COMET ASSAY FOR RAPID DETECTION OF DNA BASE DAMAGE IN PLANTS

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ABSTRACT

The objective of this study was to evaluate the genotoxic effects of exposure to environmental agent's pollutants in many doses data. The experimental data (1.553, 2.190: 1.515, 1.516) respectively, suggest that the DNA damage parameters (Tail length, Tail width l) were found higher value in exposed population when compared with the ratio of the length to width that cells exhibiting no decampment when having a ratio of one. The percentage and distribution of cells in exposed population also Proportional in values. This study demonstrates that, utilizing sensitive techniques, it is possible to detect environmental agent's risks at an early stage.

Key words:- Damage, DNA detection, Genetic testing, Environment.

التحري عن الضرر في قاعدة الدنا بالنباتات باستخدام اختبار comet

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المستخلص

الهدف من هذه الدراسة هو تقييم التأثيرات الوراثية عند التعرض للتلوث بالملوثات البيئية ضمن العديد من الجرعات والبيانات. تؤكد المعلومات المستحصلة من التجارب بان مؤشرات ضرر الدنا (طول الذيل، عرض الذيل) والذي وجد بمعدلات عالية عند التعرض إلى التلوث بالمقارنة مع نسب طول الذيل إلى عرض الخلايا التي أظهرت عدم وجود نزوح عندما تصل النسبة إلى واحد. نسبة وتوزيع هذه الخلايا المتعرضة للملوثات تتناسب طرديا مع قيم التعرض. نستنتج من هذا البحث بإمكانية استخدام هذه التقنية كتقنيات حساسة والتي من الممكن استعمالها في الكشف عن خطورة العوامل البيئية بصورة مبكرة.

الكلمات المفتاحية: التحطم، التحري عن الدنا، الفحص الجيني، البيئة.

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INTRODUCTION

Food irradiation is a method of food preservation, utilizing low levels of radiation, in order to reduce its corruption and reduce the spread of pathogens. This method utilizes specialized technologies and equipment, in addition to ensuring the health and the safety requirements and by achieving the objectives that cannot be achieved in other methods to conserve food. The amount of radioactive energy absorbed by the exposed body is known as the radiation dose, which is the most important factor in food irradiation. The unit of absorbed dose is called Gray and its symbol was duration Gy. The irradiation dose which is utilized in food processing ranges from 50 C and 10 kW. The time of the absorbed dose, the type of radioactive source, the output voltage of the radiation, its system and the speed of passage of samples should be taken into account (1). There are three kinds of the radiation are widely utilized in different applications which serve the community and the environment. The most radioactive sources utilized in the fields of the medical, the industrial and the agricultural development are Kama rays from the industrial Kama irradiation units, cobalt-60 and cesium-37. The difference between the radiated foods and the irradiated foods is the first contaminated by the radioactive elements and is therefore fatal and occurs as a result of the explosion of a nuclear reactor, as happened in 1986 in Chernobyl. Food contaminated with radiation is contaminated with alpha or beta particles or both, or foods that have been exposed to very high doses of MeV 10 by the electron BIM, the electronic accelerator or the phasetron, thus becoming radioactive material. To ensure that they are not radioactively contaminated and to ensure that they are free from radioactivity. Standards that govern the amount of natural radioactive contaminants that should accompany certain foods should be determined so that the integrated radiation dose to which the population is exposed does not exceed the internationally agreed safe level. (15).Without limiting this limit to the movement of food and food trade among the countries of the world, and generally this has been set on the basis of scientific studies and research, for example, the radioactivity of milk should not exceed 370 Becquerel kg milk and dairy products, and radioactivity should not increase any type of food Other than 600 bicryl / kg (bicryl equals one radioactive disintegration per second).(12) Peoples should know that food contamination with radioactive material can occur as a result of the natural environmental sources such as soil or groundwater content of potassium 40, thorium 232 and uranium 238. For example, radioactive isotopes released from the Chernobyl disaster in 1986 (2). That methods or methods for detecting irradiated food may be useful for determining the dose utilized in the nutrition factors and is desirable for the consumers, the regulators and the manufacturers utilizing this technique. There are several different method in order to detect the irradiated food, the Comet method is one of these methods, because it’s a sensitive, fast, uncomplicated and multifaceted technique for detecting damage to the DNA and a wide variety of the work programs to measure sensitive sites to base pH and to detect fractions of DNA in single cells), Double-rib fractures (7). Where the COMT technique is sensitive to damage in 50 fractions per cell for chromosomal and to lose sensitivity when fractures are more than 1000 fractions per cell (8)., the increase in the utilize of the radioactive sources and isotopes in various areas of human life whether agricultural, industrial, military, or medical, may increase the chances of radiation contamination as well as the amount of exposure to external and internal ionizing radiation. Therefore, there is a need to know the methods of detecting the damage of these rays in the solid and liquid food samples and determining the radiation doses that have been exposed to them. The aim of this research in order to detect the damage of the genetic material (DNA) in the irradiated food resulting from the radioactive contamination because the irradiated irradiation process (beyond the limits allowed in irradiation of food). This Research paper helps to evaluate the oxidant agent's effects of exposure to radiate contamination by using comet assay technique.

MATERIALS AND METHODS

Detection methods: A number of different models were analyzed, including (Eucalyptus

and Shafallah) obtained from several areas close to the Tammah reactor in Tawaytha area. The radioactivity was read utilizing a portable radio reading device. These readings were compared with the control models of Al-Azim as a first stage and at second stage, the samples of currants and irradiated rice were taken with gamma rays utilizing a radioactive source (cobalt 60), which was irradiated at the Tunisian Atomic Energy Organization, the Tunisian Republic and irradiated irradiation as in Table 1.

Table 1. The irradiation doses of currants and irradiated rice with gamma rays (cobalt 60).

Sample	Irradiation doses				
Irradiation dose of currants KGy	0.66	0.5	4	6	8
Irradiation dose for rice models KGy	0.66	1	4	6	8

Comet technology was utilized and followed in Gunasekarana and *et al*. (6), where dry models were grinded and transferred to 1 mL of cooled physiologic regulator solution, the mixture was mixed for five minutes and then filtered with filter papers. Mix 100 µl with 600 µl of low-melting agarose (taking 0.8 g per 100 ml of regulated solution). Dissolve 100 µl of the mixture on the pre-coated glass slide with the agarose. The slides are treated with the solution of the structured analyzer (consisting of 0.045 molars of TBE at pH 8.4, containing 2.5% of SDS solution for 15 minutes) The slides are placed in the electrode and container in the same solution as TBE, Without SDS material, under 2 volts / cm for two minutes and with 100 mA. The Beckman and Ames method (1) was utilized to examine the plant models where 300 mg of the model was added to the cooled physiologic solution for 5 minutes and then immersed in the glass-covered glass slides. The solution was then diluted for 15-60 minutes at 2.5% Dissolve sodium diodosulfate (SDS) in a dark place for 2 minutes and complete the examination as found in the above. The pigment of acrylic (15 µl of dye and 150 ml of solution) to glass the slides shall be pigmented in order to check the comet under cooling conditions (4 ° C). The models as in Figure (1) and Figure (2) were examined under a fluorescent light microscope where 50 cells are evaluated and under a wavelength (420-490 nm). The image analysis technique of single cells was utilized, based on observing the DNA-depleting model, scanning about fifty single cells.

STATISTICAL ANALYSIS

Statistical analysis was implemented by utilizing the mean ± standard error. Morphological differences were examined between the arithmetic means utilizing the Duncan test (4). To study the differences between the rates for the different models, the analysis was performed using the T test.

RESULTS AND DISCUSSION

Table 2 and Table 3 showed that the results of radioactivity in the plant samples compared with the reading of the plant samples from other regions (control). Eucalyptus samples recorded higher reading than the radioactivity of Shafallah samples and control samples, whereas radioactivity in Shaflah samples did not exceed the baseline reading for the device but higher than the radioactivity of the control model.

Table 2. Radioactivity of eucalyptus plants compared with control samples

The sample	0.79 C/s	0.078 ms/hr
Eucalyptus		
1	0.58	0.080
2	0.62	0.074
3	0.72	0.72
4	0.59	0.59
5	0.66	0.66
Control	0.64	0.64

Each value represents the rates of three replicates (readings).

Table 3. Radioactivity of Shafah samples compared with samples (control).

The sample	0.79 C/s	0.078 ms/hr
Shafallah		
1	0.51	0.074
2	0.53	0.073
3	0.59	0.076
4	0.62	0.066
5	0.58	0.059
Control	0.51	0.067

Each value represents the rates of three replicates (readings).

The methods utilized to measure the amount of migratory DNA fragments differed by this test in almost all segments, including positive and negative, although a number of scientists used this technique. Most methods are flexible in

collecting comet data including the application of photorealistic techniques for single cells. The simplest method in order to collect the comet data is to specify the proportion of cells as migration changes; however, these methods are usually determined by the conditions of the electric relay (14). Table (4) shows the number of the positive samples of the test, where the number of negative samples of the examination exceeded both plants (eucalyptus and shaflh), but the positive numbers for the examination in the models of eucalyptus higher than in the models Shaflah and this may because the Eucalyptus contains oil and fat higher than Shafallah and the higher percentage of fat higher the radiation activity than the plants that contain the proportion of lower fat, and on the other hand can be the reason for the emergence of positive results for examination in several models. Although there is no significant increase in radiation activity in the plant cells, which can cause reactive oxygen species (ROS), they interact strongly with the genetic material of the plant cells, resulting in fractures in the plant's DNA and appearance as results Positive examination of Figure 1 and 2.

Table 4. Number of positive and negative models to examine the comet of the studied models

The Sample	Numbers of Samples	Positive	Negative
Eucalyptus	15	13	2
Shafallah	15	9	6
Control	15	1	14

Each value represents rates for three replicates

The classification method of the comets is very useful in some respects, based on the length of tail and / or DNA ratio in the tail by assigning the numerical value of each migratory group, the rate of DNA migration of cells in the food or animal medium can be calculated. The metric is commonly utilized in comet studies, which are the length of the migration of DNA molecules measured by the micrometer (5). It is believed to have a direct relationship with the sizes of migratory DNA molecules represented by the rate and level of single strand breaks (SSB) or the sites of alkaline fluoride which can be attributed to fractions of DNA binding (13). In order to obtain the micrometer measurements, the micrometer is utilized for the optical microscope and the ruler determines the image of positive and negative cells for examination or by the camera's imaging screen. When the image is analyzed, several criteria are utilized in order to determine the boundaries of migratory DNA. Further, the term tail length is utilized in order to describe the length of the image of migratory DNA molecules, while other researchers use this term reveal that the measurements vary in length, width or only width compared to non-migration of a DNA molecule when the rate reaches one sideline (10).

Table 5. Differences in Metric Scale in Length-to-Width Rates of Different Models

Sample	Ratio to Length of Width Ratio ± Standard Error				
Eucalyptus	1.307± 0.19	1.06± 0.08	0.88± 0.39	0.99± 0.09	1.70± 0.49
Control	0.79±0.02	0.75± 0.03	0.80± 0.01	0.94± 0.09	0.80± 0.19
Shafallah	1.13±0.07	1.07± 0.06	0.93± 0.19	0.84± 0.29	0.60± 0.02
Control	0.65±0.02	0.74± 0.03	0.80± 0.01	0.79± 0.09	0.60± 0.19

Represents the rate of five replicates

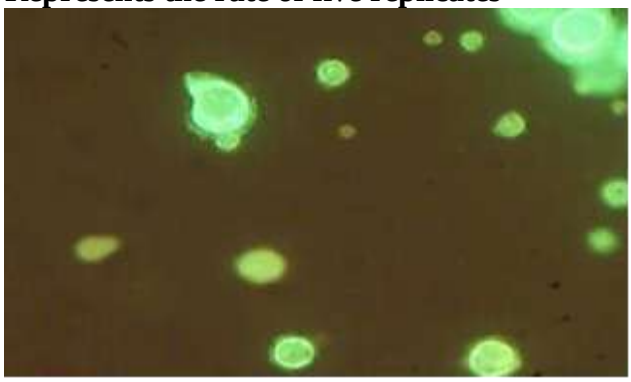

Figure 1: Comet examination of eucalyptus plant

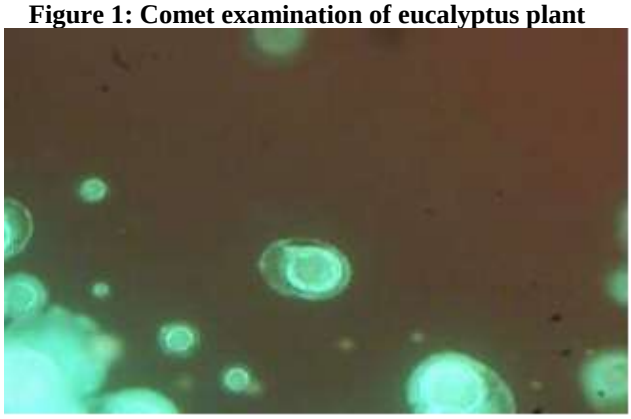

Figure 2: Comet examination of the Shafah plant

Effective oxygen species (ROS) causes damage to large molecules through its disease-related products related to aging such as heart disease, Parkinson's disease, diabetes and mitochondria (11). Theory of free radicals and human aging. Irradiation processes damage food by fracturing molecules and releasing

free radicals that kill some bacteria but infect neighboring organisms with damage (14)., as well as causing damage to vitamins and enzymes, and share chemicals such as pesticides in food to form new chemicals Unique radiolytic products (URPs), some of which are known as toxins and some irradiated foods. Since scientists have not studied the long-term effects of these new chemicals in our diet, we cannot consider them safe (9). There is no single method that can be applied in all diets. Different foods vary in physical and chemical characteristics, so the appropriate choice of detection method depends on the type of food, the dose used for irradiation, the required accuracy and cost. The method of the culprit of the methods of detection of food treated radiation, including irradiated simple and accurate, easy to use, fast and inexpensive. Detection techniques help to establish a control system and enhance consumer confidence in the acceptance of irradiated food.

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