSI) as compared with all treatments. The obtained results, suggested that the assurance should be strongly taken in the consideration for the safety, efficacy, and toxicity of the preparation derived from plants. Furthermore, iPBS assay is a powerful tool for the detection of the genotoxic effects of environmental chemicals and offer great promise for future especially for the determination of genetic damage following exposure to contaminants.

Keywords: iPBS technique**,** Plant oils**,** Cowpea seeds**,** GTS and BSI.

Introduction

Seeds are the basic and crucial input for agricultural production. During storage, the quality of seeds gets deteriorated in a number of ways; of which infestation by the storage pests contributes a bulk share. *Callosobruchus maculates* (Family Bruchidae), is one of the most devastating pests for leguminous seeds causing up to 100% loss of stored *Vigna unguiculata* (L.) (Cowpea) (Lale, 1991 and Tiroesele et al., 2015). Widespread use of synthetic insecticides for the control of storage pests has generated different problems such as environmental pollution and toxic hazards. However, if the infection is severe, the continued application could not fully suppress pests as well as led to pest resistance (Maina & Lale, 2004). Wang et al. (2015) believed that the biocontrol method for stored-product pests would have a broad application prospects in the future.

Recently, there has been an increased attention in developing new control methods or materials that are active against the target insect species, environmentally safe, biodegradable, with no residual activity or harmful effects on the stored seeds. It has reported that essential oils have a fundamental role in the plants as antiviral, antibacterial, antifungal and insecticides (Isman, 2000). Plant oils are generally exempted from toxicity data requirements by the Environmental Protection Agency, USA. The volatile compounds, of the essential oils of Trigonella foenum-graecum (fenugreek) and Origanum majorana (marjoram) seeds were tested as antimicrobial agent by Ramadan et al. (2014) who showed that the tested oils have very strong potential applicability as antimicrobial and antioxidant agents. The authors demonstrated that terpinene-4-ol, geranial, trans-cinnamyl aldehyde and linolenic acid were the dominant volatiles in these oils. The essential oil obtained from marjoram contains high percentage of polyphenols and monoterpenes which are well known as antioxidants (Misharina et al., 2009). On the other hand, *Streptomyces* spp. are one of the most important actinobacteria which can provide new bioactive compounds for use as insect-control agents, such as avermectin, doramectin and quinomycin (Huamei et al.,

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2008 and Korkmaz et al., 2015). Abamectin (ABM), a macrocyclic lactone natural product extracted from *Streptomyces avermitilis*, has been documented most recently as a bio-insecticide in many parts of the world (Burg et al., 1979; Fisher & Mrozik, 1989; Mujica et al., 2000; Mahmoud et al., 2013 and Siddique et al., 2013).

DNA damages due to toxigenesis have been recently estimated by using DNA markers approaches (Savva, 1998, 2000; Atienzar et al., 1999 and Neeratanaphan et al., 2014). Molecular markers have provided new ways of detecting DNA damage (Cenkci et al., 2009 and Esmaeili et al., 2017). Kalendar et al. (2010) has described inter primer binding site (iPBS) technique that is based on LTR retrotransposons and serve as a general marker system. Retrotransposons are one of the two main groups of transposable elements (TEs) in eukaryotic genomes. In many crops between 40 to 70% of the total DNA is covered by LTR retrotransposons (Bennetzen, 1996 and Kumar & Bennetzen, 1999). The conserved regions of primer binding sequences (PBS) in LTR retrotransposons was exploited to designed primers that was proved to be very effective in PCR amplification of eukaryotic genomic DNA (Kalendar et al., 2010, 2011).

The mechanism by which this protocol works is that retrotransposon integration sites demonstrate joints between the conserved LTR ends and flanking genomic DNA. Therefore, the retrotransposon-based marker system using PCR will amplify a segment of genomic DNA around this joint. So, it is believed that DNA fingerprints using PCR could be used as biomarkers and as a method of identifying mechanisms of toxicity. Therefore, this work aims to assess DNA fingerprinting-based iPBS approach as an alternative biomarker assay to detect the DNA damage in cowpea seeds exposed to three plant oils of *Trigonella foenum-graecum* (fenugreek)*, Panax ginseng* (ginseng), *Origanum majorana* (marjoram) and the bio-insecticide abamectin in terms of DNA patterns change and genomic template stability (GTS) evaluation values. The chemical composition of these plant oils had been previously identified (Billaud, 2001; Leeja & Thoppil, 2007 and Lü et al., 2009), but there are only limited data and there are no reports available on their insecticidal effect against stored products insect (*Callosobruchu smaculatus*) and on stored seeds.

Materials and Methods

Seeds of cowpea (cultivar Teba); three plant oils; fenugreek (family: Fabaceae), ginseng (family: Araliaceae), marjoram (family: Lamiaceae) and the bio-insecticide abamectin were used in this study. Abamectin is a mixture of 80% avermectin B_{1a} and 20% avermectin B_{1b}; derived from *Streptomyces avermitilis* (Burg et al., 1979 and Fisher & Mrozik, 1989). Seeds of cowpea and abamectin were provided from the Agricultural Research Center, Giza, Egypt, while plant oils were obtained from Cap-Pharm Company; Cairo Egypt (http://www.elcaptain.net/).

The lethal concentration (LC) for 95% and 20% mortality (LC_{95}, LC_{20}), for each oils and abamectin were determined then, three mixtures (1, 2 and 3) from these oils and abamectin were prepared. These concentrations were selected on the basis of mortality percentage of *Callosobruchus maculates* and their effect on the stored plant seeds (Essam, 2017). Seeds of cowpea were treated with the concentrations of $2LC_{95}$ of each oils; LC_{95} abamectin, and with the three mixtures (LC_{20} abamectin + 2 LC_{95} of each oil) then stored for a duration of three months with control seeds (untreated seeds), Three replicates were carried out for every treatment. All treatments were done in the laboratory conditions; 27±3°C and 65±5% RH (relative humidity).

Molecular studies

iPBS assay

Ten iPBS-primers were used in this study according to Kalendar et al. (2010). The codes and sequences of these primers are listed in Table 1 (Primers were designed at Helsinki University Finland and provided by Dr. Marwa Mahmoud, Agriculture Research Center, Cairo). The annealing temperature for all primers have been shown in Table 1.

DNA isolation and PCR amplification

Genomic DNA was isolated from seeds of the cowpea seedlings, previously treated with $2LC_{95}$ of each oil, LC_{95} abamectin and the mixtures (1, 2, and 3) following the CTAB protocol (Doyle & Doyle, 1990) with some modifications (Baloch et al., 2016).

The extracted DNA was quantified and DNA purity was determined through measuring UV

absorbance at 260 and 280nm. For prepearing PCR reactions, the following reagents were mixed; 2.5µl dNTPs (2mM), 2µl $MgCl_2$ (25mM), 5µl 5X Taq buffer, 1µl Primer, 3µl Templet DNA (20ng/µl), 0.1µl Taq DNA polymerase (5U/µl) and the reaction completed by distilled H_2O up to 25µl. Amplification was done in MJ Research PTC-200 thermal cycler PCR, using the following program: one cycle of 95°C for 3min; 35 cycles of 95°C for 20sec, 55°C for 30sec, 72°C for 90sec and one cycle of final extension at 72°C for 5 min. Then all reactions were holed on the PCR at 12 C° forever. For screening the PCR products, 1.2% of agrose gels were stained using ethidium bromide solution (0.5mg/ml). Samples were mixed with 1X loading buffer, and loaded into the wells of the gel. Electrophoresis was done in 1X TAE buffer at a constant voltage of 70Volts. Electrophoresis Gene Ruler™ DNA ladder 100-10,000 base range was used to justify the DNA band size. The DNA fingerprints of control and treated seeds may then be compared and analyzed.

Data analysis

Changes in the iPBS patterns were expressed as presence (1) or absence (0). Genomic template stability percentage (GTS %) was calculated according to Atienzar et al. (1999) as following:

$$GTS\ (\%) = 100 - [100\ (A/N)]$$

where A is the average number of polymorphic bands detected in DNA profiles of each treated sample, and N is the number of total bands in the control DNA profiles.

The band sharing index (BSI) was also calculated as a measure of similarity between two samples using the following equation:

$$BSI = 2S/(C + D)$$

where S is the number of bands shared between two samples, C is the number of bands in the first sample and D the number of bands in the second sample. A BSI value of one indicates that two samples are identical, while BSI value of zero denotes that two samples are totally different (Savva, 2000).

Results and Discussion

Monitoring of genotoxicity and DNA fingerprinting

Exposure of an organism to a genotoxic chemical may result in damages in the DNA. However, some of this damage may not be revealed by the methods used widely at present. The enormous advances and developments in molecular biology tools during the last three decades offer various new possibilities for detecting DNA damage through DNA markers (Castellani et al., 1993; Savva et al., 1994; Savva, 1996, 1998; Enan, 2006; Al-Qurainy 2010 and Pal, 2016). Consequently, in this work, we investigated the possibility of using DNA fingerprinting by iPBS technique that is based on LTR retrotransposons and serve as a general marker system to detect DNA damage in cowpea genome for alteration in response to treatments with plant oils and the bio-insecticide abamectin.

TABLE 1. Name, sequences, number of base pair of ten iPBS primers.

Serial	Primers code	Sequence 5`→3`	Number of base pair	Tm (C°)
1	2224	ATCCTGGCAATGGAACCA	18	58.3
2	2229	CGACCTGTTCTGATACCA	18	55
3	2391	ATCTGTCAGCCA	12	41.5
4	2270	ACCTGGCGTGCCA	13	56.1
5	2392	TAGATGGTGCCA	12	40.9
6	2274	ATGGTGGGCGCCA	13	56.3
7	2394	GAGCCTAGGCCA	12	46
8	2370	TCGCATCAACCAA	13	45.4
9	2415	CATCGTAGGTGGGCGCCA	18	64
10	2231	ACTTGGATGCTGATACCA	18	54.7

Rproducible electrophoretic patterns that obtained in this study were represented in Table 2 and Fig. 1. The results showed different levels of polymorphism between the retro-primers used, ranged from 37.50% polymorphism for primer 2394 to 82.35% polymorphism for primer 2270 with a total average of 52.00%. Four positive and two negative unique bands were also recorded (arrows). Similarly, Enan (2007) found change in fingerprinting patterns generated by AP-PCR in *Phaseolus vulgaris* exposed to different concentrations of para-nitrophenol (4.0-720nM), in which the highest concentration stimulated genomic alterations in more than a half, with 39% markers were polymorphic. Kalendar et al. (2010) indicated that retrotransposons are known to be activated by abiotic and biotic stress and the iPBS amplification technique is an efficient method for detection of polymorphism resulting from retrotransposon activities or recombination. Atienzar et al. (1999) reported that changes in DNA fingerprint reflect DNA alterations from single base changes to complex chromosomal rearrangements.

The quantitative analysis of iPBS patterns obtained in this work, expressed as band appeared and disappeared, shows change in band number in all tested samples as compared with the control (Table 3). Abamectin alone and the three mixtures used, recorded a maximum number of total loss and gain bands as compared with that of fenugreek, marjoram and ginseng oils. Abamectin recorded 29 total gain and loss bands followed by ginseng (28 bands) while fenugreek and marjoram oils recorded 26 bands. On the other hand, mixture 2 (LC20 abamectin + 2LC95 ginseng) recorded the highest number of loss and gain bands (37) followed by mixture 3 and 1 which recorded 33 and 31 bands respectively. Similarly, Savva et al. (1994) showed that the RAPD profiles generated from rats exposed to benzo (a)pyrene revealed the appearance and disappearance of bands in comparison to control patterns and this change in DNA fingerprints will be altered by factors such as the appearance or disappearance of priming sites, by DNA strand breaks and by the presence of DNA adducts in primer binding sites on DNA.

Exposure of an organism to a genotoxic chemical may result in the formation of covalently bound adduct between the chemical and the DNA; faulty repair of these adducts may prevent the primer from binding to those sites command to mutations and, sometimes to cytogenetic changes (Savva, 1998 and Atienzar et al., 1999, 2002). Atienzar & Jha (2006, 2004) confirmed that when the *Taq* DNA polymerase encounters a DNA adduct, there are a number of possible results including blockage, by-pass and the possible dissociation of the enzyme/adduct complex which will cause changes in RAPD profiles. Breakages which take place in the DNA template between two opposite primers may result in a loss of an amplicon whereas genetic rearrangements and point mutations may be accountable for either a loss or induction of new annealing sites which could result in the disappearance or appearance of new amplicons, respectively. The appearance of bands could be referring to the presence of priming sites which become accessible to primers after structural modulation in DNA sequence that occurred due to mutations (resulting in new annealing events) or large deletions (bringing two pre-existing annealing sites closer) or recombination; while the disappearance of bands may be attributed to the presence of DNA adducts, which can act to block or reduce the polymerization of DNA in the PCR-reaction (Jones & Parry, 1992).

In addition to appearance and disappearance of bands in iPBS profiles, a decrease in genetic template stability (GTS%) values were recorded with all treatments as compared with the control (Table 3). Both fenugreek and marjoram recorded higher value (70.79%) than ginseng and abamectin which recorded 68.54% and 67.42%, respectively. On the other hand, mixture 1 (LC_{20} abamectin + $2LC_{95}$ fenugreek) recorded GTS value of 65.17%, followed by mixture 3 (LC_{20} abamectin + $2LC_{95}$ marjoram), then mixture 2 (LC_{20} abamectin + $2LC_{95}$ ginseng) which recorded 62.93 and 58.43% respectively. These data showed that GTS values of sample treated with mixture 2 (LC_{20} abamectin + $2LC_{95}$ ginseng) is recorded the lowest values between all treatments. GTS effect is ascribed to the multiple changes in iPBS profiles (loss or addition of bands) which tend to counterbalance each other. High GTS value indicates that the genome is less prone to alterations in its DNA, whereas low GTS value indicates greater chances of DNA alteration. These results are consistent with earlier studies. Ciğerci et al. (2016) showed that GTS % values was significantly decreased in onion bulbs treated with *Thermopsis turcica* extract for 24h. Changes in bands may be the consequences of genomic template instability,

related to the level of DNA damage, the efficiency of DNA repair and replication (Atienzar et al., 2002; Atienzar & Jha 2004 and Ciğerci et al., 2016).

TABLE 2. Number of total, monomorphic and polymorphic bands, negative and positive unique bands and percentage of polymorphism of iPBS markers.

Primers names	Total number of bands	Monomorphic bands	Polymorphic bands	Negative unique bands	Positive unique bands	Polymorphic percentage
1- 2224	19	8	10	----	1	57.89
2- 2229	14	6	8	----	----	57.89
3- 2270	17	3	13	1	----	82.35
4- 2391	13	6	6	---	1	53.85
5- 2392	7	4	3	---	---	42.86
6- 2274	12	5	6	---	1	58.33
7- 2394	8	5	3	---	---	37.50
8- 2370	13	5	8	----	---	61.53
9- 2415	13	6	5	1	1	53.85
10- 2231	9	6	3	---	---	53.60
Average	125	54	65	2	4	52.00

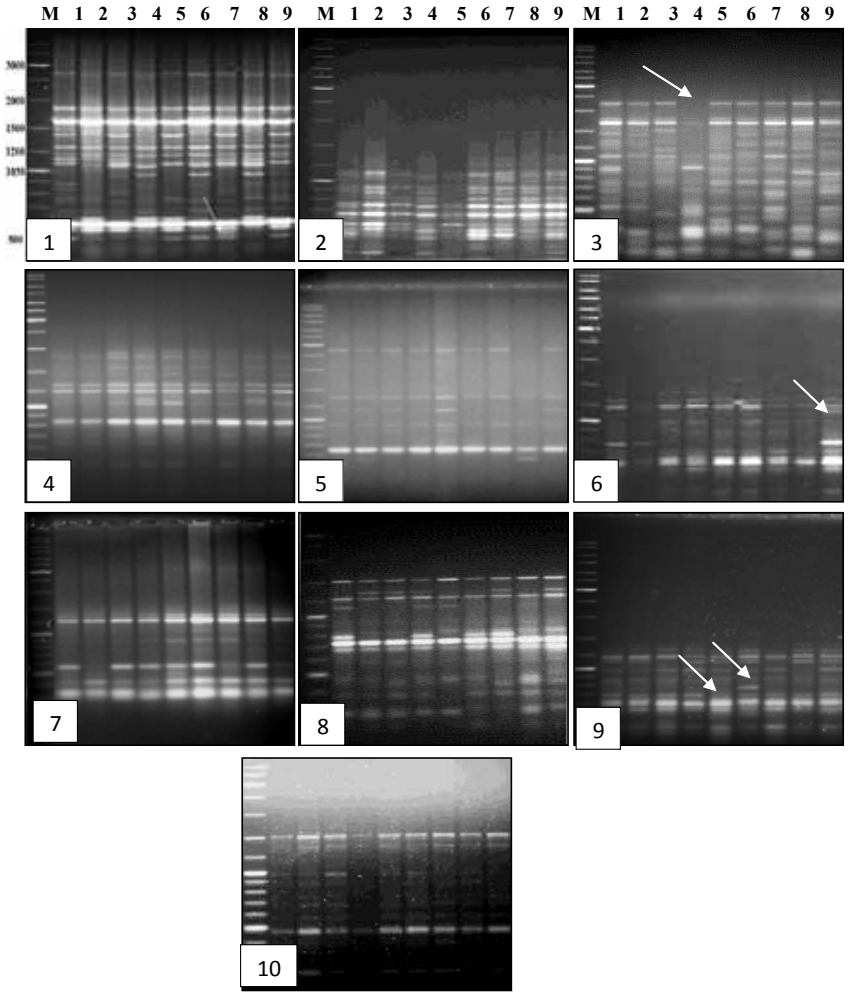

Fig. 1. iPBS profile of *Vigna unguiculata* using primers (1- 10): 2224, 2229, 2270, 2391, 2392, 2274, 2394, 2370, 2415 and 2231 [M= Ladder DNA, 1, 2= Negative and positive control, 3-5= 2LC95 of fenugreek, ginseng and marjoram, 6= LC95 of abamectin, 7- 9= Mixture 1, 2 & 3].

TABLE 3. Band Sharing Index (BSI), number of new bands appeared (a), bands disappeared (b), as related to control and Genome Templet Stability (GTS) percentage in *Vigna unguiculata* L. seeds treated with the tested materials using iPBS markers.

Number	Primers name	Control	$2LC_{95}$ Fenugreek			$2LC_{95}$ Ginseng			$2LC_{95}$ Marjoram			LC_{95} Abamectin			Mixture 1 (LC_{20} abamectin + 2 LC_{95} fenugreek)			Mixture 2 (LC_{20} abamectin + 2 LC_{95} ginseng)			Mixture 3 (LC_{20} abamectin + 2 LC_{95} marjoram)		
			BSI	a	b	BSI	a	b	BSI	a	b	BSI	a	b	BSI	a	b	BSI	a	b	BSI	a	b
1	2224	15	0.89	1	1	0.79	2	4	0.93	0	1	0.90	2	1	0.84	3	2	0.76	3	3	0.97	0	1
2	2229	9	0.75	1	3	0.80	3	1	0.88	0	2	0.80	3	1	0.74	2	3	0.67	5	1	0.78	5	0
3	2270	11	0.70	2	4	0.74	1	4	0.67	3	4	0.59	3	3	0.75	4	2	0.85	4	0	0.57	4	5
4	2391	8	0.89	1	1	0.90	3	0	0.95	2	0	1.00	1	1	1.00	1	0	1.00	2	0	0.93	1	2
5	2392	5	0.91	1	0	0.89	0	1	0.73	1	0	0.89	0	1	0.89	0	1	0.80	1	1	0.91	1	0
6	2274	9	0.84	2	1	0.84	2	1	0.84	1	1	0.84	1	1	0.82	0	2	0.75	1	3	0.84	1	1
7	2394	6	1.00	0	1	0.83	1	1	0.92	1	0	0.77	2	1	0.77	2	1	0.77	2	1	0.90	0	1
8	2370	9	0.59	2	2	0.95	1	0	0.88	1	2	0.72	1	1	0.78	2	2	0.78	2	2	0.67	3	3
9	2415	9	0.95	1	0	0.95	1	0	0.74	3	2	0.80	2	2	0.89	1	1	0.78	2	2	0.84	2	1
10	2231	8	0.88	1	1	1.00	0	2	0.88	1	1	0.88	1	1	0.88	1	1	0.88	1	1	0.86	0	2
GTS %		100	70.79			68.54			70.79			67.42			65.17			58.43			62.93		

The genomic template stability, as a qualitative measure of genotoxic effect, could be directly compared with variations in other parameters. The three tested oils and the bio-insecticide abamectin used in this study showed previously significant decrease effect on mitotic index and induced chromosomal aberrations in root cells of *Vicia faba* plant (Essam, 2017). Altwaty et al. (2016) documented a reduction in mitotic activity and different types of chromosomal aberrations in root tips of *Vicia faba* by extract of *Dipterygium glaucum*. In addition to that there are some newly induced DNA fragments and other disappeared detected by RAPD assays which could be attributed to the modifications in genomic template stability induced by chemicals found in this extract. Moreover, Labra et al. (2003) reported the higher sensitivity of the DNA marker techniques to the classical genotoxic tests like comet and micronucleus assay since DNA markers was capable of detecting temporary DNA changes at lower concentration of pollutants.

The value of Band Sharing Index (BSI) for each treatment compared with the control was calculated and the results showed that abamectin BSI has lower percentage (0.82%) as compared with 0.84, 0.87, and 0.84% that recorded with fenugreek, ginseng and marjoram, respectively. On the other hand, mixture 2 (LC_{20} abamectin + $2LC_{95}$ ginseng) showed the lowest Band Sharing Index as compared with all treatments (Table 3). Comparing BSI values of all treated samples, it can be reported that effects of fenugreek and marjoram are similar while that of abamectin and ginseng are totally different. These data are in the same trend with GTS values, although the BSI is not as valuable as GTS index, because it does not take into consideration the absence of bands in the DNA profiles. The data obtained from GTS% and Band Sharing index showed that the amount of dose is an important factor in genotoxicity and hence should be taken into consideration. Pal & Kundu (2015) showed that *Alternanthera philoxeroides* herb treated with Cd exogenously, resulted both appearance and loss of bands in RAPD assay and GTS values at 1mM Cd, recorded 94%, but at 1.5mM the value decreased to 42.5% and at 1.8mM, the value dipped to a lowest of 27.3% indicating subsequent loss of repair and replication mechanism of the damaged DNA.

Conclusion

From the results obtained, emphasis should be given on the safety, efficacy, and toxicity of the preparation derived from plants. Furthermore, iPBS assay is a useful procedure for the detection of the genotoxic effects of environmental chemicals and offer great promise for future especially for the determination of genetic damage following exposure to contaminants.

References

Al-Qurainy, F. (2010) Application of inter simple sequence repeat (ISSR marker) to detect genotoxic effect of heavy metals on *Eruca sativa* L. *Afr. J. Biotech.* **9**(4), 467-474.

Altwaty, N., El-Sayed, O.E., Aly, N.H., Baeshen, M.N. and Baeshen, N.A. (2016) Molecular and cytogenetic assessment of *Dipterygium glaucum* genotoxicity. *Ann. Brazilian Acad. Sci.* 1: 12. http://dx.doi.org/10.1590/0001-3765201620150208

Atienzar, F.A. and Jha, A.N. (2004) The random amplified polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgenerational effects in B(a)P exposed *Daphnia magna*. *Mutat. Res.* **552**, 125-140.

Atienzar, F.A. and Jha, A.N. (2006) The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review. *Mutat. Res*. **613**, 76-102.

Atienzar, F.A., Cordi, B. and Evenden, A.J. (1999) Qualitative assessment of genotoxicity using Random Amplified Polymorphic DNA: Comparison of genomic Template Stability with key fitness parameters in *Daphnia Magna* exposed to benzo[a] pyrene. *Environ. Toxicol. Chem.* **18**, 2275-2282.

Atienzar, F.A., Venier, P. and Jha, A.N. (2002)Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. *Mutat. Res.* **521**, 151-163.

Baloch, F.S., Alsaleh, A., Andeden, E.E., Hatipoğlu, R., Nachit, M. and Özkan, H. (2016) High levels of segregation distortion in the molecular linkage map of bread wheat representing the West Asia and North Africa region. *Turk. J. Agric. For.* **40**, 352-364.

Bennetzen, J.L. (1996) The contributions of retro-elements to plant genome organization, function and evolution. *Trends Microb.* **4**, 347-353.

Billaud, C. (2001) Composition, nutritional value and physiological properties. *Adrian J. Fenugreek. Sciences-des-ailments*. **21**(1), 3-26.

Burg, R.W., Miller, B.M., Baker, E.E., Birnbaum, J. and Currie, S.A. et al. (1979) Avermectins, new family of potent anthelmintic agents: producing organisms and fermentation. *J. Antimicrob. Agents Chemother.* **15**, 361-367.

Castellani, S., Mattei, N., Renzoni, A., Walker, C.H. and Savva, D. (1993) Preliminary studies on the use of PCR and DNA fingerprints to detect the genotoxic effects of environmental chemicals. *First SETAC World Congress on Ecotoxicology and Environmental Chemistry*, Lisbon, Portugal, Abstract Book, pp. 246-7.

Cenkci, S., Yildiz, M., Cigerci, I., Konuk, M. and Bozdag, A. (2009) Toxic chemicals induced gentoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. *Chemosphere,* **76**, 900-906.

Ciğerci, I.H., Cenkci, S., Kargioglu, M. and Konuk, M. (2016) Genotoxicity of *Thermopsis turcica* on *Allium cepa* L. roots revealed by alkaline comet and random amplified polymorphic DNA assays. *Cytotechnology*, **68**, 829-838.

Doyle, J.J. and Doyle, J.L.(1990) Isolation of plant DNA from fresh tissue. *Focus,* **12**, 13-15.

Enan, M.R. (2006) Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnol. *Appl. Biochem.* **43**, 147-154.

Enan, M.R. (2007) Assessment of genotoxic activity of para-nitrophenol in higher plant using arbitrarily primed polymerase chain reaction (AP-PCR). *Am. J. Biochem. Biotech.* **3**(2), 103-109.

Esmaeili, T., Majd, S., Irian, S., Nabiuni, M. and Ghahremaninejad, F. (2017) Assessment of DNA damage using random amplified polymorphic DNA (RAPD) in vegetative stage bean (*Phaseolus vulgaris* L.) grown under a low frequency electromagnetic field. *Applied Ecology and Environmental Research,* **15**(4), 729-739.

Essam, R.S. (2017) The safe storage for seeds using some natural products. *Ph. D. Thesis*, Fac. Sci. Ain Shams University, Cairo, Eygpt.

Fisher, M.H. and Mrozik, H. (1989) Chemistry. In: "*Avermectin and Abamectin*", Campbell, W.C. (Ed.), pp 1-23. Springer, New York USA.

Huamei, L., Sheng, Q., Yongxia, W., Wenjum, L. and Jie, Z. (2008) Insecticidal action of quinomycin A from *Streptomyces* sp. KN-0647, isolated from a forest soil. *World J. Microbial. Biotech.* **24**, 2243-2248.

Isman, M.B. (2000) Plant essential oils for pest and disease management. *Crop Prot.* **19**, 603-608.

Jones, N.J. and Parry, J.M. (1992) The detection of DNA adducts, DNA base changes and chromosome damage for the assessment of exposure to genotoxic pollutants. *Aqua. Toxic.* **22**, 323-344.

Kalendar, R., Antonius, K., Smýkal, P. and Schulman, A.H. (2010) iPBS: A universal method for DNA fingerprinting and retrotransposon isolation. *Theo. Appl. Genet.* **121**, 1419-1430.

Kalendar, R., Flavell, A.T., Ellis, T.H., Sjaksts,T., Moisy, C. and Schulman, A.H. (2011) Analysis of plant diversity with retrotransposon-based molecular markers. *Heredity,* **106**, 520-530.

Korkmaz, M., Ertürk, Ö. and Gürel, D. (2015) Insecticidal effects of some *Streptomycetes* strains isolated from soil samples against the larvae and adults of the *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). *Bitkikoruma Bul.* **55**(1), 73-84.

Kumar, A. and Bennetzen, J.L. (1999) Plant retrotransposons. *Ann. Rev. Genet.* **33**, 479-532.

Labra, M., Fabio, T., Grassi, F. and Regondi, S.M. et al. (2003) AFLP analysis as biomarker of exposure to organic and inorganic genotoxic substances in plants. *Chemosphere,* **25**, 1183-1188.

Lale, N.E. (1991) The biological effects of three essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Afr. Zool.* **105**, 357-362.

Leeja, L. and Thoppil, L.E. (2007) Antimicrobial activity of methanol extract of *Origanum majorana* L. (Sweet marjoram). *J. Environ. Biol.* **28**, 145-146.

Lü, J.M., Yao, Q. and Chen, C. (2009) Ginseng compounds: An update on their molecular mechanisms and medical applications. *Current Vascular Pharmacology,* **7**(3), 293-302.

Mahmoud, M.A., Hamadttu, A.E. and Ibrahim, A. (2013) Toxicity of bio-insecticides abamectin on red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Int. J Agric. Sci. Res.* **2**, 107-115.

Maina, Y.T. and Lale ,N.E. (2004) Efficacy of integrating varietal resistance and neem (*Azadirachta indica*) seed oil for the management of *Callosobruchus maculatus* infesting Bambara groundnut in storage. *Nigerian J. Entom.* **2**, 94-103.

Misharina, T.A., Terenina, M.B. and Krikunova, N.I. (2009) Antioxidant properties of essential oils. *Prikl. Biokhim. Mikrobiol.* **45**(6), 710-716.

Mujica, N., Pravatiner, M. and Cisneros, F. (2000) Effectiveness of abamectin and plant-oil mixtures on eggs and larvae of the leaf miner fly, *Liriomyza huidobrensis* Blanchard. CIP Program Report, pp.161-166.

Neeratanaphan, L., Sudmoon, R. and Chaveerach, A. (2014) Assessment of genotoxicity through ISSR marker in *Pistia stratiotes* induced by lead. *Environment Asia*, **7**(2), 99-107.

Pal, S. (2016) Detection of environmental contaminants by RAPD method. *Int. Cur. Microbiol. App. Sci.* **5**(8), 553-557.

Pal, S. and Kundu, R. (2015) Heavy metal induced genotoxicity detection by RAPD in Alligator weed. *Int. J. Eng. Technol. Sci. Res.* **2**(9), 1-12.

Ramadan, M.M., Yehia, H.A., Shaheen, M.S. and Abed EL-Fattah, M.S. (2014) Aroma volatiles, antibacterial, antifungal and antioxidant properties of essential oils obtained from some spices widely consumed in Egypt. *Am. Eurasian J. Agric. Environ. Sci.* **14**, 486-494.

Savva, D. (1996) DNA fingerprinting as a biomarker assay in ecotoxicology. *Toxicol. Ecotoxicol. News Rev.* **3**, 110-114.

Savva, D. (1998) Use of DNA fingerprinting to detect genotoxic effects. *Ecotoxicol. Environ. Safety,* **41**,103-106.

Savva, D. (2000) The use of arbitrarily primed PCR (AP-PCR) fingerprinting detects exposure to genotoxic chemicals. *Ecotoxicology,* **9**, 341-353.

Savva, D., Castellani, S., Mattei, N., Renzoni, A. and Walker, C.H. (1994) The use of PCR and DNA fingerprints to detect the genotoxic effects of environmental chemicals. In: *Environmental Contamination, Proceedings of the 6th International Conference,* S.P. Varnavas (Ed.), Delphi, Greece, CEP Consultants, Edinburgh, pp. 105-110.

Siddique, S., Syed, Q., Adnan, A., Nadeem, M., Muhammad, I. and Qureshi, F.A. (2013) Production of avermectin B_{1b} from *Streptomyces avermitilis* 41445 by batch submerged fermentation. *Jundishapur J. Microbiol.* **6**(8), 71-98.

Tiroesele, B., Thomas, K. and Seketeme, S. (2015) Control of cowpea Weevil, *Callosobruchus Maculatus* (F.) (Coleoptera: Bruchidae), using natural plant products. *Insects*. **6**, 77-84.

Wang, X., Mavrodi, D.V., Linfeng, K., Mavrodi, O.V., Yang, M. and Thomashow, L.S. et al. (2015) Biocontrol and plant growth-promoting activity of rhizobacteria from Chinese fields with contaminated soils. *Microbial Biotechnol.* **8**(3), 404-418.

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إستخدام تقنية البصمة الوراثية للحمض النووى iPBS-DNA لتقيم التأثيرات الوراثية لثلاثة من الزيوت النباتية الطبيعية والمبيد الحيوى ابامكتين

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إستخدمت في هذا البحث تقنية inter Primer Banding Sequences (iPBS) المستندة على التراكيب الوراثية للجينات المتنقلة Transposable elements من نوع LTR Retrotransposons والتى تتأثر بظروف الإجهاد المختلفة سواء كانت حيوية او غير حيوية، وذلك لتقييم مدى التغير على مستوى الـ DNA في البذور المعاملة بثلاثة من الزيوت النباتية المستخلصة من نباتات الحلبة (*Trigonella foenum-graecum*) والجنسنج (*Panax ginseng*) والبردقوش (*Origanum majoran*a) بالإضافة إلى المبيد الحيوي أبامكتين(Abamectin) المستخلص من *Streptomyces avermitili*s، كما إمتدت الدراسة أيضاً لتشمل تقييم سمية هذه الزيوت في حالة خلطها مع المبيد الحيوي .إستخدم لذلك عشرة بوادئ (iPBS- primers) مع الـ DNA المستخلص من بذور اللوبيا المعاملة بتركيز 2LC95 لكل من الزيوت الثلاثة وتركيز LC95 للمبيد أبامكتين بالإضافة لثلاثة تركيزات أخرى إستعمل فيها تركيز مخفف من المبيد الحيوي (LC20) مع واحداً من الزيوت الثلاثة حتى نهاية فترة التخزين. أظهرت هذه البادئات تغيراً في البصمة الوراثية لـ DNA في جميع المعاملات عند مقارنتها بالبذور الغير معاملة، حيث سجلت هذه البادئات مستوى مختلف من التباين تراوح من 37.50 % للبادئ 2394 إلى 82.35 % للبادئ 2270، كما أظهرت خمسة بوادئ عدد ستة حزم فريدة منها أربعة حزم موجبة. وأظهرت النتائج عدداً من الأشرطة الجديدة وإختفاء عدداً آخر منها، اشتملت الدراسة أيضاً تقدير نسبة Genomic Template Stability (GTS) وأوضحت النتائج أن أعلى قيمة تم تسجليها لكل من العينات المعاملة بزيت الحلبة أوزيت البردقوش وكذلك الخليط (LC_{20} abamectin–LC_{95} fenugreek) مما يدل على أن جينوم البذور المعاملة بهما أقل عرضة للتغيير بينما زيت الجنسج مفرداً أو مختلط مع المبيد الحيوي أبامكتين هو الأكثر تأثيراً في حدوث تغييراً على مستوى الجينوم. وأن أقلها تأثيراً هو زيت الحلبة مفرداً أو مشتركاً مع المبيد. من النتائج المتحصل عليها نوصي بإستخدام الخليط المكون من المبيد والحلبة أو الخليط المكون من المبيد والبردقوش حيث أن الزيوت النباتية وحدها لا تقدم الاستبعاد الكلي لسوسة اللوبيا بل أنها خفضت فقط أعدادها وأن إضافة الزيوت النباتية إلى المبيد الحيوى تحسن فعاليتة بالإضافة إلى خفض التركيزات المستعملة منه حيث أن كل من الخليط (LC_{20} abamectin - $2LC_{95}$ fenugreek) والخليط (LC_{20} abamectin - LC_{95} marjoram) سجلا فاعلية في منع ظهور حشرات الجيل الأول في نهاية مدة التخزين بالإضافة لتسجيلهما نسبة GTS مرتفعة، حيث تدل النسبة المرتفعة على إمكانية حدوث تغيراً طفيفاً في المادة الوراثية للبذور.