## GAMMA RADIATION AS A CONTROL OF MYCOTOXINS AND THEIR PRODUCING FUNGI IN MEDICINAL PLANTS

By

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#### ABSTRACT

The current study was done on 54 samples of medicinal plants mostly used in public medicine as a treatment of kidney affections, common cold, cough and constipation, these plants are Cymbopogon proximus Ambroxia maritima , Artemisia , Telio , Ginger, Cinnamomum , Nigella sativa and Cassia Senna and were collected randomly from 3 different localities in Egypt (Moshtohor, Tukh & Cairo). The incidence and concentrations of some mycotoxins (Ochratoxin A and Atlatoxin B1) were determined in these plants. Also, some mycotoxins producing fungi were isolated from these plants (Basidiobatrys sp. A.flavus : A.niger : A.parasiticus : A.ochraceus and Fusarium sp. ) . All samples were subjected to gamma radiation at 5 kGy to investigate the possibility of controlling mycotoxins and fungi in medicinal plants . Our results indicated that gamma radiation destroyed the majority of mycotoxins and fungi in gamma irradiated medicinal plants, however, some mycotoxins and fungi still present in medicinal plants even after exposure to gamma radiation at the recommended dose. We concluded that gamma radiation is a useful control method for mycotoxins and fungi in medicinal plants but other control measures either in preharvest, harvest or storage period of these plants should go hand in hand,

#### INTRODUCTION

During the past fifty years, several reports have appeared in the literature discussing the different uses of some common spices obtained from plants as drug. A large section of the Egyptian population unquestionably believes in the efficacy of herbal drugs, and this belief has gained impetus in other countries in recent years.

Wanherweghem et al., (1993) investigated association between rapidly progressive interstitial renal fibrosis in young women and slimming regimen including Chinese herbs (Stephania tetrandra and Magnolia officinalis). They stated that the striking relation between a specific type of fibrosing interstitial nephritis in young women and a slimming treatment involving Chinese herbs adds support to the arguments against uncontrolled therapy with herbal preparation.

Mycotoxins elaborated in various feedstuffs and agricultural commodities is a major problem in tropics and subtropics where climatic conditions; agricultural and storage practices are considered conductive to fungal growth and toxin production. Mycotoxins are the most notorious toxic metabolites of mould fungi identified in many agricultural products screened by numerous toxigenic moulds (Aziz, 1987).

Misra (1981) isolated A. Flavus, A. Fumigatus, A. Ochraceus, A. Sydow chaetomium dolicholrichum, C.biopiculatum, P. oxalicum and Rhizopus from seeds of Amomum subulatum, Coriandrum sativum, cuminum cyminum, Foeniculum vulgare, Trachyspermum and Cinnamoum zeylaonicum which were used as common drug plants.

Saowarase et al., (1987) reported that 15% of thirty nine medicinal herbs were found to be contaminated with aflatoxin B<sub>1</sub>(AFB<sub>1</sub>), 20 to 150 ppb and 5% contained AFB1 20 to 90 ppb. Similarly Roy et al., (1988) investigated 158 isolates of A. Flavus obtained from many samples of drug plants and they found that 49 isolate were found to be toxigenic producing 0.86 to 5.24 ug/ml of culture filtrate.

Roy et al., (1988) examined mycologically 15 common drug plants collected from store houses in Bihar, India. The investigators observed that A.flavus, A. Candidus, A.niger, A.luchuensis, A.ochraceus, A. Nidulans, F.moniliforme, F.oxysporum, Alternaria alternata, Curvularia funata, Chaetomium sp., Penicillium citrnum and Rhizopus stolonifer were the most common fungi isolated from the plant samples taken. Similarly Eggbo, Zeinab (1990) found that liquorice was more highly contaminated with moulds than other plants of medicinal value (tea, fenugreek and anise).

Radiation is defined as a physical phenomenon in which energy travel through the space without the aid of a material medium. The radiant energy is a form of energy that travel through space in a wave motion. Electromagnetic radiations may include radio waves, infra-red, ultra-violet and x-ray, the last of which have the highest frequency. The gamma radiation 600

is a form of energy resembling x-rays, but released from nuclei with very high energy and very high frequency. Both x-rays and gamma rays are ionizing radiation (Lawrence, 1971).

The ability of radiation to kill microorganisms has been the object of investigations since the late 19th century. Research directed toward the use of radiation for the preservation of food began in 1945. The doses of radiation necessary for complete sterilization of a product usually used without rising temperature (Weiser et al.,1971). Also, it is important to stress that gamma rays from cobalt 60 did not induce any radioactivity in the processed materials (Ley et al.,1969). Many workers reviewed the industrial application of nuclear energy for preservation of foods for man and

animals.

El-Gendy, Hoda (1979) reported that irradiation dose 5 kGy inactivated all natural fungal flora contaminated medicinal and pharmaceutical products. Similarly, El-Bazza, Zeneb (1983) found that by increasing irradiation doses up to 5.0 kGy, the fungi contaminated certain Egyptian foods decreased. Also, El-Tablawy, Scham (1993) observed that fungal counts of survivors /g of herb samples decreased with increasing the irradiation doses from 0.0 to 5.0 KGy . Also, El-Bazza, Zeneb (1983) mentioned that there was a complete inhibition of mycelial growth of A Parasitious and A. Ochraceus and mycotoxin production at 5.6 and 4.0 KGy for aflatoxins (B and B2) and Ochratoxin A, respectively. While Hassaucin, Wesam (1987) observed complete inhibition for growth of A flowus and aflatoxin production at dose level 3 KGy but toxigenic moulds of Allams were eliminated with 5 KGy irradiation as recorded by Aziz et al., (1996). Meanwhile complete inhibition of mycelial development of A Parasiticus and aflatoxin production occurred at 2.0 KGy as observed by Eggbo, Zeinab (1990).

Levels of gamma irradiation ranged from 6-10 kGy were effective on the microbial load of Cinnamon and Ginger (Toofanian and Stegeman, 1988). However, El-Zawahry et al., (1991) reported that the dose required to inhibit completely the natural fungal flora ranged from 4 to 6 KGy.

Moussa (1994) observed 10 genera of contaminating moulds in a survey of eighty four samples of different medicinal plants collected from different locations in Cairo markets. The most common fungal species isolated were A. flams, A. Parasiticus, A. Niger, P. viridicatum and F.oxysporum. The natural contamination of mycotoxins in medicinal plants revealed the detection of aflatoxin in 17 out of 84 samples whereas

ochratoxin A (OTA) was detected in 3 samples.

El-Bazza et al.,(1996) exposed twenty four samples of medicinal herbs to increasing doses of gamma radiation (from 0.0 to 6.0 KGy). Five fungi isolated of Aspesgillus species could produce aflatoxins and one isolate could produce OTB.

In a survey carried out in the UK by Patel et al., (1996) to determine the levels of mycotoxins in a range of ethnic foods, the highest mycotoxin levels and frequency of occurrence were in chili powders, curry powder and ginger.

Halt (1998) analyzed the level of toxigenic moulds in 62 samples of medicinal plants. The most predominant fungi detected were Aspergillus, pencilium, Mucor, Rhizopus, Absidia, Alternaria, Cladosporium and Trichoderma, A.flavus, a known producer of aflatoxin was present in 11 (18%) of the samples. The contaminated samples with A.flavus were also analyzed for ochratoxin and zeralenone. Ochratoxin was found in one of the 7 samples analyzed.

Owing to the hazardous toxic effect of some mycotoxins we aimed in this study to analyze some medicinal plants (as a row materials used in drug manufacturing) for their contamination with moulds an presence of aflatoxin B<sub>1</sub> and ochratoxin A, and trying to nullify or reduce the fungal count and mycotoxin concentration by using gamma radiation.

## MATERIALS AND METHODS

Sampling:

54 samples of 9 types of medicinal plants (cymbopogon proximus, Ambrosia maritima, Artemisia, barley, Nigelia sativa, Telio, Cinnamomum, Ginger and Cassia senna) each group of 6 samples were randomly collected from 3 locations in Egypt, being Moshtohor (rural area), Tukh (urban area) and Cairo (more civilized area). All

Samples were grounded, classified into 2 parts. From the first part 0.02 gm were diluted with 2 ml sterilized water for fungal count according to Johnson (1957), then identification of isolated fungi were done in Agricultural Research center by using the keys of Alexopoulas and Mims (1979); 5 gm were used for quantitative determination of ochratoxin A by using Veratox® quantitative ochratoxin A·ELISA kits, NeogenTM corporation using automatic ELISA reader which was kindly available from Dept. of Animal Science, Fac. of Science, Zagazig University, Benha Branch, and AFB; by using supracritical Fluid Extraction (SFE) (Taylor et al.,1992). The concentrations of OTA and AFB; were determined using standard calibration curve (Figs a & b) respectively. Twenty five grams from the second part of each sample in polyethylene pouches were irradiated at

dose of 5 kGy according to (Moussa, 1994) by using Cobalt 60 gamma cell-220 located at the National Center for radiation Research and Technology, Naser City, Egypt with a dose rate of the gamma source 27 rad/ second at the time of experiment.

All previously mentioned steps for counting and identification of fungi and quantitative determination of AFB<sub>1</sub> and OTA were repeated on all irradiated samples. Statistical analyses were conducted after **Snedecor and Cochran (1967)** where variable means for data showing high significant differences in the ANOVA were compared. All statements of significance were based on the 0.05 level of probability.

## RESULTS

Results of this study can be illustrated in the following Tables (1-4) and Figures (1-5).

Table (1): Total fungal colony count in some medicinal plants before and after

radiation and the percentage of their reduction.

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Cymbopogon proximus	15	72	82	53.2 5	175	71	104	59.4	22	83	14	63.2
Ambrosia martima	15	72	78	52.0 0	196	80	116	59.1	14	61	84	57.9
Artemisia	16	64	10 5	62.1	161	59	102	63.3 5	23	91	14	61.9
Barley	16 4	68	95	58.5 4	202	84	118	58.4 2	19	61	13	68.5
Nigella sativa	14	62	87	58.3	196	65	131	66.8 4	18	43	14	77.2
Telio	21 4	85	12	60.2 8	289	12	169	58,4	27	11	16	60.0
Cinnamontu m	22 4	88	13	60.7	258	11	145	56.2 0	22 6	10	12	55.7
Ginger	15 9	64	95	59.7 5	223	87	136	60.9 9	20 2	97	10 5	54.9
Cassia senna	15	69	86	55.4 8	195	10 0	95	48.7	20	74	12	63,5
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ANOVA test was used

B= Before

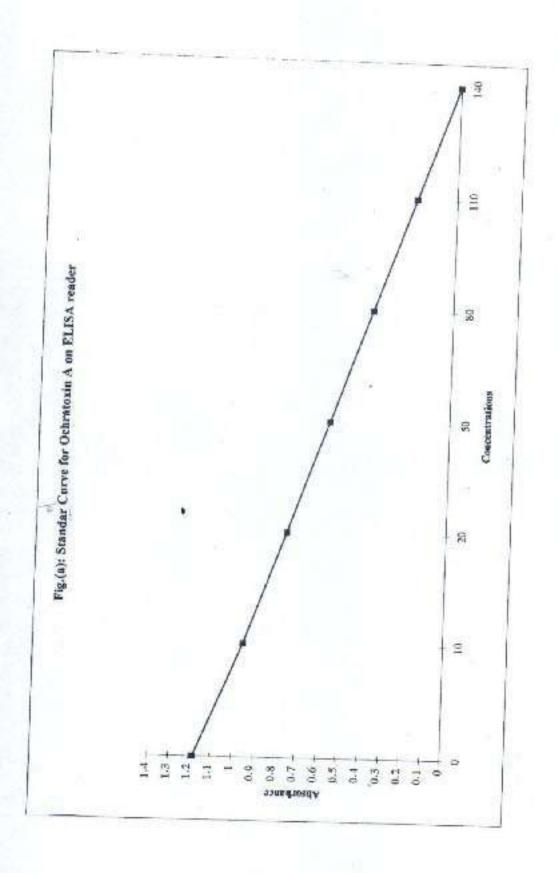
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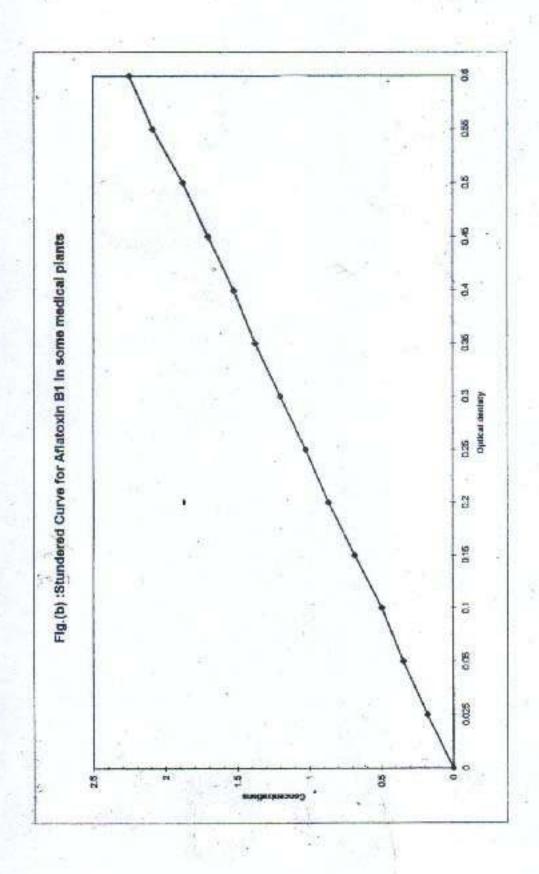
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<sup>=</sup> percentage

<sup>\*\*</sup> high significant differences between different localities ( p≤0.01)

high significant differences after exposure of examined medicinal plants to radiation (p≤0.01)





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ile (2): Total fungal colony count * in individual experiment medic	Moshtoher**

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Table (3): Conscritations of allacasin B1 in some medical plants before and after exposure to radiation with the percentage of reduction.

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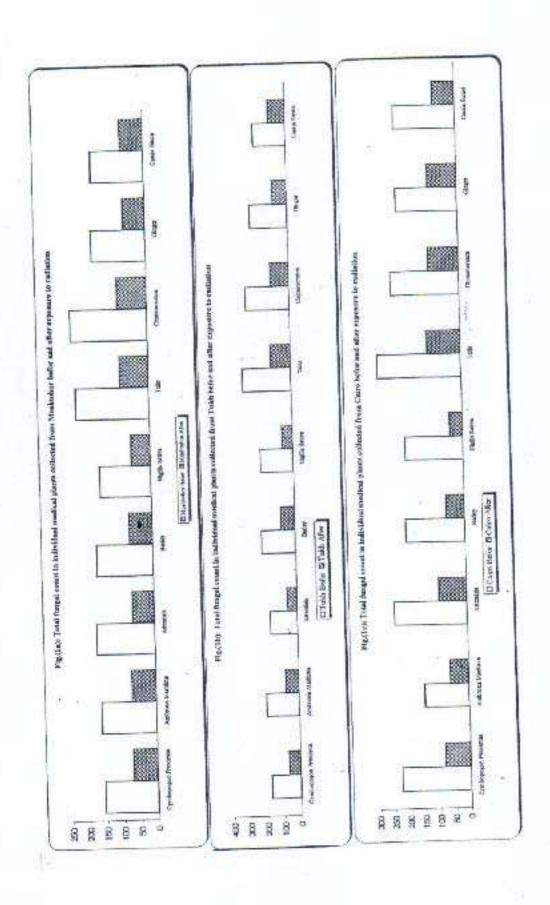
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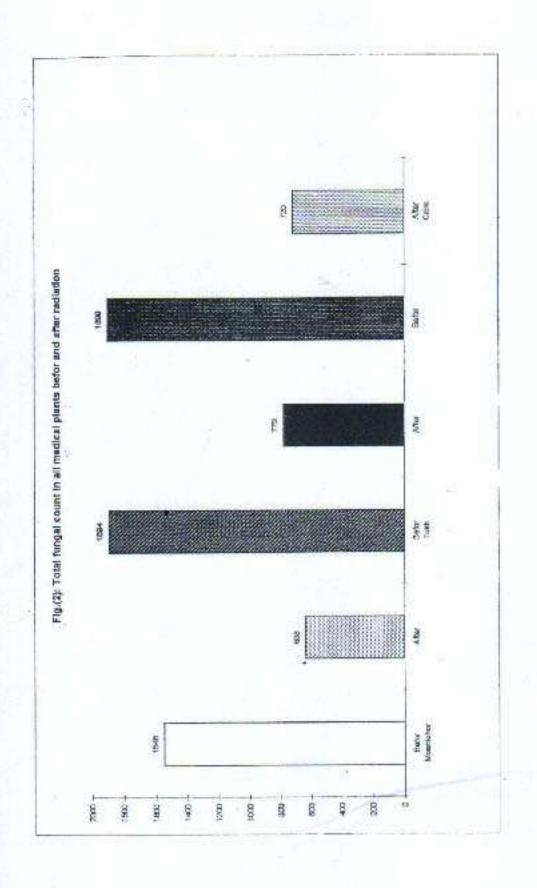
Table (4);Concentrations of othertraxia A in some medical plants before and alter exposure to radiation with the percentage of reduction.

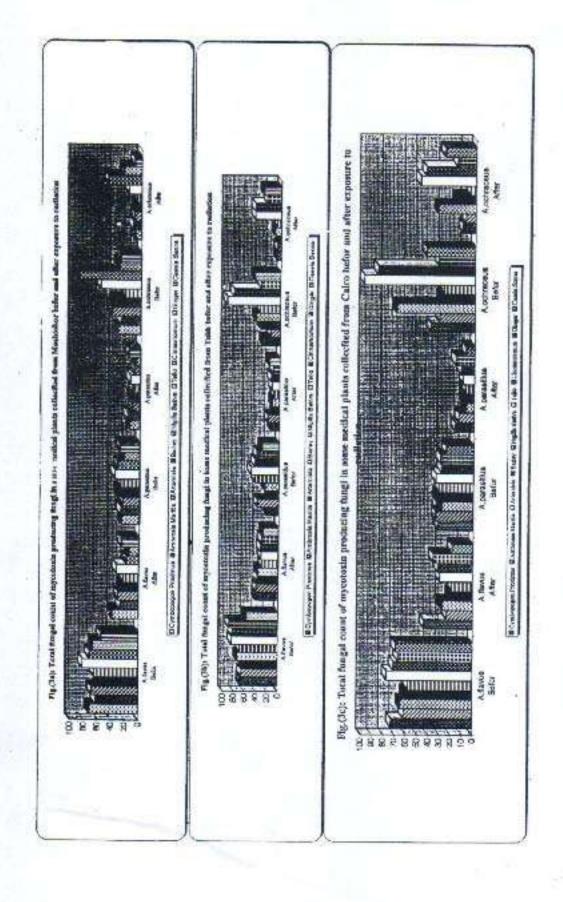
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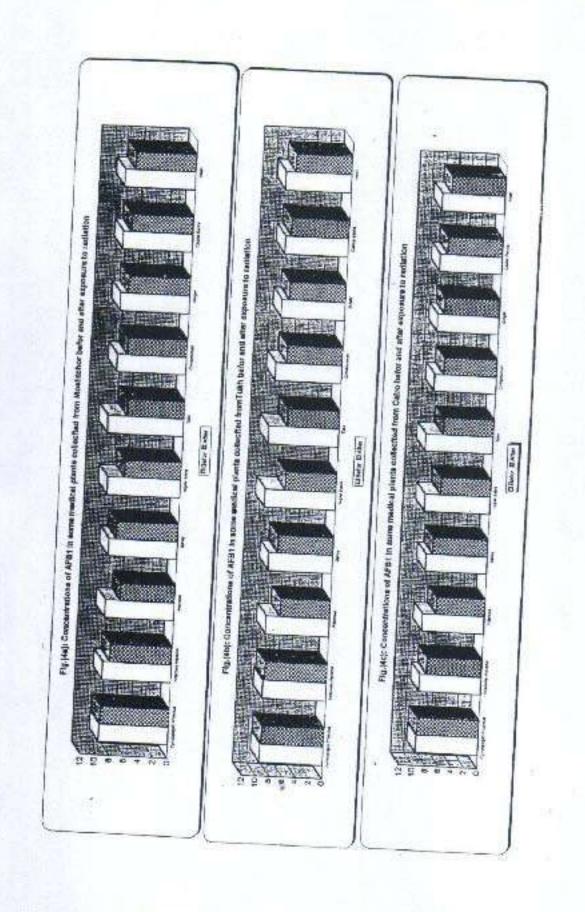
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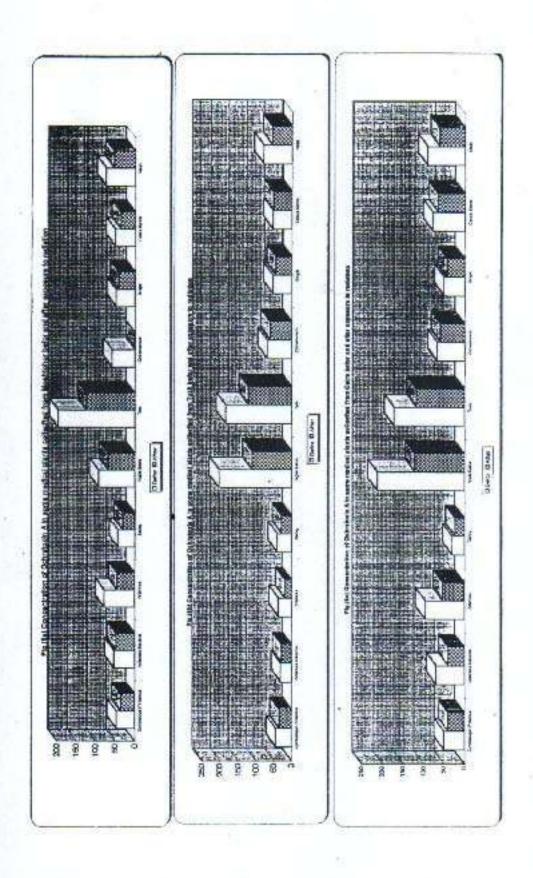
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## DISCUSSIONS

Medicinal plants either in crude forms or being involved in the medicament prepared from herbs are used for treating a number of human diseases. The traditional unscientific method of collection, transporting, sorting and marketing of herbal drugs has promoted their association with several microorganisms.

The total fungal count in the examined medicinal plants before and after exposure to radiation was presented in table (1) and figures 1(a,b,c) where the highest fungal count before and after radiation was recorded in Tukh (289 and 120, respectively) in Telio, while the lowest fungal count before and after radiation was present in Cairo (145,43 in Ambrosia maritima and Nigella sativa, respectively). The highest fungal count was present in Tukh which is a small city may be partially due to bad storage conditions in markets (sporadic small and retail markets) from which our samples were collected, also may be partially due to less hygienic, unclean conditions under which the samples were stored.

The lowest fungal count present in Cairo, which is a big city may be partially due to the better storage conditions in the markets from which these medicinal plants, were collected (big shops and supermarkets). In this respect, Kiran et al.(1985) observed that the natural contamination of agricultural products with different microorganisms was a result of natural extraneous contamination by dust followed by holding under humid conditions. Similarly, Moussa (1994) mentioned that the highest mould count in medicinal plants are usually due to contamination with dust from soil which is considered the main habitat of moulds.

Gamma rays induced reduction in total fungal colony count and the percentages of this reduction were 58.13%, 58.99%, 62.09% in medicinal plants collected from Moshtohor, Tukh and Cairo respectively. In this respect we agree with several authors reported the destructive effect of gamma radiation on mycotoxin producing fungi e.g El-Gendy, Hoda (1979); El-Bazza, Zeneb (1983); Eggbo, Zeinab (1990) El-Zawahry et al., (1991); El-Tablawy, Seham (1993); Aziz et al., (1996) and Osborne et.al., (1996).

Regarding the locations from which these medicinal plants were collected, in Moshtohor, the highest and lowest fungal count were present in cinnamoum and Nigella sativa, respectively, in Tukh, the highest and lowest fungal count were present in Telio and Artemisia, respectively and in Cairo were present in Telio, Nigella sativa respectively.

Concerning the count of mycotoxin producing fungi table (2) and figures 3 (a ,b ,c) showed that there was a highly significant differences in

Bazza, Zeneb (1983) who reported that there was a complete inhibition of mycotoxin production at 5.6 and 4 KGy for aflatoxins B<sub>1</sub>; B<sub>2</sub> and OTA respectively; El-Hady (1986) who noticed that increasing doses of gamma irradiation up to 5 KGy, caused reduction in the aflatoxin production and finally complete inhibition and El-Far et al., (1992) who observed that by increasing the irradiation doses, the viable population of Aflavus NRRL 5520 as well as AFB<sub>1</sub> production decreased greatly.

The influence of gamma rays either in production of aflatoxin B<sub>1</sub> or its destructive effect on the toxin itself have been reviewed by many authors e.g. Ogbadu,(1980) who recorded that aflatoxin B<sub>1</sub> production decreas with increase of gamma irradiation. Also we refer to the study of Mutluer and Erkoc, (1987) who found that AFB<sub>1</sub> was the most radiosensitive of the four aflatoxin compounds. They also concluded that irradiation was found to be suitable for the destruction of aflatoxins in solution.

Regarding the concentrations of aflatoxin B<sub>1</sub> in the examined medicinal plants within different localities, Table (3) showed that Telio has the highest AFB<sub>1</sub> concentration in Moshtohor and Tukh, while Cinamomum has the highest concentration in Cairo. This is in accordance with the data of Table (2) concerning the count of A.flavus and A.parasitius where Telio has the highest fungal count in Moshtohor, Tukh and Cairo, but Cinamomum has fungal count slightly lower than Telio in Cairo. The highest percentage of reduction was in Artemisia, Telio and Cinamomum in Moshtohor, Tukh and Cairo, respectively.

Concerning to the concentrations of ochratoxin A in some medicinal plants before and after irradiation, table (4), fig. 5 (a,b,c) illustrated that the highest concentrations of ochratoxin A before radiation in the examined medicinal plants was 91.49 ± 11.57 ug/kg in Cairo and the lowest one was 79.10 ± 9.35 in Moshtohor, meanwhile, exposing these medicinal plants to irradiation lowered the OTA concentrations with the highest percentage of reduction (32.23%) in Cairo and the lowest percentage (28.12%) in Moshtohor. Also, table (4) indicates that irradiation resulted in a more pronounced effect on the ochratoxin A content of artemisia which showed the highest percentages of reduction in all localities (43.44%; 49.45% and 45.23%) in artemisia collected from Moshtohor, Tukh and Cairo respectively, while Ginger had the lowest percentage of reduction in Moshtohor and Cairo while Cassia senna has the lowest percentage of reduction in Tukh. Also we notice that Telio has the highest concentration of OTA in Tukh and Cairo, but Cinamomum has the highest concentration of OTA in Moshtohor. This is in agreement with the data of Table (2) concerning the fungal count of A.ochraceus where Telio was the highest

fungal count in Tukh and Cairo and Cinamomum has the highest fungal count in Moshtohor

Many authors previously indicated the inhibitory effect of irradiation on the production of ochratoxins e.g. Applegate and Chipley, (1976); El-Bazza, Zeneb, 1983; Hassanein, Wesam (1987) and Chelack et al., (1991).

Moussa (1994) detected AFB<sub>1</sub> and OTA in 20 out of 76 medicinal plant samples analyzed ,the highest level of AFB<sub>1</sub> was detected in Fennel, Camel (Cymbopogon proximus), but Ginger and Cinnamon were free of AFB<sub>1</sub>. OTA was second from black cumin (Nigella sativa), Fennel and Absinthium (Ambrosia maritima). Many authors documented the occurrence of mycotoxins in some common drug plants as El-Kady et al., (1990 and 1995) and Roy and Chourasia (1990) who detected aflatoxins in a variety of medicinal plants, but also recorded that the frequency of occurrence of OTA and Zeralenone was comparatively very low or not detected.

## CONCLUSION AND RECOMMENDATIONS

The effect of radiation on spices has been studied over many years for potential toxicity, teratogenicity, carcinogenicty and follow-up studies have focused on bone malformation, litter size, susceptibility to diseases etc... No adverse effects have been found so far (Farkas,1983). Similarly, extensive toxicological studies of almost every type of food commodity have produced no evidence of adverse effects of irradiation (Diehl,1983).

It is of interest to mention that radiation treatment up to 10 KGy are non-toxic and the quality of the product is much preserved as stated by FAO/WHO (Welt,1983).

Our results indicated that gamma radiation destroyed the majority of mycotoxins and fungi in gamma irradiated medicinal plants. However, some mycotoxins and fungi still present in these plants even after exposing to gamma radiation at the recommended dose. So, we can conclude that gamma radiation is a useful control method for mycotoxins and fungi in medicinal plants, but other control measures either in prehervest, harvest and storage period of these plants should be considered.

The pharmaceutical companies should encouraged to apply gamma radiation at the dose recommended by this study to all medicinal plants used in drug manufacturing for controlling mycotexins.

All herbal drug plants must be tested for their moulds biosynthesizing mycotoxins capacities before use.

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# الملخص العربى أشعة جاما كوسيلة للتحكم في السموم الفطرية ونمو الفطريات المفرزة لسا في النباتات الطبيـة

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أجريت هذه الدراسة على ٤ و عينة من النباتات الطبية المعتاد إستخدامها كعلاج شعبي في حالات إصابات الكلى ، البرد ، الكحة والإمساك وهذه النباتات وهي الحلفا بر ، دمسيسة ، الشيح ، التينيو ، الزنجبيل ، القرقة ، حية البركة ، العسنامكي والتي تم تجميعها عشوانيا من ٣ أماكن مختلفة من جمهورية مصر العربية (مشتهر -طوخ - القاهرة). وقد تم قياس معدل التلوث ببعض السموم القطرية (اوكراتوكسين أو أقلاتوكسين ب١) وتركيزها في هذه النباتات . كما تم عزل انفطريات المفرزة للسموم القطرية من هذه النباتات (جنس بالسيديوياترس ، أسبرجليس فلافيس ، أ. نيجر ، أ. بار ازيتيكس ، أ. أوكر اسيس وجنس الفيوز اريوم ) . وتم تعريض كل العينسات الأشعة جاما عنده كيلوجراى لاستكشاف مقدرتها عنسى التحكم فهي السموم القطرية والقطريات المفرزة لها في النباتات الطبية . وتوصلت نقائجنا إلى أن التعرض الأشعة جاما أدى إلى تكسير معظم السموم الفطرية وكذلك القطريات المفرزة نها في النباتات الطبية المعرضة لها ، ولكن بعض من السموم القطرية والقطريات ما زالت موجودة في النباتات الطبية حتى بعد التعرض الشعة جاسا عند الجرعة المستخدمة. ومن ذلك نستنتج أن أشعة جاما طريقة مفيدة للتحلم في السموم الفطرية والقطريات في التباتات الطبية ولكن مع بتضاذ باقى الطرق الوقانية في مرحلة قبل الحصاد ، الحصاد أو التخزين ثهده النباتات.