Review of Literature

REVIEW OF LITERATURE

Radiation

Radiation is the process of energy transport without the necessary intervention of a transporting medium. This may be accomplished either by electromagnetic waves or by particles, e.g. electrons, neutrons, or ions **(Kiefer, 1990)**. Radiation, in general, includes both ionizing and non-ionizing radiation. Non-ionizing radiation include ultraviolet radiation (UV), visible light, infrared, radio-wave and microwaves. The amount of energy carried by UV radiation which sometimes used in practical application is not large and its lethal activity is of a relatively low order, its penetrating ability is much smaller than those of energetic radiation **(Mc-Eldowney *et al.*, 1993)**. It causes less damage to microbial DNA. This coupled with its poor penetration to the normal packing materials so UV light unsuitable for sterilization of pharmaceutical dosage forms.

Ionizing radiation includes α-particles, β-particles (electrons), γ-rays, X-rays, neutrons and protons. Ionizing radiation has received their name owing to their ability of ionizing atoms and molecules in the irradiated substance. There are essentially only two ways to produce ionizing radiation; the acceleration of charged particles, which may then react with suitable targets to yield secondary radiation, and the use of radioactive nuclei. X-rays are generated when accelerated electrons interact with matter, while γ-rays are emitted as part of nuclear disintegration. Electrons are also emitted as part of radioactive decay (β-radiation), missions created by interaction of protons, neutrons or α-particles with atomic nuclei, α-particles are helium nuclei consisting of two protons and two neutrons which are produced by radioactive decay. Neutrons are produced by number of nuclear reactions **(Kamp, 1986; Goodhead, 1987 and Kiefer, 1990)**.

Radiation units

There are five types of radiation units **(Diehl, 1995)**.

Radiation intensity:

The radiation intensity of any radioactive source is measured in unit of Curie (Ci) "Curie is a quantity of radioactive substance in which 3.7×10^{10} radioactive disintegration occur per second", the new unit of radiation intensity according to the International System (IS) now is the Bacquerel (Bq).

$$1Ci = 3.7 \times 10^{10} Bq.$$

Radiation exposure:

The radiation exposure is measured in unit of Rőentgen ®. "A Rőentgen is a unit of measure used for expressing exposure dose of X-rays or gamma radiation". The new unit for radiation exposure is the Coulomb.

$$1 C/kg = 3876 R$$

Radiation absorbed dose (rad):

The amount of radiation energy absorbed in a unit mass of material (such as food) is measured in unit of rad. This unit is the most important unit in food irradiation. It is defined as "a unit equivalent to the absorption of 100 erg/g of matter". The new unit according to (IS) is the Gray (Gy); it is equal to the absorption of 1J/kg, i.e. 1Gy = 100 rad.

Radiation equivalent man (rem):

The dose equivalent is used to assess the radiation hazard of the chronic action of radiation. The new unit is the Sievert (Sv).

$$1Sv = 100 rem.$$

Dose rate

The amount of radiation energy absorbed in a unit of time. It is measured in Gy/s.

Radiation sources used for food irradiation

There are three types of ionizing radiation permitted for treating foods as reported by **Vankooij (1982) and WHO (1988)**. These types are summarized as follows:

a) **Electron beams:** which are negatively charged particles produced from electron accelerators machine sources operated at or below an energy level of 10 Mev.

b) **X-rays:** which are electromagnetic radiation of short wavelength, generated from machine sources operated at or below an energy level of 5 Mev.

c) **Gamma rays:** which are electromagnetic radiation of very short wavelength emitted by the nuclei of radioactive substance during decay from radioisotopes, mainly cobalt-60 and cesium-137. Gamma rays are usually used in most applications because of its high penetrating power and low costs.

Dose survival curves

When a suspension of a microorganism is exposed to incremental doses of radiation and determine in each dose the survival cells forming unit (cfu), a dose-survival curve can be conducted. Hence, dose-survival curve illustrates the relationship between numbers of survive cells forming units and radiation dose **(Ley, 1973)**. There are three types of survival curves:

1. Exponential in activation (Type A)

A typical survival curve for most microorganisms represents a straight line or exponential rate kill Curve (A), so it gives a constant slope over the whole dose range (Fig. i); it indicates that the organisms needs only one hit to be inactivated (single hit theory). This type of curve exhibited by different kinds of bacteria such as *Bacillus brevis* **(Ingram & Roberts, 1980)**.

2. Non-exponential inactivation (Type B)

With certain microorganisms "shoulder" may appear in the low dose range before the linear stopetype (B). This shoulder may be explained by multi targets and /or certain repair processes being operative at low doses. The sigmoidal curve revealed that the organisms needs more than one hit to be inactivated. This type of curve may be described by the so-called multi-target single hit expression **(Ingram & Roberts, 1980)**.

3. Resistant tail (Type C)

The response depicted by curve C in Fig. (i) in which an exponential rate of kill is followed by a decreasing rate of spore in activation, is encountered less frequently. Similar tailing-off phenomena have been noted for some sporing and non-sporing bacteria. The reason of this tailing-off is not known, but the production of radiation-resistant mutants remains as a possible reason for the tailing phenomenon. Repeated passage of resistant survivors of vegetative cell suspensions through successive sub-lethal irradiated doses has produce a 4-5 increase in resistance. This increasing being accompanied by an increase in spore resistance and eventually by the loss of ability to produce spore **(Russell, 1982)**.

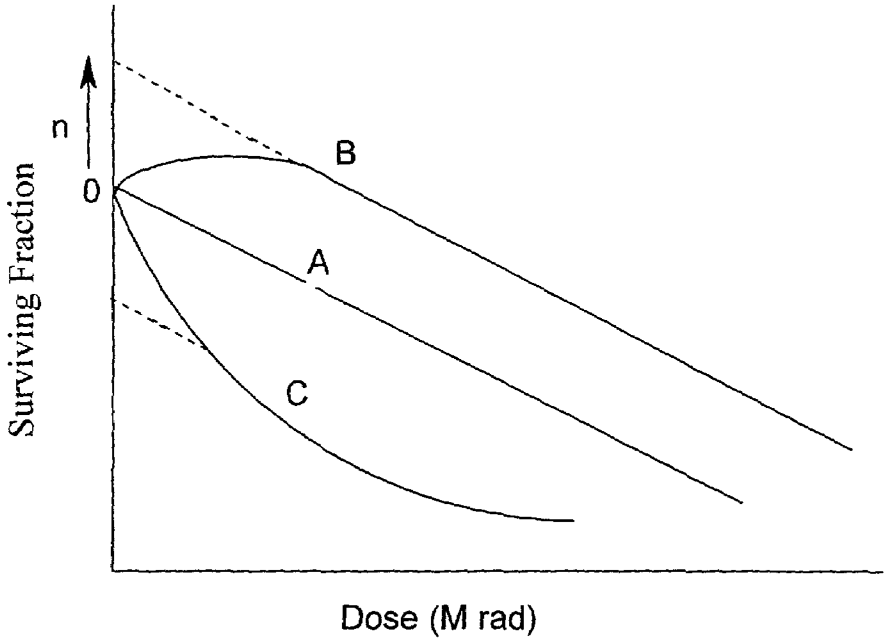

Fig. (i): Types of radiation inactivation curves.

Radiation decimal reduction dose (D_{10}-value)

The sensitivity or resistance of microorganisms including fungi to ionizing radiation is measured by the so-called "D_{10}-value" which is defined either as "the radiation dose necessary to reduce microbial population by a 10 fold (only one log cycle)" or as "that required to kill 90 % of a population". **El-Zawahry & Rowley (1979)** reported that D_{10}-value was taken as criterion for radiation resistance of microorganisms.

Ingram & Roberts (1980) reported that determination of the D_{10}-values for microorganisms using survival curves (dose-response curves) are very important and useful in:

- Calculating the lethal and sublethal dose of a microorganism.
- Knowing the relative resistance of a particular microorganism to ionizing radiation.
- Determining the optimum irradiation dose required for practical application to eliminate a particular microorganisms.
- Calculating the 5-log reduction for a particular pathogens.

Dose response curve constructed by plotting logarithmic of the surviving counts on the ordinate and radiation dose on the abscissa. It usually result in a first order death rate. From the straight-line plots the so-called decimal reduction dose (D_{10}-value) for the microorganisms can be calculated.

Practical applications of food irradiation

Radiation processing involves the application of sufficient ionizing energy in the forms of X-rays, γ-rays or electron beam, to render an article free from viable microorganisms **(Toru & Setsuko, 1992)**. The main objectives of food irradiation are to reduce post-harvest losses and enhance the safety and quality of foods. Among the general potential application of food irradiation are shelf-life extension (radurization) of different types of food by partial elimination of spoilage microorganisms, and ensure hygienic quality (radicidation) of fresh and frozen foods, raw or frozen poultry and meat, dried and semi-dried foods by elimination of pathogenic bacteria and mycotoxigenic fungi **(Rowley & Brynjolfsson, 1980; Farkas, 1981; Vankooij 1982 and Giddings, 1984)**.

Generally, there are three potential applicationsof food irradiation as reported by several investigators **(Farkas, 1980; Vankooij, 1982; WHO, 1988; Atallah, 1997 and WHO, 1999)**.

A- Low-dose applications (up to 1kGy) including:

1- Sprout inhibition of potatoes, onion and garlic using irradiation doses ranging from 0.05 to 0.15 kGy.

2- Insect disinfestations of cereals and pulses, spices, fresh and dried fruits using an irradiation doses ranging from 0.2 to 0.8 kGy.

3- Control of parasites in fresh meat, poultry and fish using an irradiation doses ranging from 0.1 to 1.0 kGy.

4- Delay of physiological processes (e.g. ripening) of fresh fruits and vegetables using an irradiation doses of 0.5 to 1.0 kGy.

B- Medium-dose applications (1-10 kGy) including:

1- Shelf-life extension of perishable food items (meat, poultry, fish, fruits and vegetables) using an irradiation doses ranging from 1.0 to 5.0 kGy.

2-Reduction and/or elimination of spoilage and pathogenic micro-organisms of fresh and frozen sea foods, raw or frozen meat and poultry using an irradiation doses ranging from 3.0 to 10.0 kGy.

3- Improving technological properties of some foods such as increasing juice yield in grapes (2.0 kGy) and reduction of cooking time in dehydrate vegetables (7.0 kGy).

C- High-dose applications (10-50 kGy) including:

1- Industrial sterilization of meat, poultry, sea foods, hospital diets, etc. using high radiation dose (combined with mild heat) of 30-50 kGy.

2- Decontamination of certain food additives and ingredients such as spices, enzyme preparation, natural gum, etc, using an irradiation doses ranging from 10-50 kGy.

Advantages of radiation treatment:

Radiation offers a number of advantages that make it an attractive choice in a number of situations **(Ingram & Roberts, 1980 and De-Risio, 1986)**:

1- Radiation cause no significant temperature rise, which permits sterilization of heat sensitive drugs and articles of low melting-point plastics, and keeps freshness to food and its physical state (Frozen or dried commodities).

2- It is certainly the best, and often the only method of sterilizing biological tissues and preparation of biological origin.

3- Due to its high penetrating ability, gamma-radiation reaches all parts of the object to be sterilized, this also means that irradiation can be applied to sterile the packaged materials, thus avoiding recontamination or reinfestation.

4- It is highly lethal, but the dose may be adjusted to yield pasteurizing or sterilizing effects.

5- At low level (< 5 kGy) it produces virtually no organo-leptically detectable changes in the product.

6- New application of radiation processing include radiation synthesis of substance, radiation catalysis, the application to battery separator, new deodorant, cross-linked cable insulator, heat shrinkable material and foamed polyethylene, curing of surface coating radiation treatment of foods (Radio-sterilization), application of radiator technologies in agriculture and radiation chemistry **(Giusti *et al.*, 1998)**.

7- New radiation processing technologies for environment conservation has been conducted, including the removal of sulfur oxides and nitrogen oxides from stack gas, water treatment and sewage sludge treatment by radiation **(Machi, 1990; Musilek, 1992 and Ming-HO, 2001)**.

8- Using of radiation to produce mutants for microbiological, biochemical and genetic analysis studies for scientific research to study the repair mechanisms of these mutants and their resistivity toward radiation **(Root *et al.*, 1985; Simic *et al.*, 1986; Thacker, 1987 and Radford, 1988)**.

Safety and wholesomeness of irradiated foods

The safety and wholesomeness of irradiated foods have been extensively studied in many countries. **Pauli & Tarantino (1995)** prepared a comprehensive review on the information of Food and Drug Administration (FDA) requires establishing the safety of proposed applications of irradiation. The review covered four broad areas, radiological safety, toxicological safety, microbiological safety and nutritional adequacy. The most recent review of the safety and nutritional adequacy of irradiated foods concluded that food irradiation will not changes the composition of food and as a result would have not toxicological effect on human health; in addition, irradiation will not lead to nutrient losses so will not affect nutrition status of people **(WHO, 1994)**. Furthermore, a recent meeting of the Food and Agriculture Organization (FAO) of the united nations, International Atomic Energy Agency (IAEA), and the World Health Organization (WHO) concluded (on the basis of knowledge derived from over 50 years of research) that irradiated foods are safe and wholesome at any radiation dose **(WHO, 1997)**These organization came to the following conclusion: foods treated with doses greater than 10 kGy can be considered safe and nutritionally adequate when produced under Good Manufacturing Practice (GMP).

Action of ionizing radiation on microorganisms

There has been increasing interest in use of ionizing radiation for inactivating or inhibiting growth of microorganisms including fungi in different foods, livestock, feed products and animals diets **(El-Fouly *et al.*, 1989; Erhart, 1990; El-Far *et al.*, 1993; Youssef *et al.*, 1999 and Aziz & Youssef, 2002)**.

Many hypothesis have been proposed and tested to know how can radiation kill a microbial cell`. Some scientists, especially in the Soviet Union, thought "radiotoxin" (toxic substances produced in the irradiated cells) were responsible. Others, proposed that radiation was directly damaging to cellular membranes. Radiation effects on enzymes or on the energy metabolism were postulated **(Yarmonenko, 1988)**.

Generally, the death of microorganisms (bacteria, mold, and yeast) is a consequence of the ionizing action of high energy radiation. Most studies indicate that lethal damage to microbial DNA resulting from losses of ability to multiply. Ionizing radiation can affect microbial DNA in two ways, directly by ionizing rays energy known as direct hit or indirectly by the effect of primarily water radicals $\dot{H}$, $\dot{OH}$, e^- and other reactive molecules which are formed as a result of ionizing radiation on the cell water (water radiolysis) **(Ingram & Roberts, 1980)**. The most important of these three radicals on DNA damage is $\dot{OH}$ radical as reported by **Goldblith (1971)**. Therefore, microorganisms are more resistant to radiation in the dry state than in the presence of water. **Desrosier, (1970)** and **Wright, (1998)** reported that the effect of gamma radiation on microorganisms leads to a variety of detectable effects, i.e. gene mutation, various types of growth inhibition, alteration in nutrient requirements, changes in membrane permeability and lethality. The lethal dose of radiation on the microorganisms depends upon many factors among of which are the presence of oxygen, water content, type of organisms, concentration of organisms, physical and chemical properties of the medium, stage of development (e.g. vegetative cell, spores) and post irradiation treatment as reported by **Rowley & Brnjolfesson (1980)**.

Early, in **1958, Howard** had established that the damage to cells produced by ionizing radiation can be divided into three categories:

- Lethal damage, which is irreversible, irrepairable and by definition leads to cell death.
- Sub-lethal damage, which under normal circumstances can be repaired unless additional sublethal damage is added.
- Potentially lethal damage, this component of radiation damage can be influenced by environmental conditions (oxygen, temperature, chemicals, etc.).

Ionizing radiation can affect DNA either by direct or indirect action. The event may be ionized or excited in the target molecule initiating the chain events that lead to a series of biological changes.

1. Direct action

In such processes, the energy is directly deposited in the target molecule of biological system without intervention radical species derived from water radiolysis, or other system of environment.

In dry cells, the action of ionizing radiation is predominantly a direct one. Ionization occurs at random in the molecules of which the cells are composed, chemical change ensures directly as a result of this ionization (**Chu & Vandyk, 1993 and Roger *et al.*, 1998**).

2. Indirect action

In moist cells chemical change occur indirectly through the ionization of water. The water molecule split to form free radicals, which are extremely reactive, these free radicals are relatively short-lived. They can interact with biologically important materials causing determined effects, or conversely can react innocently to revert to their former state (**Yuring *et al.*, 2000**).

Water is the main constituent of all biological systems in vegetative cells, its fraction lies between 40 and 70 % but even in bacterial spores, it is still around 20 % **(Chu & Vandyk, 1993).**

In **1968, Casarett** had found that in the absence of oxygen, water molecules absorb energy from ionizing radiation; it is either ionized or excited. Excitation is often followed by a hemolytic splitting of the molecule:

Note: the dot [•] means exciting (has excess energy) or unpaired electron in the radical.

1. $H_2O \longrightarrow H_2O^{\bullet}$ (excitation)
2. $H_2O^{\bullet} \longrightarrow \dot{H} + \dot{O}H$ (splitting)
3. $H_2O \longrightarrow H_2O^{+} + e^{-}_{aq}$ (ionization)
4. $H_2O + e^{-}_{aq} \longrightarrow H_2O^{-}$

The positive and negative free radicals of water ions are both unstable and each dissociates to form stable ions and free radicals.

5. $H_2O^{+} \longrightarrow OH^{\bullet} + H^{+}$
6. $H_2O^{-} \longrightarrow OH^{-} + H^{\bullet}$

The unstable [H^{+}, OH^{-}] ions combine to form water.

7. $OH^{-} + H^{+} \longrightarrow H_2O$

But the primary free radical [$\dot{H}$, $\dot{O}H$, e^{-}_{aq}] are very reactive and during the course of diffusion, they distribute the absorbed energy to solute molecules either organic or inorganic with high efficiency and may give rise to secondary free radical (somewhat less reactive) and molecules which in turn are capable to attaching macromolecules.

If there is O_2 in the environment [$\dot{H}$ and e^{-}_{aq}] react with it.

8. $O_2 + H^{\bullet} \longrightarrow HO_2^{\bullet}$
9. $O_2 + e^{-}_{aq} \longrightarrow O^{-}_2$

The primary free radicals can also react with each other

10. $\dot{H} + H^{\bullet} \longrightarrow H_2$

11. $H^{\bullet} + OH^{\bullet} \longrightarrow H_2O$

12. $OH^{\bullet} + OH^{\bullet} \longrightarrow H_2O_2$

H_2O_2 may also form by:

13. $HO_2^{\bullet} + HO_2^{\bullet} \longrightarrow H_2O_2 + O_2$

The ionized water molecules [H_2O^+] may react with another natural water molecule to form:

14. $H_2O^+ + H_2O \longrightarrow H_3O^+ + OH^{\bullet}$

The products of water radiolysis could be summarized in the follow:

15. ($H^{\bullet}$, $OH^{\bullet}$, H_3O^+, e^-_{aq}, OH^-, H^+, H_2O_2)

Target theory

The target theory states that the effect of ionizing radiation on some particular molecules or molecules is responsible for the measured effect, the production of an effective event in the target is often called hit. The target may be the whole cell, part of cell, or critical molecule. Generally, the measured effect in a system may be cell death or in ability to grow or to divide.

In the simplest form of the target theory, one hit is sufficient to produce the measured effect in associated organisms with very small radiation dose, the number of affected targets will be directly proportional to the amount of radiation **(Root *et al.*, 1985 and Roger *et al.*, 1998)**. It is now universally accepted that the deoxyribonucleic acid (DNA) in the chromosomes represent the most critical "target" of ionizing radiation **(Grecz *et al.*, 1983)**.

Relative radiation-resistance of microorganisms:

Under comparable conditions, microorganisms differ greatly in their resistance to ionizing radiation. There are differences in resistance from genus to genus and from species to species, and even among strains of the same species. Although the range of resistance among strains of the same species is usually narrow enough to be ignored (**Anellis *et al.*, 1973**).

The differences in radiation resistance of microorganisms are related to:

1- Chemical and physical structure of the microbial cells.
2- The number, nature and longevity of radiation induced reactive chemical changes causing cell injury.
3- Inherent ability of the microbial cells to recover from radiation injury (ability to repair the different damage caused by ionizing radiation).

Most microorganisms are able to repair single strand breaks of DNA but it is generally believed that the more sensitive organisms to radiation can not repair double strand breaks (**Moseley, 1968**).

From the reported data resulting from the numerous investigations carried out on the effect of ionizing radiation on microorganisms (**Thornley, 1963; Anellis *et al.*, 1973; Gaughran & Goudi, 1974; Ingram & Roberts, 1980 and Stegeman, 1981**). It can be concluded:

1- Bacterial spores are considered more radiation resistant than vegetative bacteria.
2- Among vegetative bacteria, Gram-positive bacteria are more resistant to radiation than Gram-negative bacteria.
3- Vegetative cocci are more resistant than vegetative bacilli.

4- The radiation resistance of moulds is of the same orders as that of the vegetative bacteria.
5- Yeasts are more resistant to radiation than moulds and vegetative bacteria.
6- The radiation resistance of viruses is much higher than that of bacteria even its spores.

Relative radiation-resistance of fungi:

Fungi are of particular importance in foods for two reasons:

1- They are major causes of food spoilage.
2- Certain species are able to produce in food specific secondary metabolites called mycotoxins which are toxic and carcinogenic to man and animals.

Several studies have indicated that medium doses of gamma radiation greatly reduced or completely eliminated, the fungi presented on most types of food either fresh or dried, hence extending the shelf-life of these products to several times their normal storage life and preventing mycotoxin formation.

Most studies of the inactivation of fungi by irradiation have been made on a sexual spores. Germinating spores, mycelia and other morphological structure of fungi might have different radiation responses **(Sommer, 1973)**. The radiation resistance of fungi is influenced not only by genetic factors but also by the number of cells in a spore (effect of multicellularity), the number of nuclei per cell (effect of multinuclearity). The haploid yeast cells are more sensitive than diploldones. Yeast appear to be about as sensitive as non-spore forming bacteria. He also added that the D_{10}-values for *Penicillium expansum*, *Mucor* sp, *Botrytis cinerea, Rhizopus stolonifer*, *Alternaria citri*, *Cladosporium herbarum* and

Alternaria tenius were approximately 0.5, 0.6, 0.8, 0.9, 1.0, 1.1 and 1.4 kGy, respectively.

Roy & Mukewar (1973) reported that a dose of 2.0 kGy of gamma radiation has little fungicidal effect in vitro on the growth of *Aspergillus niger* and *Fusarium coeruleum.*

Lorenz (1975) found that radiation doses of the order of 2kGy and above are required to eliminate bacterial and fungal deterioration of grains.

Sadi (1978) reported that very low doses of gamma radiation enhanced the activities of microorganisms and may be stimulative for their growth. In the meantime increased doses of gamma radiation exerted an inhibitory effect on the enzymatic activity of microorganisms.

Aziz (1982) reported that molds are relatively sensitive to radiation as compared to the other microorganisms especially spore- former bacteria, where *Paecilomyces variottii* isolated from poultry diet exhibited the highest D_{10}-value (3.30kGy) followed by *P. cyclopium* (2.40 kGy) and *A. fumigatus* (2.20 kGy).

Hussein (1984) found that the D_{10}-values for *Cunninghamella elegans* and *Alternaria alternata* were 1.0 kGy and 1.8 kGy, respectively.

Ragab *et al.* (1986) found that the D_{10}-values for *Aspergillus niger, Fusarium solani, Penicillium citrinum, Aspergillus terreus, P.chrysogenum* and *A. flavus* which were isolated from garlic were 1.0, 0.61, 0.53, 0.45, 0.43 and 0.31 kGy, respectively.

Thomas (1986) reported that radiation doses in the range of 0.5-2.0 kGy have been shown to control fungal spoilage depending on type and size of fungal population, and initial microbial load of the fruit.

Generally, *Aspergillus* and *Penicillium* species are relatively sensitive to ionizing radiation with a D_{10}-values ranged from 0.25 to 0.65

kGy. Whereas, *Fusarium* and *Alternaria* are more resistant to radiation, with D_{10}-values ranged from 0.65 to 1.5 kGy **(Saleh *et al.*, 1988)**. They also examined ten species of fungi representing the genera: *Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium and Penicillium* for their relative resistance to gamma radiation from Cs-137 source. Inactivation doses of dematiaceous fungi in agar medium ranged from 6 to higher than 17 kGy, whereas those for moniliaceous fungi were less than 3 kGy. They found that D_{10}-values for *Curvularia geniculata* were greater than 2.9 kGy and exceed those for controlling spores of *Bacillus pumilus* (1.5 kGy). They also added that spores of *Alternaria and Curvularia* exhibited higher resistances compared to the *Penicillium* and *Aspergillus*. The presence of multicelled thick walled macroconidia which contain melanine pigment may impart radiation protection to these fungi. *Cladosporium* which also produces multicellular thick-walled spores **(Malloch, 1981)** appeared to be radiation resistant within the applied dose range.

Halasz *et al.* (1989) inoculated *F. graminearum* and *F. tricinctum* on moisted corn and rice. The inoculated substrates were exposed to gamma irradiation and the growth rate together with mycotoxin production were measured. They observed a delay in mycelium growth and an increase in these toxin production after irradiation with 1.0 and 3.0 kGy. But at 9.0 kGy neither growth nor toxin production could be detected in any inoculated corn and rice substrate.

Aziz & Abd El-Aal (1990) found that the D_{10}-values for *A. ochraceus, P. chrysogenum* and *F. moniliforme* were 0.13, 1.60 and 1.32 kGy, respectively.

Aziz & El-Halfawy (1991) reported that the fungal genera: *Aspergillus, Penicillium, Fusarium* and *Alternaria* were predominatd in wheat, cow peas and rice samples collected from different stores in

Egypt. *A. flavus* isolated from all stored grains was characterized by its ability to produce aflatoxins. When stored grains were exposed to gamma irradiation doses of 0.4-0.6 kGy and 2.0-4.0 kGy, both insect and mould growth were greatly suppressed. The growth of aflatoxin producing *A. flavus* which contaminated stored grains were inhibited completely at 4.0 kGy and the stored grains became totally free from the toxigenic mould.

Shahin (1993) found that doses of 2 kGy of gamma radiation reduced fungal population of sesame seeds by 90 %, while irradiation dose of 4 kGy caused complete elimination of fungal contamination.

Norberg & Serra-Freire (1993) reported that *Penicillium citrinum* is a fungus which produce mycotoxin responsible for intoxication in humans and animals as a result of eating contaminated food. Radiation resistance of *P. citrinum* increased when exposed to lower dose levels of radiation. The minimum lethal dose (MLD) of gamma irradiation for *P.citrinum* was 2.2 kGy.

El-Far *et al.* (1993) found that a radiation dose of 4 to 6 kGy completely destroyed fungal flora contaminating different food and feed products.

Blank & Corrigan (1995) reported that the D_{10}-gamma values for the *Aspergillus* spp. ranged from 0.245 (*A. niger*) to 0.319 kGy *(A. echinulatus)* and from 0.198 (*A. ochraceus, A. fumigatus*) to 0.243 kGy (*A. glauaus*) for electron beam treatment. For the *penicillium* spp., the D_{10}- gamma values ranged from 0.236 (*P. aurantiogriseum*) to 0.416 kGy (*P. roqueforti*) and from 0.194 (*P. aurantiogriseum*) to 0.341 kGy (*P. roqueforti*) for electron beam treatment. Indicating that the source of radiation may affect radiation resistance of fungi. The D_{10}-values for both

Curvularia geniculata and *Alternaria* were at least 3 times greater than all of the aforementioned organisms.

Adam *et al.* (1995) found that *Fusarium oxysporum, Aspergillus flavus, Penicillium chrysogenum* and *Alternaria citri* isolated from the hot-lab exhibited the highest D_{10}-values being 2.00, 1.40, 1.15 and 0.95 kGy, respectively, these values were about 1.67, 3.10, 1.92, and 1.36 folds as the D_{10}-values of the same isolate recovered from soil indicating that the source of isolation may affect radiation resistance of fungi.

Hammad (1995) found that 1.75 kGy was the minimum dose required for effective inhibition of post-harvest fungi in strawberies.

Hammad *et al.* (1995) determined D_{10}-values for two local isolates of *A. flavus* (No.1 from smoked herring and No. 184 from raisins). They found that the D_{10}-values for *A. flavus* isolate No.1 was 0.43 and 0.50 kGy in physiological saline solution and in smoked hearings, respectively. While, D_{10}-values for *A. flavus* isolate No.184 was 0.53 and 0.62 kGy in saline solution and raisins, respectively. This indicates that the source of isolate may affect the sensitivity of fungi to radiation.

Aziz *et al.* (1997) studied the radiation resistance of some fungi isolated from medicinal plants. They found that the resistance of studied fungi to gamma radiation was in the following order: *Fusarium solani* > *F. oxysporum* > *Aspergillus fumigatus* > *A. flavus* > *A. parasiticus* > *A. ochraceus.*

Hegazi *et al.* (2000) showed that irradiation dose of 1.0 kGy slightly affected the growth diameter of *Alternaria alternata* isolated from grapes fruits. Meanwhile, the highest irradiation dose used (3kGy) reduced the growth of *Alternaria alternata*. On the other hand, no growth was observed with *Botrytis cinerea* exposed to the same irradiation doses used. These results indicated that *A. alternata* was relatively resistant to gamma irradiation while *B. cinerea* was sensitive.

Hammed (2001) recorded that *Aspergillus flavus* was sensitive toward gamma radiation and its D_{10}-value was 0.52 kGy.

El-Fouly *et al.* (2002) reported that the D_{10} value of *Aspergillus flavus* was 0.64 kGy in saline solution whereas it was 0.72 kGy for *A. terreus*, this indicating that *A. terreus* was relatively more resistant to gamma radiation than *Aspergillus flavus*.

Barkai-Golan *et al.* (2002b) inoculated irradiated suspension (10^5 spores /ml) of the main fungi in Israel that cause rot instored melons, on potato dextrose agar and into melon fruits. They found that 4.0 kGy dose was lethal to *Trichothecium roseum*. A 1.0 kGy dose was lethal to *Penicillium cyclopium* and *P. viridcatum* in vitro, while 1.5 kGy was the lowest lethal dose tested in vivo. The incubation of *Fusarium* sp. was inhibited on melons by 2.0 kGy, *Alternaria tenius* was radiation resistance with 3.0 kGy being sublethal both in vitro and in vivo.

Richter & Barnard (2002) reported that the Ascospores of *Pyronema domesticum* were more resistant to radiation than sclerotia. The D_{10}-value for sclerotia of two strains were 0.79 and 1.09 kGy and the D_{10}-value for wild type ascospores was 2.83 kGy.

Mironenko *et al* (2002) reported that the natural (field) strains of the filamentous fungus *Alternaria alternata* were extremely variable in response to gamma irradiation ranging from supersensitive to highly resistance to radiation. At the same time nearly all strains originating from the highly radiation polluted reactor of the Chernobyl Nuclear power plant possessed high radiation resistance.

Sommer *et al.* (2004) compared the sensitivity of conidia, mycelia and sclerotia of *Botrytis cinerea* to gamma radiation. They found that young mycelia and sclerotia were more resistant and mature mycelia more sensitive than conidia.

Kulik & Justice (2004) inoculated seeds of *Allium cepa L.*, *Raphanus salivus L.*, *Poapratensis L.*, *Trifolium incarnation L.*, *Sorghum vulgare pers.*, *Triticum aestivum L.* and *Zea mays L.* with spores of *Aspergillus amstelodami* and spores of *A. flavus* and exposed them to doses of 0.00, 0.05, 0.10, 0.20, 0.40 or 0.80 kGy Some destruction of *A. amstelodami* and *A. flavus* apparently occurred at 0.80 kGy. At this exposure, however, the seedling-production potential of all crop species, with the possible exception of *Trifloium incarnatum* and *Raphanus sativus*, was greatly reduced. In general, the fungi were found to be more resistant to radiation injury than the seeds.

Swelim (2004a) reported that *Fusarium solani* was significantly resistant to radiation as compared with *Fusarium verticilliodes* recording D_{10} values of 1.90 and 0.54 kGy, respectively.

Abd El-Rahman (2005) determined the D_{10}-values of 5 species of *Fusarium* which isolated from corn, garlic, onion and animal feed. She found that the D_{10}-values for *F. tabacinum*, *F. solani*, *F. verticilliodes*, *F. oxysporum* and *F. dimerium* were 1.36, 1.33, 1.45, 1.0 and 0.89 kGy, respectively, indicating that *F. verticilliodes* was the most resistant to radiation in comparison with other *Fusarium* species.

Factors influencing radiation resistance of microorganisms

There are many factors affecting resistant of microorganisms to ionizing radiation, i.e. influencing the shape of survival curves. In other words, the extent to which an organism is resistant to radiation is dependent upon the following general factors:

1-Type of the organism

Beuchat (1981) revealed that resistance to death and injury was greatly affected by the type of organisms. Also, **Koshikawa *et al.* (1993)** reported that, multi-cellular organisms are most sensitive to radiation than unicellular organisms, Gram-negative bacteria are more sensitive than Gram-positive one and bacterial spores are more resistant than vegetative forms. In general, it is accepted that viruses are more resistant than bacterial spores, resistance increasing with decreased particle size and in turn spores are more resistant than vegetative organisms, yeast and molds.

2-Initial counts

The higher the initial population of organisms, the greater is the dose required to kill them. As the inactivation follows a statistical law, the initial cell density does not appear to affect the radiation resistance of spores **(IAEA, 1973 and Whitby & Gelda, 1979)**.

3- Size of DNA:

Size and structural arrangement of DNA in the microbial cells are considered among the factors affecting radiation sensitivity **(Moseley, 1990)**.

4- Compounds associated with DNA:

The DNA in a microbial cell is associated with basic peptides, nucleoproteins, RNA, lipids, lipoproteins and metal ions. In different species of microorganisms these substances may modify indirect effect of radiation differently **(Moseley, 1990)**.

5- Presence or absence of oxygen:

The role of oxygen in enhancing radiation damage is well established in radiobiology. It was shown several years ago that dried *B. subtilis* spores were more sensitive when irradiated in air than under reduced pressure, the highest level of inactivation occurred with post irradiation storage in oxygen and the lowest with post irradiation storage for 15 min in nitric oxide before exposure to oxygen **(Russell, 1982)**. Generally, the presence of oxygen during the radiation process increase the lethal effect of radiation on microorganism. Under completely anaerobic conditions the D_{10}-values of some vegetative bacteria increase with a factor of 2.5 to 4.7 in comparison with aerobic conditions.

6- Temperature:

Temperature is one of the most important factors to be considered in radiation inactivation of microorganisms. The temperature during irradiation was shown to alter radiosensitivity of microorganisms. It has an effect on the function of free radicals, which in turn affects the essential DNA targets directly **(Sztanyik, 1974)**. The freezing temperature protected the cells during irradiation by abolishing at least 50 % of the indirect effect. This is because in the frozen state the diffusion of the free radicals is very much restricted.

The use of ionizing radiation on combination with heat as a potential method of food preservation has received considerable attention **(Sankaranarayanan, 1982)**. Some undesirable entities in food, e.g. viruses and enzymes, are highly radiation resistant but are easily destroyed by mild heating therefore, combination of heat and radiation has the potential to reduce the sensitivity of microorganisms in the treatment of foods without compromising safety and quality **(Root *et al.*, 1985)**.

7- Water content:

Water content is considered the most important factor affecting radiation resistance of microorganisms. **Silliker *et al.* (1980)** reported that, because of the significance of the radiolysis of water and the production of highly reactive hydrogen and hydroxyl radicals, it is anticipated that the radiation resistance of organism is greatest in the dry state, where in the dry state killing of organisms is due mainly to direct hits, while the effect in moist conditions is due to both direct and indirect effects of radiation. Thus, irradiation of foods in the frozen state increases the radiation resistance of many vegetative bacteria by a factor of about 2.

8- Physical state of medium:

The fluidity of the irradiated substrate may have significant effects on the ability of the treatment to destroy cells. When free radical diffusion is retarded or prohibited, the indirect effects of ionizing radiation will not function, hence larger doses are required for an equal kill **(Desrosier & Rosenstock, 1960).**

9-Composition of medium:

The composition of the medium (substrate) in which the microorganism is suspended has a marked effect. The substrate in which cells are exposed has marked influence on the destruction of cells. Protein in the medium is protective to cells, and consequently higher dose levels are required to destroy cells in the presence of protein than in a neutral buffer solution or in destilled water. A large number of chemical additives are protective to cells, including sodium hydrosulfite, sulfhydryl compounds, alcohol, aldehydes, glycols, carboxylic acid, and sugars. Cysteine has pronounced protective effects, it is to be expected that compounds capable of reacting with free radicals will be protective to cells. The effect of reducing and non-reducing sugar in various concentration and their protective effect to bacterial spores has received attention, glucose actively reacting with $\overset{\bullet}{OH}$. While more than ten times the survivors appear in sugar solutions, more than 90 percent of the spores are killed by 5 kGy. A one molar solution is nearly 20percent sugar **(Desrosier & Rosenstock, 1960)**. Many of these effects attributed to media may, at a very basic level, also be attributed to the availability of water in the medium **(Molins, 2001)**.

10-Age of culture:

young cells appear more radiation resistant than aged cells. Maximum resistance has been found at the end of the lag phase of growth, an increased sensitivity to irradiation during the logarithmic phase, followed by a gradual recovery of resistance in the stationary phase of growth **(Desrosier & Rosenstock, 1960)**.

11-Type of radiation:

The work of **Kiefer (1990)** on the germicidal efficiencies of gamma rays, X-rays and cathode rays has indicated that the effects of these radiations are essentially identical when evaluated in terms of the killing effect of equal doses.

Effect of ionizing radiation on fungal cell contents:

Many major molecules are of particular importance in the structure and function of microbial cells. Among of which are protein and amino acids, lipids, carbohydrates, enzymes, DNA... etc. **Lawrence (1971)** reported that different chemical and then metabolic or physiological changes were induced in these major molecules by exposure of cells to ionizing radiation. Ionizing radiation exhibits a chain of reactions leading to over stress to the cells which tends to disturb their organisms. Some changes are temporary and others are permanent. Cell disturbance will depend partly on the amount of chemical changes, which in turn will depend on the absorbed dose.

Habbs & Mccellam (1975) and Zaider & Brenner (1984) reported that, the mechanisms responsible for the effect of radiation on the cellular systems involve injury to cellular substructures (protein and amino acids, lipids, carbohydrates, enzymes, nucleic acid) all of which may have marked effect.

1-Effect on protein and amino acids content:

Generally, ionizing radiation cause a splitting in the peptide bonds in protein molecule as well as deamination, decarboxylation and hydroxylation of the amino acids. Sometimes, irradiation cause cross-linking between two peptides or more.

Naslund *et al.* (1976) reported that doses of the order of mega rads are required to split the peptide bonds. They added that, sulfur containing amino acids (SH-compounds) as cysteine, glutathione and cysteamine have radioprotective action. These amino acids acts as scavengers which they react more readily with free radicals. Thus, restricting the indirect action of ionizing radiation.

El-Zawahry (1976) reported that, twice irradiation of *Rhizobium leguminosarum* with high doses of gamma rays, specially 0.5 kGy, accelerated protein building and accumulation of higher amounts of total soluble-N, represented mainly by the peptide fraction particularly in the presence of $(NH_4)_2SO_4$.

Awny (1982) and **El-Sherbeny (1982)** indicated a preliminary stimulating effect for amino-N and protein-N at low irradiation doses, followed by inhibition at higher doses.

Abo El-Khair (1986) concluded that, gamma irradiation at certain experimented doses most probably altered the genetic code for amino acid and protein synthesis of *Paecilomyces violacea* that led to the disappearance of some amino acids and the appearance of others from culture medium of developed mats.

Salama *et al.* (1989) reported that irradiation of fungal inocula with gamma rays inhibited protein synthesis in developed mats.

Mohamed (2003) found that induction of radiation resistance in bacterial strains had caused an increasing or decreasing in some amino acids according to their sensitivity to radiation.

2-Effect on lipids content:

Irradiation of microbial cells by ionizing radiation led to lipid peroxidation that may result in disturbance of structured organization of cells and membrane associated enzyme activity **(Mead, 1976)** and makes the membrane leaky **(Nakazawa *et al.*, 1981)**. Considerable evidence has accumulated indicating that the end products of lipid peroxidation are carcinogenic **(Shamberger *et al.*, 1974)**, toxic **(Pryor, 1980)** and mutagenic **(Yonei & Furui, 1981)**.

Radiation induced lipid peroxidation was previously studied in systems including biological membranes **(Grezelinska *et al.*, 1979)**, fast mixed with other components as starch **(Wills, 1980)**, and lipid material in different aqueous or nonaqueous solvents **(Barclay & Ingold, 1981)**.

Swelim (2004a) recorded that doses (0.25-6.0 kGy) caused obvious increase in total lipid content of *Fusarium solani* especially at dose level of 0.5 kGy which induced a shift in the fungal metabolism resulting 32.5% increase in its total lipid content. This could be explained by an attempt from the organism to increase its ability to resist the harmful effect of radiation.

3-Effect on carbohydrates content:

During the studies of **Kirn *et al.* (1968)** on the effect of gamma-rays on yeast cells, they found that carbohydrates leak out and losses increase proportionally to radiation dose.

Abd El-Rahman (1973) and **El-Sherbeny, (1982)** found a significant decrease in the polysaccharide content and a slight increase in the total soluble sugars in the mycelia mats that developed from the irradiated inocula. The changes were a function of the radiation dose, such inhibition in carbohydrate content could be due to lower enzyme activities concerned with sugar absorption and utilization and/or polysaccharide synthesis **(Salama *et al.*, 1977)**.

Ghaly (1986) reported that, the amounts of reducing sugars were not affected by different radiation doses, but polysaccharides increased at dose of 0.1 kGy after 8 days incubation, lower doses or higher than (0.1 kGy) decreased polysaccharide content of *Streptomyces lipmoni.*

Swelim, (2004a) found that carbohydrate content inhibited as radiation dose increase and recorded that the total carbohydrate of *Fusarium solani* was higher than that of *F. verticillioides* due to higher resistance of *F. solani.*

4-Effect on enzymes activity:

Since enzymes are essential for the maintenance of the vital process, it was necessary to review the effect of radiation on the synthesis and activity of cellular enzymes. The influence of radiation on dilute enzyme solutions is mainly an indirect effect in which the radio-damage is brought about via the water radicals **(Hutchinson, 1961)**.

Enzymes are noted for their high radiation resistance. **El-Zawahry *et al.* (1982)** showed a clear drop of the enzymatic activity for all the enzymes that were examined after bacterial exposure to gamma radiation. The enzymatic activity decreased by increasing irradiation dose and the intracellular enzyme L-asparagenase was more sensitive at low irradiation doses than the extracellular amylase and protease.

Mostafa *et al.* (1982) indicated that the activity of all the tested enzymes was generally lower in the gamma irradiated cells than that in the control cells and the enzymatic activity decreased by increase in the radiation dose, and the bacterial enzymes had lower sensitivity to the inactivation by gamma irradiation than fungal enzyme.

Tohamy (1991) found that urease production of *Strepmyces. orientalis* was completely inhibited by all irradiation doses used (0.1-4.0 kGy) while for *Strep. alboniger* and *Strep. citrous* were gradually inhibited by low irradiation doses. On the other hand, low irradiation doses up to 0.5 kGy were stimulator for enzyme production by *Strep. sclerotialus* and *Strep. corchorusii.* Also, she added that, the higher doses 1, 2, 3 and 4 kGy inhibited enzyme production for all isolates except *Strep. corchorusii.*

5-Effect on nucleic acids content:

The inactivation of cells, viruses and biological active bacteriophage DNA by X- or gamma radiation is discussed in relation to the chemical alteration in DNA **(Blok & Loman, 1973)**. They concluded that, DNA damage after irradiation in vivo was due to both direct and indirect action leading to production of DNA breaks. The same results were reported by **Billen, (1984).** It is also possible that the inhibition of DNA synthesis is a secondary consequence of damage to some enzymatic system of the cell **(Bresler *et al.*, 1979 and Schaefer *et al.*, 1980)**.

Ultraviolet light and X-rays are produce structural defects in DNA, which, unless repaired, are likely to inhibit DNA synthesis or cause some error in protein synthesis, which leads to cell death and there are no indication that DNA is the only target relevant to inactivation, although it seems that it is the principal target of ionizing radiation **(Richmond & Zim brick, 1975 and Hieda *et al.*, 1984)**.

El-Zawahry (1976) illustrated that, DNA and RNA content of irradiated *Rhizobium leguminosarum* were decreased, also RNA/DNA ratio decreased in most mutants except for the 2nd irradiation group at 0.4 and 0.5 kGy and the 3rd group at 0.4 kGy where an increase in that ratio was evident.

Giusti *et al.* (1998) mentioned that the most sensitive target for lethal effect of ionizing radiation is DNA molecule. Because of their presence in several copies and their turnover, and of the high dose required to produce a detectable damage, proteins have been considered of minor biological relevance. In more quantitative terms, a dose of 0.5 kGy produces observably DNA damage, chromosomal aberrations and a decrease of the surviving fraction of cells in culture. Detectable radiation damage in protein is reported for a dose greater than 10 kGy.

Farrag & Saleh (1996) reported that, gamma radiation induced three type of damage in DNA, single strand breaks, double strand breaks and nucleotide damage include base damage and damage in sugar moiety, can cause mutations and disappearance of some oral activities.

Radiation may cause alternation in DNA as following **(IAEA, 1970 and Kiefer, 1990)**:

1- Rupture of the hydrogen bonds, which link the base pairs adenine, thymine, cytosine, guanine (denaturation).
2- Introduction of breaks in either or both of the DNA chains between sugar and phosphate group (SSB, DSB, base damage, and base loss).
3- Formation of links between adjacent thymine residues on a chain, to form a dimmer.
4- Cross linking between the single strand of the helix, or between a strand and the protein (histone) associated with DNA in the chromosome.

Role of cellular structure and intracellular constituents in radiation resistance:

There are several mechanisms which may be responsible for radiation resistance of microorganisms through their cellular structure and intracellular constituents. These include, spore structure **(Wayne *et al.*, 2000)**, cell wall **(Sanders & Maxcy, 1979)**, protein and amino acids **(Kohler & Marahiel, 1997)**, enzymes **(Minton, 1996)**, DNA and its capability to repair **(Daly & Minton, 1997)**, guanine / cytosine (G/C) ratio **(Ito *et al.*, 1983)**, sulphyhydryl compounds **(Jacqueline *et al.*, 1996)**, lipid **(Swelim, 2004a)**, carbohydrates **(Melin *et al.*, 1986)** and pigments **(Saleh *et al.*, 1988)**.

1-Spore structure:

Resistance of spore-former bacteria to ionizing radiation may be related to spore content. **Mohamed, (2003)** found that spore former cells were much resistant than that of vegetative cells by a factor of 5-15.

Many factors in spore structure involved in spore radiation resistance, with major contributors being:

- The decrease core water content so reduces the oxidative damage of DNA by free radicals **(Setlow, 2000)**.
- The mineralization of the spore core due to the accumulation of high divalent cations **(Paidhungat *et al.*, 2000)**.
- The small acid soluble spore proteins (SASSP) which bind to spore DNA to protect it **(Setlow, 2000)**.
- The spore contains some enzymes as found in the vegetative cell, responsible for more than one DNA repair mechanisms **(Wayne *et al.*, 2000)**.

- Presence of initiate chemicals substance that protect against radiation damage such as dipicolonic acid (DPA) which rich in S-S structure **(Paidhungat *et al.*, 2000)**.

Kamat & Pardhan (1987) investigated the role of dipicolonic acid (DPA) in determining the resistance of *Bacillus cereus* to U.V. and gamma-radiation. Spores of *B. cereus* containing 6% DPA were compared with those containing 0.8% DPA. The later were found to be far more sensitive to U.V. radiation. Similar UV radiation sensitivity was also found in respect to DPA-less mutant of *B. cereus*. Pretreatment of DPA deficient spores with DPA or the presence of DPA less mutant of *B. cereus* result in increased resistance of those to UV radiation.

2- Cell wall:

Sanders & Maxcy (1979) studied the division system and cell wall of highly radiation-resistant *Moraxella–Acinitobacter* (M-A) group, *Pseudomonas radiora*, *Deinococcus radiodurans* and they found that the most resistant M-A possessed unusually thick cell walls, indicating a possible role of the cell wall in radiation resistance in the M-A. Thick septation was present in most of the bacteria studied except in *Pseudomonas radiora* thus excluding this as a necessity for high resistance. They also found that, the highly resistant M-A divided in multiple plans and had base composition of 54.0-57.5%.

3- Proteins and amino acids:

Proteins and amino acids, as intracellular major compounds, may play a role in radiation resistance of microorganisms. **Hartman *et al.* (1988)** reported that there are many amino acids that play role in radiation resistance such as histidine, cysteine through scavenging the free radicals. **Smith *et al.* (1992)** found two new induced proteins in the radio-resistant mutant of *Deinococcus radiodurans,* and were not detected in sensitive strain, and suggested that these special induced proteins might be directly responsible for radiation resistance. **Kohler & Marahiel (1997)** also found 6 new proteins induced in the radio-resistant mutant of the same strain but not found in the wild one, the author supposed that these proteins play important role in the DNA repair mechanism.

Increased sensitivity to gamma-radiation in bacteria lacking heterodimeric protein (HU protein) suggesting that HU protein protects DNA against cleavage by gamma radiation, **(Boubrick *et al.*, 1995)**.

Tanaka *et al.* (1996) found induction of 10 proteins and reduction of 15 proteins after gamma radiation in *Deinococcus radiodurans*. One of these proteins is glycoproteins since it reacts with lectin. The amino acids sequence of N-terminal and internal region indicated that this protein is homologous to EF-TU protein of *E. coli*, the other protein was seems to be a new enzyme since it had no homology to known proteins, no accumulation of these proteins were observed in radiation sensitive strain of *D. radiodurans* and in both *E. coli* and *B. pumilus* suggesting that induction of these two proteins would be specific for high radio-resistant strains.

4- Enzymes:

Many enzymes (repair enzymes) present in some microorganisms play significant role in radiation resistance, such as catalase, hydrogen peroxidase, superoxide dismutase, glutathione peroxidase **(Wang & Schellhorn, 1995)**.

In addition, there are enzymes responsible for synthesis of certain substance act as a protector such as glutathione synthetases **(Moore *et al.*, 1989)**.

Anaerobic bacterial cell was less radio-sensitive than the aerobic one, so that it might be expected that increasing the intracellular concentration of superoxide dismutase enzyme in the bacterial cell would result in a reduction in radio-sensitization by oxygen. Since depletion of oxygen results in radiation resistance (protection) **(Tallentire, 1985)**. He added that, depletion of oxygen would be speeded up by substance that increased the rate of respiration, so that substance might be deemed "protective" cystamine otherwise known as β-mercaptoethylamine.

After gamma radiation, *D. radiodurans* expresses relatively high levels of catalase and superoxide dismutase which react with free radicals that cause an oxidative damage to cell membrane, protein and nucleic acid **(Wang & Schellhorn, 1995 and Lye *et al.*, 1999)**.

5- Nucleic acids and its capability to repair:

Radio-sensitivity of various organisms may be correlated with their total DNA contents, DNA base composition and radiation dose. **Morse & Carter (1949)** found that the RNA content/cell of irradiated *E. coli* increased 5 folds and DNA and nitrogen increased two to three folds. **Stapleton (1965)** recorded that the radioresistant strains of *E. coli* were composed of large cells with excess DNA, RNA and protein than the sensitive strains. **Swez & Pollard, (1966)** denoted that the DNA of *E. coli* was degraded randomly by ionizing radiation and no part is preferentially selected.

The repair of DNA damage has been shown in many organisms related closely to cell survival, and cells that are lacking the ability to repair DNA are sensitive to radiation **(Edward, 1990)**.

DNA repair of damage caused by ionizing or UV radiation can be responsible for the resistance of some bacteria **(Bridges, 1976)**. However, the type of damage inflicted by ionizing and UV radiations are different, the former induces single-strand or double-strand scission of DNA, where as the UV induces the formation of thymine dimmers in the DNA single-strand breaks which can be repaired in spores during post-irradiation germination **(Frankenberg, 1981 and Frankenberg *et al.* 1984).**

Deinococcus radiophillus is more resistant to UV and ionizing radiation than *D. radiodurans*, fenestrated peptidoglycan layer present in *D. radiophillus* and not present in *D. radiodurans*, and their percentage of G + C in DNA is 70, 62 respectively and 59, 66 for *D. proteolyticus* strain **(Moseley & Evans, 1983 and Ito *et al.*, 1983).**

Radiation resistant *D. radiodurans* can repair completely almost all of the DNA damage including double strand breaks induced by gamma rays **(Sjarief, 1990).**

The dose response curve of *Deinococcus* show a large shoulder due to its resistivity to radiation because of their effective DNA repair mechanism as excision repair and recombination, enzymatic repair mechanisms **(Minton, 1996).**

6- Guanine / Cytosine ratio:

Kaplan & Zavarine (1962) found in a series of bacteria a linear relationship between their radiosensitivity and the guanine-cytosine content of DNA. However, **Alexander *et al.* (1965)** reported that there are several bacteria which do not fit at all into the relationship proposed by **Kaplan & Zavarine (1962).**

A study of the nitrogenous base composition in 8 strains of bacteria and their resistance to irradiation suggested an insures relationship between radiation resistance and guanine-cytosine (GC) content, i.e. resistance increased with decreasing (GC) content **(Yamazaki, 1971).** He also, added that preliminary experiments showed reverse and that might be true. However, *Deinococcus radiodurans*, the most resistant bacterium to radiation has high (GC) content, the same as the very sensitive *Pseudomonas*. He also reported that the radio-resistance of the spores in some groups of 46 strains of *Bacillus subtilis* correlated to their (GC) content but no correlation was found in other spores.

7- Sulphyhydryl compounds:

Moore *et al.* (1989) showed that *E. coli* enriched its content of gamma-glutamethylcysteine synthetase and glutathione synthetase activities by recombinant DNA technique is more resistant to the total effect of gamma-radiation than the corresponding wild strain.

Williams, (1989) reported that increased capacity for glutathione synthesis enhances resistance towards radiation in *E. coli.*

8-Lipids:

Carbonneau *et al.* (1984) and **Counsell & Murray (1986)** found that radiation-resistant species belonging to the genus *Deinococcus (D. radiodurans, D. radiophilis, D. proteolyticus and D. radiopugnans* and *all strains of D. fradiadurans)* had unusual numbers of polar lipid (mostly phosphoglycolipids) and did not have phosphatidy glycerol (PG) or its derivatives, while *D. erythromyxa* had a normal form of phospholipids profile, including PG.

Swelim (2004a) found that existence of high percentage of lipids partially protect other essential components in the living cell such as enzymes and nucleic acids from the deleterious effects of gamma radiation. Lipids usually contain some antioxidants such as toxophenols and carotenoids, which are very sensitive to radiation and subsequently gave other resistance to the cell components.

9- Carbohydrates:

Melin *et al.* (1986) showed that there were a relation between high carbonhydrate-containing lipid and a high resistance to physical and chemical agents, in particular to radiation. They also concluded that radio-resistant strains of *Micrococcaceae* exhibit a higher carbohydrate / phosphorus index than other strains. This seems to be relationship between high carbohydrate-containing lipid content and a high resistance to physical and chemical agents in particular to radiation.

10- Pigments:

Fungal melanins are brown to black pigments, they are not considered essential for growth and development of fungal cells, but they enhance the survival and competitive abilities of fungi in certain environments **(Bulter & Day, 1998).**

Saleh *et al.* (1988) recorded that fungi with melanin pigment *Alternaria alternata, Cladosporium cladosporioides, Curvularia lunata* and *Curvularia geniculata* exhibit high radiation resistance, the D_{10}-value of *Curvularia geniculata* (> 2.9 kGy) exceeded those for control spores of *Bacillus pumilus* (1.5 kGy).