Discussion

DISCUSSION

The radiation resistance of microorganisms varies widely: different types, species and strains exhibit greatly different sensitivity. Certain environmental factors are also able to influence the radiation response such as temperature, oxygen, water and chemical agent **(IAEA, 1973 and Russell, 1990)**. Cellular structure and intercellular constituents affect the radiation resistance of microorganisms including fungi.

In the present study, fifty three isolates of *Curvularia, Alternaria* and *Fusarium* were collected from different sources. *Fusarium* spp. were isolated from wheat, potato, tomato, chicken feed and soil while *Curvularia* spp. were isolated from soil, air, orange and lupine, meanwhile *Alternaria* spp. were isolated from tomato, chicken feed, mandarine, fenugreek and bread. Several other investigator isolated these fungi, and others from different sources. **Domesch *et al.*, (1981)** and **Aziz (1982)** reported that species of *Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium,Helminthosporium, Curvularia, Epicoccum* and *Verticillium* were the most common and widespread fungi, in air, soil and different agricultural commodities.

Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, Curvularia, and *Verticillium* are also common and widespread in many food and feed ingredients as recorded by **Aziz & Saleh (1995), Refaie *et al.*, (1996)** and **Rustom, (1997)**.

Many species of *Curvularia, Alternaria* and *Fusarium* were isolated from samples of wheat, cowpeas and rice collected from different sources **(Aziz & El-Halfawy, 1991)**. The same above isolates were isolated from maize, groundnut and soybean **(Bhattacharya & Raha, 2002)**, from animal feeds **(Swelim, 2004b)**, and from soil **(Nasrat, 2005)**.

Hammad *et al.* **(1995)** isolated *Alternaria alternata, Alternaria tenuissima* from strawberries. Also, **Hegazi** *et al.* **(2002)** isolated *Alternaria alternata* from graps and tomatoes, while **Wade & Beuchat (2003)** isolated it from tomatos. **Barkai-Golan (2002a,b)** isolated *A. tenuis* from pears and melon fruits.

Moubasher *et al.* **(1982)** isolated several *Fusarium* isolates from 25 samples of wheat, barely, maize and sorghum collected from different places in Egypt. They found that *Fusarium oxysporum* was the most frequent species in the tested samples. **El-Kady** ***et al.*** **(1988)** also isolated several *Fusarium* species from wheat grains, and identified them as; *F. solani, F. equiseti, F. verticillioides, F. oxysporum* and *F. semitectum*. **Bao** ***et al.*****, (2002)** isolated pathogenic and nonpathogenic *F. oxysporum* from tomato plants. **Abd El-Rahman, (2005)** also isolated many *Fusarium* species from garlic, corn, animal feed, wheat grains, barely and peanut.

Five isolates from each genus of *Curvularia* , *Alternaria* and *Fusarium* were chosen for further studies. They were selected according to their differences in the morphological characters and source of isolate. The five isolates of *Fusarium* were taken from soil, wheat, tomato, potato and chicken feed; the five isolates of *Alternaria* were taken from tomato, chicken feed, mandarine, fenugreek and bread; while one *Curvularia* sp. were selected from each of soil, air, orange and two isolates from lupine.

For studying the effect of gamma irradiation on the rate of growth of the chosen isolates, the results indicated that the unirradiated isolate (wild isolate) of *Curvularia* (Cl_1) isolated from lupine was the faster one in its growth comparing with other *Curvularia* isolates since it reached the edges of the petri-dishes after 6 days, while *Curvularia* samples (Cs) isolated from soil was the slower one of its growth and reached the petri edge after 8 days.

On the other hand, Cl_1 isolate also showed highest resistance to gamma rays since its growth diameter recorded 7.0 cm at the 6^{th} day after exposure to dose level of 10.0 kGy. While Co isolates (isolated from orange) also continued to grow even after exposed to dose level of 10.0 kGy but its growth diameter recorded only 4.0 cm at the 7^{th} day of incubations. Meanwhile, Cl_2 (isolated from lupine seeds) showed highest sensitivity to gamma rays and its growth completely inhibited after exposure to dose level of 6.0 kGy. The growth of Cs and Ca isolates (isolated from soil and air) inhibited after exposure to dose levels of 9.0 and 8.0 kGy respectively.

The data of the present work, showed that *Alternaria* isolates were more sensitive to gamma rays than *Curvularia* spp. Dose level of 5 kGy inhibited the growth of two isolates of *Alternaria* A_b (isolated from bread) and A_t (isolated from tomato), while A_c sample (isolated from chicken feed) remained alive after exposure to dose level of 9.0 kGy.

Among the *Fusarium* isolates (Fw) isolated from wheat was the faster in its growth than the other *Fusarium* isolates since its mycelia reached the petri-dishes edge after 5 days of incubation, while the other four isolates reached the edges after 7 days. Exposure the above five isolates of *Fusarium* spp. to increasing doses of gamma rays affected greatly their mycelium growth. Fc sample which isolated from chicken feed showed highest resistant to radiation among the other *Fusarium* isolates and tolerated dose level of 8.0 kGy followed by (Fs) and (Ft) isolates, while (Fp) was completely inhibited after exposure to dose level 4.0 kGy.

In this respect, **El-Ashmaway (1982)** recorded that there was a significant decrease in the mycelium growth of *Fusarium oxysporum,* *F. equiseli* and *Mucor* spp. with the increase of the radiation doses from 2.5 to 10.0 kGy.

On the other hand, **Sadi (1978)** and **Urbain (1986)** reported that low doses of gamma radiation enhanced the activities of microorganisms and may be stimulative for their growth, but increased doses of gamma radiation exerted an inhibitory effect on the enzymatic activities of microorganisms.

The six isolates, which represented the highest and lowest radiation resistant isolates, of *Curvularia*, *Alternaria* and *Fusarium,* i.e. Cl_1, Cl_2, Ac, A_t Fc and Fp were identified as *Curvularia lunata, Curvularia tuberculata, Alternaria alternata*, *Alternaria tenuissima*, *Fusarium oxysporum* and *Fusarium semitectum,* respectively.

These results were in agreement with that obtained by several investigators. **Hussein (1984)** recorded that *Alternaria alternata* was relatively resistant to gamma rays. **Saleh *et al.* (1988)** found that spores of *Alternaria* and *Curvularia* exhibited higher resistances compared to the *penicillium* and *Aspergillus*. **Aziz *et al.* (1997)** found that the resistance of studied fungi to gamma radiation was in the following order: *Fusarium solani, F. oxysporum, Asperagillus fumigatus, A. flavus, A. parasiticus* and *A. ochraceus*. **Hegazi *et al.* (2000)** indicated that *A. alternata* was relatively resistant to gamma irradiation. **Barkai-Golan *et al.* (2002a)** found that *Alternaria tenuis* and *Stemphylium botryosum* were gamma radiation resistant. **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. Also, **Swelim (2004a)** reported that *Fusarium solani* was significantly radiation resistant as compared with *Fusarium verticilliodes*. **Abd El-Rahman (2005)** found that *Fusarium verticilliodes* was the most radiation resistant fungi with compared with other *Fusarium* species isolated from different cereal grains, garlic, onion and animal feed.

The response of the selected fungal species to gamma radiation was studied by determining their mycelium dry weight (biomass) as indication of growth. The spore suspensions of the six previous selected fungi were exposed to increasing dose levels of gamma rays up to 8.0 kGy for determining the effect of radiation on their growth (biomass). The results revealed that there was a gradual decrease in the biomass content of *C. lunata* with increasing irradiation doses up to 6.0 kGy. Meanwhile, irradiation dose of 8.0 kGy sharply decreased the biomass content. On the other hand, there was a sharp decrease in the biomass content of *C. tuberculata* at all irradiation doses used. Irradiation dose of 8.0 kGy almost inhibited the growth of *C. tuberculata*.

In case of *Alternaria alternata* there was an increase in its dry weight biomass (3.6 %) by exposure to the low dose level (0.25 kGy) followed by gradual decreases as irradiation dose increased. Meanwhile, exposure *Alternaria tenuissima* to dose level of 0.25 kGy decreased its biomass by 23.1 % and gradual decreases occurred thereafter by increasing the dose levels of gamma rays recording 72.1% decrease at dose level of 6.0 kGy and 92.0 % at dose level of 8.0 kGy. Gradual decrease in the dry weight biomass of *F. oxysporum* was occurred by increasing the dose levels of gamma ray recording 58.5% and 61.2% at 6.0 and 8.0 kGy. Meanwhile, exposure *F. semitectum* to low dose level of gamma rays i.e. 0.25 kGy caused slight increase in its dry weight biomass (1.2 %), as occurred in case of *A. alternata.* Thereafter gradual decreases occurred by increasing the dose levels of gamma rays. The rate of decreasing in the biomass were relatively higher than that observed in *F. oxysporum* where the decreasing of the biomass recorded 77.3 % after exposure to dose level of 6.0 kGy, and its growth almost inhibited after exposure to 8.0 kGy as occurred in case of *C. tuberculata*.

In this respect, **Aziz *et al.* (1989)** reported that gamma irradiation delayed the mycelial growth of *Aspergillus ochraceus* and the growth inhibited when the fungal conidia were exposed to 3.0 kGy. Also, **El-Far *et al.* (1993)** noticed that by increasing the radiation doses, the viable population of *A. flavus* decreased greatly and no growth occurred after treatment with 6.0 kGy. **Shahin (1993)** reported that as gamma irradiation dose increased mycelial dry weights of *A. flavus* were decreased. **Swelim (2004a)** reported that the increasing dose levels of gamma rays delayed the mycelium growth of *F. verticillioides* and *F. solani*, where the dry weights obtained from *F. verticillioides* sharply decreased after exposure to dose level of 3.0 kGy. He added that the dry weight of *F. solani* was higher than that obtained from *F. verticillioides* at the same dose level indicating the relative resistance of *F. solani* than *F. verticillioides*.

Several authors also reported that low doses of gamma radiation enhanced the activities of microorganisms and may be stimulative for their growth, and high doses of gamma radiation exerted an inhibitory effect on the enzymatic activities of microorganisms **(Sadi, 1978; El-Zawahry *et al.*, 1983; El-Zawahry *et al.*, 1988; Urbain, 1986; Shalaby, 1999 and Swelim, 2004a)**.

Microorganisms differ greatly in their resistance to gamma radiation. The descending order of resistance for various microorganisms was as follows: bacterial spores > yeast > moulds > gram positive bacteria > gram negative bacteria **(Stegeman, 1981)**. Moreover, radiation resistance of the same microorganisms differ with species and even with strains, although the range of resistance among strains of a single species is usually small enough to be ignored for practical purpose (**Anellis *et al.*, 1973**). The resistance of microorganisms to ionizing radiation is usually measured by the so called "D_{10}-value".

The experimental fungal species *Curvularia lunata, Curvularia tuberculata, Alternarria alternata, Alternaria tenuissima, Fusarium oxysporum* and *Fusarium semitectum* were studied for their resistance to gamma radiation through determination of their D_{10}-values. The dose response curve of each fungus of these fungi was drawn in both saline solution and substrate. D_{10}-value of *C. lunata* and *C. tuberculata* were determined in lupine seeds since the genus *Curvularia* was the only genus excited in lupine seeds in the present work and both of the above isolates were isolated from this substrate. The D_{10}-value of the other four isolates were evaluated in chicken feeds where this substrate was highly infected with different species of genera *Alternaria* and *Fusarium.* The results obtained from the present investigation indicating that the D_{10}-values of (*Curvularia lunata, C. tuberculata, Alternaria alternata, A. tenuissima, Fusarium oxysporum* and *F. semitectum* respectively) in physiological saline solution (0.85 % NaCl) were 1.92, 1.25, 1.47, 0.47, 1.31 and 0.70 kGy, respectively. The corresponding D_{10}-values in lupine seeds for *C. lunata* and *C. tuberculata* were 2.25 and 1.56 kGy while D_{10}-values in chicken feed for *A. alternata, A. tenuissima, F. oxysporum* and *F. semitectum* were 1.70, 1.30, 1.83 and 1.23, respectively. This means that, in saline solution, the most radiation resistant fungus among the six tested fungi was *C. lunata* followed by *A. alternata* then, *F. oxysporum* while *Alternaria tenuissima* was the most sensitive fungus to gamma radiation. It is clear from the results that the substrates either lupine seeds or chicken feed give protection to the fungus against gamma rays so the D_{10}-values of the six fungi in substrate were relatively higher than in saline solution.

Similar results were reported by many other investigators. **Hussein (1984)** found that D_{10}-value of *Alternaria alternata* was 1.8 kGy. **Saleh *et al.*, (1988)** reported that the two genera of *Aspergillus* and *Penicillium* are relatively sensitive to ionizing radiation, while the genera *Fusarium* and *Alternaria* were more resistant. They also added, that the fungi with melanized hyphae such as *Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia lunata* and *Curvularia geniculata*, survived the higher radiation doses. Also, **Osman (1973)** mentioned that the most radiation resistant species of the tested fungi were *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium notatum* and *Alternaria humicola* as compared with other isolates. **Orabi and Hammad (1989)** found that the D_{10}-value of *Fusarium solani* in macerated potatoes was 0.87 kGy. **Adam *et al.* (1995)** reported that the D_{10}-value of *Fusarium oxysporum* was 2.00 kGy.

Hegazi *et al.* (2000) found that at relatively higher irradiation dose (2 kGy) only three fungi *Alternaria alternata, Cladosporium herbarum* and *Stemphylium herbarum* were isolated, indicating that these fungi were more resistant to irradiation doses used. The resistance of *Alternaria alternata* and *Cladosporium herbarum* to irradiation has been also reported by **Sommer & Fortlage (1966)** and **Hammad (1995)**. **Aziz *et al.* (1997)** studied the radiation resistance of some fungi and recorded them in the following order *Fusarium solani, F. oxysporum, Aspergillus fumigatus, A. flavus, A. parasitious* and *A. ochraceus*.

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 resistant to radiation. **Swelim (2004a)**, reported that *Fusarium solani* was significantly radio resistant as compared with *Fusarium verticilliodies* recording D_{10}-values of 1.90 and 0.54 kGy. **Abd-El Rahman (2005)**, found that D_{10}-value of *Fusarium tabacinum*, *F. solani*, *F. verticilliodes*, *F. oxysporum* and *F. dimerium* were 1.36, 1.33, 1.45, 1.00 and 0.89 kGy, respectively, indicating that *F. verticilliodes* was the most resistant to radiation in comparison with other *Fusarium* species.

The present results exhibited that the D_{10}-values in saline solution of the six tested fungi were less than that in the substrate. This may explained by the indirect effect of primary water free radicals ($\dot{O}H$, $\dot{H}$, e^-) resulting from water radiolysis, which are formed much more in saline solution than in substrate (lupine seeds and chicken feed). In addition, lupine seeds and chicken feed as substrates may contain certain compounds that act as protective agents or scavengers which protect the fungal spores against damage by irradiation. **Rowley & Brynjolfsson (1980)** reported that radiation resistance of any particular fungus is influenced by many factors, among which, the availability of water and suspending medium. **Schubert (1981)** mentioned that the protective agents (scavengers) may react with given free radical (from water radiolysis), hence protecting or reducing the radiation damage to the cells normally attacked by these radicals. The effect of radiation on living cells are described by the summation of the direct effect of radiation on the vital centers in the cells plus the indirect effect on the same centers caused by chemical changes outside these centers **(Lawrence, 1971)**. Resistance to injury and death is greatly effected by the type of organisms as well as by the nature of the environment in which the organisms is stressed **(Beuchat, 1981)**. **Sjarief, (1990)** and **Lye *et al.* (1999)** found that the resistance of microorganisms can be explained in view of its ability of having an efficient DNA repair mechanism of all DNA damage including double strand breaks.

The variation in response to gamma irradiation can vary from one organism to another. **Sommer & Fortlage (1966)** explained the high radiation resistance of *Alternaria* and *Fusarium* by multicellularity of spores, while **Salama *et al.* (1977)** revealed that variation in the radio resistance of fungi is most probably due to the effect of inherent characters connected with mycelial water content and production of natural radio-protecting substance.

On the other hand, **Mostafa *et al.* (1983)** stated that the high resistance to gamma radiation may be due to the presence of an efficient repair mechanisms. Cultures in agar provide a heavier and more diversified bioburden, along with nutrient conditions that favor the function of repair systems. Also, organic compounds in the medium compete with the fungus for the hydrolytic products of irradiation **(Silverman, 1983 and Urbain, 1986)**.

Blank & Corrigan, (1995) reported that spores of *Alternaria* and *Curvularia* exhibited higher resistance compared to the *Penicillia* and *Aspergilli*, regardless of radiation source.

In order to study the role of protein and amino acids contents in radiation resistance, the amount of protein and the composition of the amino acids of the tested fungi were determined.

It worth to mention that the relatively higher resistance to gamma radiation which gave higher D_{10}-value were also contained higher percentage of protein i.e. *Curvularia lunata* (D_{10}-value 1.92 kGy) contain 76.88 % protein, followed by *Alternaria alternata* (D_{10}-value 1.47 kGy) contain 72.69 % protein, *Fusarium oxysporum* (D_{10}-value 1.31 kGy) contain 69.83 % protein and *C. tuberculata* (D_{10}-value 1.25 kGy) contain 70.13 % protein. While the strain which showed lowest D_{10}-value contain also the lowest protein, *A. tenuissima* (D_{10}-value 0.47 kGy) contain 64.06 % protein and *F. semitectum* (D_{10}-value 0.70 kGy) contain 46.88 % protein. This indicate that there was a positive correlation between radiation resistance of fungal species and their content of total protein, i.e. the higher radiation resistance, the higher total protein content.

In this respect, **Moore *et al.* (1989)** and **Wang & Schellhorn (1995)** reported that some proteins as protective enzymes present in some bacteria played a significant role in radiation resistance or these enzymes may be responsible for the synthesis of certain polypeptide acting as radiation protector which induced by radiation.

In contrary to our results, **Swelim (2004a)** reported that protein was obviously high in *Fusarium verticillioides* (less resistant) compared with *Fusarium solani* (more resistant).

Several investigators reported that certain amino acid specially those contain sulpher bond (cysteine, cystine and methionine) and those contain double bond (histidine) gave the organism highly resistance to gamma radiation since they work as a scavenger to the free radicals occurred due to the effect of ionizing radiation on the water molecules **(Urbain, 1977; Winters *et al.*, 1995; Milligan *et al.*, 1995 and Jacqueline *et al.*, 1996)**. The results of the present work clearly indicated that the fungal species which showed high resistance to gamma radiation contained high percentage of cysteine, methionine and histidine in comparison with those showed sensitivity to gamma radiation. *Curvularia lunata* which was the highest radiation resistant in the tested fungi (D_{10}-value 1.92-2.25 kGy) contained 0.4, 1.2 and 3.8 mg/g of cysteine, methionine and histidine, respectively. Also, *Alternaria alternata* (D_{10}-value 1.47-1.70 kGy) contained 0.6 and 0.3 mg/g of cysteine and histidine, respectively. Meanwhile, *A. tenuissima* and *F. semitectum* which exhibited sensitivity to gamma radiation didn't contain any sulfur amino acid (cysteine and methionine) while they contain only relative low content of histidine (1.8 mg/g).

In this respect, **Swallow, (1977)** and **Urbain, (1977)** reported that the sulphur amino acids such as cysteine, cystine and methionine may act as free radical-scavengers, thus ameliorating the degradative effects of other of the amino acid components of protein. **Dahl *et al.*, (1988)** reported that, histidine and N-alanyl-methyl histidine had a protective effect through lessing lethality and single strand break in DNA scavenging free radicals making the bacterial strain bear the induction of radiation resistance. High concentration of sulfur amino acids in bacterial cells enable them to tolerate the effect of high doses of radiation through scavenging the free radical so protect DNA against oxidative damage of radiation **(Jacqueline *et al.*, 1996)**. Under the stress of radiation some bacteria strains induced the production of specific proteins containing certain amino acids such as (histidine, methionine and cysteine) as a response to survive, consequently they became resistance **(Winters *et al.*, 1995 and Milligan *et al.*, 1995)**.

In the present work, the results of intercellular constituents of studied fungal species revealed that *C. lunata* and *A. alternata* which showed the highest radiation resistance contained the highest content of total lipids, while *A. tenuissima* and *F. semitectum*, which showed sensitivity to gamma radiation had the lowest content of total lipids. This indicates that the total lipids content in a fungal cells may play an important role in its radiation resistance, i.e. the higher content of total lipids, the more resistant to radiation. This role could be partially explained by susceptibility of poly unsaturated fatty acids found in the lipids to oxidation by free radicals produced upon water radiolysis protecting the sensitive molecules of biological importance in the living cells such as DNA and certain enzymes.

Diehl, (1995) recorded that lipids are more susceptible to oxidation by radiation than proteins and carbohydrates especially due to its content of high percentage of poly unsaturated fatty acids, **(Swelim, 2004a)** added that the existence of high percentage of lipids partially protect other essential components in the living cell such as enzymes and nucleic acids from the deleterious effect of gamma radiation. Lipids usually contain some antioxidants such as toxophenols and carotenoids, which are very sensitive to radiation and subsequently gave another resistance to the cell components.

In the present work, the saturated and unsaturated fatty acids were also analyzed. For the six studied fungal species, four saturated fatty acids were identified as lauric, myristic, palmitic and stearic. The four saturated fatty acids were found in *Alternaria tenuissima* and *Fusarium oxysporum*, while lauric fatty acid was not found in *Curvularia lunata*, *C. tuberculata* and *A. alternata*. Meanwhile, myristic fatty acid was not found in *F. semitectum*.

Regarding the unsaturated fatty acids, four fatty acidswere also detected and identified as oleic, linoleic, linolenic and erucic. The four unsaturated fatty acid were found in *A. tenuissium* and *F. semitectum*. While two of them , oleic and linoleic were found only in *C. lunata* and *C. tuberculata*. Meanwhile, linolenic was not found in *F. oxysporum* .

It was found that the percentage of unsaturated fatty acids were relatively higher in radiation resistance species, i.e. *C. lunata*, (73.48 %), *A. alternata* (71.68%) and *F. oxysporum* (70.11 %) compared with 69.11%, 68.67 % and 53.38 % for the corresponding relative radiation sensitive species i.e. *C. tuberculata*, *A. tenuissima* and *F. semitectum*, respectively.

Radiation resistance of various microorganisms may be correlated with their nucleic acids content. So, the total DNA and RNA in selected resistant and sensitive tested fungi were determined. The present work indicated that the highest radiation resistant tested fungal species, i.e. *Curvularia lunata* contained the highest total nucleic acids either RNA (24.5 mg/g) or DNA (12.13 mg/g) followed by *Alternaria alternata* RNA (23.63 mg/g) and DNA (11.50 mg/g). Meanwhile, the relative radiation sensitive species (*C. tuberculata, A. tenuissima* and *F. semitectum*) contained the lowest values of total nucleic acid (29.60, 28.17 and 28.46 mg/g), respectively.

In this respect, **Stapleton (1965)** recorded that the radio-resistant strains of *E. coli* were composed of large cells with excess DNA, RNA and protein than the sensitive strains. **Swelim (2004a)** found that the radio-resistant *Fusarium solani* contained total nucleic acids (either RNA or DNA) higher than that of the radiation sensitive strain *F. verticillioides*.

After exposure the studied fungal species to dose level of 4.0 kGy, the protein percentage decreased in all the fungi but the decreasing in the total protein of the most radiation resistant species, (*Curvularia lunata*) was lower (only 6.5 %) in comparison with the most radiation sensitive species (*A. tenuissima)* since the decreasing in protein content reached 15.55 %.

Osman, (1973); Salama *et al.* (1977) and **El-Sherbeny (1982)** found that irradiation with γ- or X-rays results in the inhibition of protein synthesis which leads most probably to the increase in the amino acid content of the fungal cells due to the possible inhibition in the process of nitrogen absorption and utilization. **Salama *et al.* (1989)** reported that irradiation of fungal inocula with gamma rays inhibited the protein synthesis in the developed mats. The inhibition was parallel to the dose levels.

Boubrick *et al.* (1995) mentioned that radiation caused induction and reduction of some proteins in bacteria. They added that the expression and production of certain protein as HU-protein in *E.coli* and *Deinococcus radiodurans* increased the radiation resistance and enable them to survive and adapted the increase of radiation resistance. **Swelim (2004a)** found that apreliminary stimulating effect of protein synthesis in *Fusarium solani* at lowest irradiation dose (0.25-0.50 kGy), followed by a decrease in protein content in the remaining higher irradiation doses (1.0-8.0 kGy).

The present results indicating that gamma irradiation caused decreasing or increasing in both total and certain amino acids. Some amino acids completely destroyed after exposuring the tested fungi to 4.0 kGy. The percentage of certain amino acid increased after irradiation in *Fusarium oxysporum* and *F. semitectum*. Serine, glycine and valine increased in *F. oxysporum* while tyrosine and arginine increased in *F. semitectum*.

It is also clearly obvious from the results that cysteine, methionine (which contain sulpher bond), and histidine (which contain double bond), were highly affected by gamma irradiation in most studied fungi either resistant or sensitive to radiation.

Gamma irradiation may alter the genetic code of amino acid and proteins **(Abo El-Khair, 1986** and **Setlow 2000)**. This observation was in agreement with that obtained in the present investigation since, the exposure of the six tested fungi to gamma rays had caused an increasing or decreasing in some amino acids according to their sensitivity to radiation.

Mohamed (2003) found that the increase in the radiation doses were accompanied by an increase or decrease in the total and fractionated protein concentrations of tested bacteria. She also added that some amino acids were decreased or increased as a result of the increasing in the radiation dose level (cysteine and Histidine).

The total nucleic acid contents of all tested fungi decreased after exposuring to 4.0 kGy gamma rays, but the percentage of decreasing were higher in DNA than that occurred in RNA. Also, the percentage of decreasing in DNA of the radiation sensitive species were more than that of the resistant ones. It is well known that, radiation induced DNA damage include single and double strand breaks, basis modification and DNA protein cross links as reported by **(Ming-Ho, 2001)**. Radiation damage can be mediated by DNA repair mechanisms and it can be also mediated by the progression or regression of certain proteins and amino acids that involved itself in radiation resistance mechanism **(Boubrick *et al.*, 1995)**. **Lawrence (1971)** mentioned that the loss of proliferate capacity is usually caused by damage within DNA molecules which controlling all aspects of metabolism, structure and development. **Glazunov & Kapul'tsevich (1991)** recorded that yeast cell inactivation regularities can be explained by DNA double-strand breaks processing during the post-irradiation cell division. Also, **El-Fouly *et al.* (2002)** found that the total nucleic contents of wild type and gamma irradiated isolate of *Aspergillus terreus* were more than that of wild type and gamma irradiated isolates of *Asperagillus flavus*. They added that this could be explained the relatively sensitive of *A. flavus* than *A. terreus* against gamma radiation. This finding agreed with irradiation of *Paecilomyces violace* **(Abo El-Khair, 1986 and El-Zawahry *et al.*, 1988)**. **Hassanein (1994)** studied the effect of gamma radiation on the DNA and RNA contents of *Bacillus megaterium* and *E. coli* and found

that the synthesis of DNA and RNA decreased after irradiation treatment with 6 and 1 kGy for *B. megaterium* and *E. coli* respectively.
Risk *et al.* (2000) recorded that radiation doses (10 and 15 kGy) decreased the amounts of both DNA and RNA extracted from *B. megaterium*. **Swelim (2004a)** found that a preliminary stimulating effect of RNA and DNA in *Fusarium solani* and *F. verticillioides* at lowest irradiation doses followed by decreased in RNA and DNA content at higher irradiation dose.

Prevention of fungal growth during storage of grains and other agricultural crops are of ultimate importance to prevent spoilage fungi and to avoid mycotoxin formation and accumulation. Researches have focused primarily on the control of fungi during storage. It was found that ionizing radiation is very promising mean for controlling and preventing the growth of fungi contaminating agricultural crops, processed food, feed product and animal diets **(Erhart, 1990 and Loaharanu, 1995)**.
In the present work, the moisture content of the contaminating lupine seeds with *Curvularia lunata* and chicken feed contaminated with *Alternaria alternata* and *Fusariumoxysporum*were adjusted at 15 % and exposed to different levels of gamma radiation.
The data indicated that, generally irradiation caused great reduction in all the fungal counts and this decreased was proportional with irradiation dose. Irradiation dose of 5.0 kGy decreased C. lunata contaminating lupine seeds by more than 2.0 log cycles, while about 4.0 log cycles reduction was achieved by exposuring to 10.0 kGy and complete destruction was obtained at 12.5 kGy. During storage, the counts slightly increased after one month of storage. These results similar to that obtain from *Alternaria alternata* and *Fusarium oxysporum* where 12.5 kGy was quite sufficient to eliminate *Alternaria alternata* completely from chicken

feed either after irradiation or during the storage period. Meanwhile, *Fusarium oxysporum* spores showed less resistant than *Curvularia lunata* and *Alternaria alternata* since dose level of 10 kGy freed the chicken feed from its spores all over the storage period. It could be concluded that dose level of 10 kGy consider enough to treated the highly contaminated food and feed with the resistant fungi species since it either freedom the food from their spores or kept their counts under the safety limit (10^3 cells/g).

El-Fouly *et al.* (1990) reported that animal diets such as yellow corn, bran, cotton seed cake and corn remaining usually composed of different carbohydrate and protein sources. They also added that the slight decrease in the microbial flora occurring during storage could be attributed to the increase in the microbial death rate than the proliferation rate due to the low water activity in the investigated animal diets since the water content was only between 10 and 13% for the different investigated samples.

Hammad *et al.* (1995) found that application of gamma irradiation greatly decreased the microbial load of strawberries (total bacterial count, mould and yeast counts) and this decreases was proportional to irradiation dose and this results proved that irradiation acted as an effective antimicrobial agent.

The results also in agreement with that obtained by **Aziz (1982)** stated that an irradiation dose of 5 kGy was enough to kill all fungal microflora contaminated poultry diets. **El-Fouly *et al.* (1989)** reported that 2.5 kGy was quite sufficient to eliminate the fungal flora naturally contaminating animal diets or their main components. They also added during storage the count of fungi tended to slightly decreased during the storage period, although there was slight increase in sample III after two months following by another decreased as the storage period increased. The calculated D_{10}-values for different fungi contaminated the diets were also found to range between 0.96 and 1.2 kGy in the complete diets.

El-Fouly *et al.* (1990) also reported that the count of bacteria contaminated the completed animal diets slightly decreased by increasing the storage periods, this could be attributed to the increase in microbial death rate than the proliferation rate due to the low water activity in the investigated animal diets, they also added dose level of 10 kGy is quite sufficient to eliminate all pathogens artificially contaminate animal diets or their components. In addition, it decreases the microbial count to minimum counts and hence increases the diet shelf life.

Also, several investigations recorded that dose required for complete elimination of natural fungal flora from foods and agricultural commodities ranged between 4.0 and 6.0 kGy **(Shahin & Aziz, 1997; Molins, 2001 and Aziz & Youssef, 2002)**.

Abd El-Rahman (2005) found that during storage the total count of *Fusarium verticillioides* inoculated in corn increased in the unirradiated samples than in those exposed to relatively low radiation doses (2.5-5.0 kGy), indicating that these doses slightly retarded the growth of the fungal spores, while irradiation dose of 12.5 kGy completely prevented the growth of *Fusarium verticilliodies* in corn throughout all the storage period which lasted for three months.

It worth to mention that, a slight increases in the total count of the tested fungi were occurred during the first month of storage either in the control or in the irradiated samples. These slight increasing could be due to the increase of the growth rate than the death rate at the first period since the moisture content was still sufficient to enable to the fungi to proliferation (15%). Thereafter with the decreasing of the moisture, the death rate become offer the growth rate. Repairing in the DNA of the injured fungal cells due to the radiation is another reason for increasing the microbial counts in the irradiated samples. These injured cells couldn't appeared after irradiation but by the time and after repaired the effect of radiation, they started to appear again.

Several investigator wearing from these injured cells especially if they are from the parthogenic ones. **Shahin (1998)** and **(Aziz *et al.* 1999)** reported that gamma radiation caused injury to microorganisms and has been widely reported to prevent or delay fungal growth. **Moustafa *et al.* (1983)**, **Sjarief, (1990)** and **Lye *et al.* (1999)** found that the resistance of microorganisms can be explained in view of its ability of having an efficient DNA repair mechanism of all DNA damage including double strand breaks.