DISSERTATION-II
REPORT ON

"EFFECT OF ELECTROMAGNETIC RADIATIONS ON THE VARIOUS GROWTH PARAMETERS OF DIFFERENT SPECIES OF *ASPERGILLUS*"

SUBMITTED TO

Department of Biotechnology
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CERTIFICATE

This is to certify that **Saquib Husain Khan** (Reg no. **11308813)** have completed dissertation-II project report (BTY 698) entitled **“Effect of Electromagnetic radiation on the various growth parameter of different species of *Aspergillus*”** under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study. No part of the report has ever been submitted for any other degree at any university. The report is fit for submission and the partial fulfilment of the conditions for the award of **M. Tech. Biotechnology.**

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DECLARATION

I hereby certify that the work which is being presented in this report entitled **"Effect of Electromagnetic radiation on the various growth parameter of different species of *Aspergillus*"** by **Saquib Husain Khan** in partial fulfilment of requirement for this award of degree of M. Tech. Biotechnology submitted in Lovely Professional University Punjab is an authentic record of my own work carried out under the supervision of **Dr. Leena Parihar.** The matter presented in this report has not been submitted by me in any other University/Institute for the award of any Degree.

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ABSTRACT

There exist several important fungi in stored grains namely *Aspergillus flavus, Aspergillus niger* and *Aspergillus fumigatus* etc which produce aflatoxins, a toxic and carcinogenic compound. This fungus can grow on diverse substrates and represents a serious public health and animal nutritional problem. Therefore, the study of techniques that can be applied to the control of aflatoxins is of greatest importance. The aim of the present study was to determine the effects of electromagnetic radiation at a frequency of 6.41GHz at a different time exposure of 2, 4, 5, 7 and 8min respectively on the growth of pure culture of *Aspergillus* species to determine the effect of growth parameters and aflaoxin production. Irradiation was found to be effective in reducing the spore formation at a maximum exposure of 8min and same was observed in aflatoxin production. To analyse the presence of toxic aflatoxin compound Thin Layer Chromatography was done which shows the blue and green florescence under UV light. The chromatography results were also supporting the morphological results. Increasing the radiation dose can also suppress the growth of fungi up to sufficient level.

Keywords: Microwave radiation, *Aspergillus* species and aflatoxin etc.

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INDEX

CONTENT PAGE No.

CERTIFICATE

DECLARATION

ABSTRACT

ACKNOWLEDGEMENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

1. INTRODUCTION **1-5**

1.1. ELECTROMAGNETIC RADIATION

1.1.1. MECHANISM OF MICROWAVES RADIATION ON LIVING ORGANISMS

1.1.2. EFFECTS OF MICROWAVE RADIATION ON MICROORGANISMS

1.1.3. HARMFUL EFFECTS OF EMR ON HUMANS

1.1.4. HARMFUL EFFECTS OF EMR ON MICROBES

1.2. FUNGAL SPECIES

2. TERMINOLOGY **6**

3. REVIEW OF LITERATURE **7-16**

4. SCOPE OF STUDY **17**

5. AIMS AND OBJECTIVES **18**

6. MATERIALS AND METHODOLOGY **19-23**

6.1. EXPERIMENTAL SITE

6.2. MATERIALS

6.2.1. INSTRUMENTS

6.2.2. MEDIA

6.2.3. CULTURES

6.3. METHODOLOGY

6.3.1. FUNGAL CULTIVATION

6.3.2. RADIATION EXPOSURE

6.3.3. MICROSCOPIC STUDY OF FUNGI

6.3.3.1. FUNGAL STAINING

6.3.4. METHOD OF AFLATOXIN PRODUCTION

6.3.5. DETECTION OF AFLATOXIN COMPOUNDS BY TLC

6.3.5.1. THIN LAYER CHROMATOGRAPHY

7. RESULTS AND DISCUSSION 24-39

7.1. GROWTH OF MICROBIAL STRAINS

7.1.1. GROWTH PARAMETERS OF *A.NIGER*

7.1.2. GROWTH PARAMETERS OF *A.FLAVUS*

7.1.3. GROWTH PARAMETERS OF *A.FUMIGATUS*

7.2. MICROSCOPIC EXAMINATION OF MICROBIAL STRAINS

7.3. EXTRACTION AND ANALYSIS OF AFLATOXIN

7.4. GRAPHICAL ANALYSIS

7.5. EFFECT OF MW ON AFLATOXIN PRODUCTION

8. CONCLUSIONS 40

9. REFERENCES 41-48

LIST OF FIGURES

S.No	Figures	Page No.
1	Sporing structure of *Aspergillus* species	4
2	Molecular structure of Aflatoxin	11
3	Sample of pure culture	21
4	Klystron microwave test bench	22
5	Growth parameters of *Aspergillus* species	24-27
6	Microscopic examination of Aspergillus species	27-28
7	Liquid broth for aflatoxin extraction	29-31
8	TLC analysis for aflatoxin	29,31,32

LIST OF TABLES

S.No	Tables	Page No.
1	Frequency and wavelength of electromagnetic radiations	2
2	Mycotoxins produced by *Aspergillus* sp. and their toxin effect	5
3	Instruments and Equipment’s	19
4	Media	20
5	Culture	21
6	Table for graph between radiation time and Rf values	33
7	Table for graph between radiation time and absorbance	35
8	Effect of MW on aflatoxin production	37
9	Quantification of aflatoxin by Thin Layer Chromatography	38

ABBREVIATIONS

ºC	degree centigrade
mL	Millilitre
Nm	Nanometer
gm	Gram
GHz	Gigahertz
kGy	Kilogrey
MW	Microwave
EMR	Electromagnetic radiation
A.	*Aspergillus*
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
NCIM	National collection of industrial micro organism
MTCC	Microbial type culture collection

CHAPTER 1

INTRODUCTION

1.1. ELECTROMAGNETIC RADIATION

Electromagnetic waves are generally produced by the motion of electrically charged particles. These types of waves are called "electromagnetic radiation" as they radiate from the electrically charged particles. These waves are also travel through empty space as well as through air and many other substances. Radio waves, x-rays, microwaves and visible light are all the examples of electromagnetic waves that differ in their wavelength from each other.

According to (Foster and Schwan, 1996) the human body is always exposed to the EMR (electromagnetic radiation) of varying intensity which is dependent on the location, either in open space or inside the building. The human body is a complex function of different parameters like electrical density, conductivity and its complex permittivity. The electromagnetic radiation is characterized by its magnetic and electric field, their polarization and direction in free space. (Pathak *et al*, 2003) demonstrated that when an electromagnetic field (EMF) falls on the human body, it penetrates into the body and attenuated by the body tissue and some of its parts are absorbed by the tissue.

Microwaves are described as non-ionizing electromagnetic waves between frequencies from 0.3 and 300 GHz (i.e., with wavelengths from 1meter to 1mm respt) (Balbani and Montovani, 2008). “One of the most important sources of microwave radiation that are encountered by humans are various industrial microwave generators used for various purpose of communications, that speed up chemical reactions or may used for heating (896 or 915 MHz); microwave ovens (915 and 2450 MHz); cellular phones (824–850, 900, 1800 or 1900 MHz); cordless phones (from 46 to 5800 MHz)”.

1.1.1. MECHANISM OF MICROWAVES RADIATION ON LIVING ORGANISMS

According to Michaelson, 1974 when irradiating living organisms, a microwave transmits 2 types of effects: thermal effect and non-thermal effect. Thermal effects are the consequence of absorbing microwave energy by cell molecules and therby cause them to produce heating of the cell and causing the cells to vibrate at a much faster rate. The absorption of microwave radiation inside the cell depends on its electrical conductivity and dielectric constant. The concept of non-thermal microwave effect belongs to the experiments in which bacterial strain

destroyed by microwave heating rather than by other methods by providing the same temperature and from studies which shows a rapid increase in the growth of bacteria induced by microwaves. The mechanism of the non-thermal activity of microwaves is still not unstated.

1.1.2. EFFECTS OF MICROWAVE RADIATION ON MICROORGANISMS

The effect of microwave on the microorganism's growth depends on the radiation frequency and the total amount of energy absorbed by the microorganisms. When microwave radiations are applied to a certain frequency, for a long period of time with high energy, kills yeasts and bacterial cells. In difference, when microbes were radiated with microwaves treatment at a lower temperature than the thermal destruction level; some of the effects were observed, from destruction to increased growth. A exact killing of *E.coli* from the microwave treatment, differ from the hyperthermia effect, was detected in several research.

Types of radiation	**Frequency**	**Wavelength**
Radio waves	$<3x10^{11}$ Hz	>1mm
Microwaves	$3x10^{11}$ - 10^{13} Hz	1mm - 25um
Infra red	$1x10^{13}$ - $4x10^{14}$ Hz	25um - 750nm
Optical waves	$4x10^{14}$ - $7.5x10^{14}$ Hz	750nm - 400 nm
Ultra violet	10^{15} - 10^{17} Hz	400nm - 1nm
X-rays	$10^{17-}10^{-20}$ Hz	1nm-1pm
Gamma rays	10^{20-} 10^{24} Hz	$<10^{-12}$ m

Table1.1 Frequency and wavelength of electromagnetic radiations (snow et al, 1997)

1.1.3. HARMFUL EFFECTS OF EMR ON HUMANS

It has been reported that from last few years, television, and some of the electrical devices causing a wide variety of symptoms on humans as well as on animals. Many studies correlates RF exposure with diseases such as neurological diseases, reproductive disorder, cancer and immune dysfunction. It has also an genotoxic effects and show chromosomal

variability, altered gene mutation, gene expression, DNA destruction and DNA structural break and these genotoxic effects occur in the red blood cells, lung cells, bone marrow, sperm, neurons and blood lymphocytes.(Ruediger, 2009; Phillips *et al,* 2009). (Ban and Grosse, 2010) reported that the World Health Organization (WHO) has also classified radiofrequency emissions as a group 2B carcinogen. And Cellular phones use in the rural areas was also seems to be linked with an higher risk for malignant brain tumours (Hardell *et al*, 2005) and some of the study also proves that electromagnetic frequencies, including radiofrequencies, can have the negative impact on human health**.**

1.1.4. HARMFUL EFFECTS OF EMR ON MICROBES

Electromagnetic radiation have also an negative impact on the microbial population and ability of ionizing radiation to kill the microbes has also been determined since the late 19th century and the complete removal of toxigenic molds in coffee beans or in other food commodities was also achievable with variable doses (for eg: 5 to 10 kGy) reported by (Aziz *et al*, 1990). Such radiation especially gamma radiation, completely inhibit the fungus growth in varieties of food and feed products and dose required for inhibition ranges from 4 to 6 KGy (Aziz et al, 2002). It may also inactivate the bacterial species generally by microwave radiation and there are number of reports which suggest that moulds are very complex to gamma radiation and in addition their mycotoxin production decrease after radiation.

1.2. FUNGAL SPECIES

Micheli in 1729 described that *Aspergillus* is one of oldest genus of fungi. The genus *Aspergillus* encompasses the organisms whose features are of high pharmaceutical, agricultural, pathological, industrial, scientific and social importance and plays a key role in the degradation of biological substrate, generally plant material (Samson and Varga, 2009; Bignell, 2010; Goldman and Osmani, 2008). Aspergilla are known for their unique features to secrete a wide variety of biologically active chemical compounds including immune-suppressants, mycotoxins, antibiotics and cholesterol lowering agents. Generally essential and basic tools for identification and determination of *Aspergillus* species are macroscopic characteristics such as colony diameter, exudates, conidial colour, colony reverse and microscopic characterization including vesicle, conidiophore, medullae, conidia and phial ides (McClenny, 2005). According to (Pitt and Hocking, 1997) for Aspergillus identification,

generally requires selective growth media, including Czapek dox agar, or a derivative such as malt extract agar and Czapek yeast extract agar (CYA).

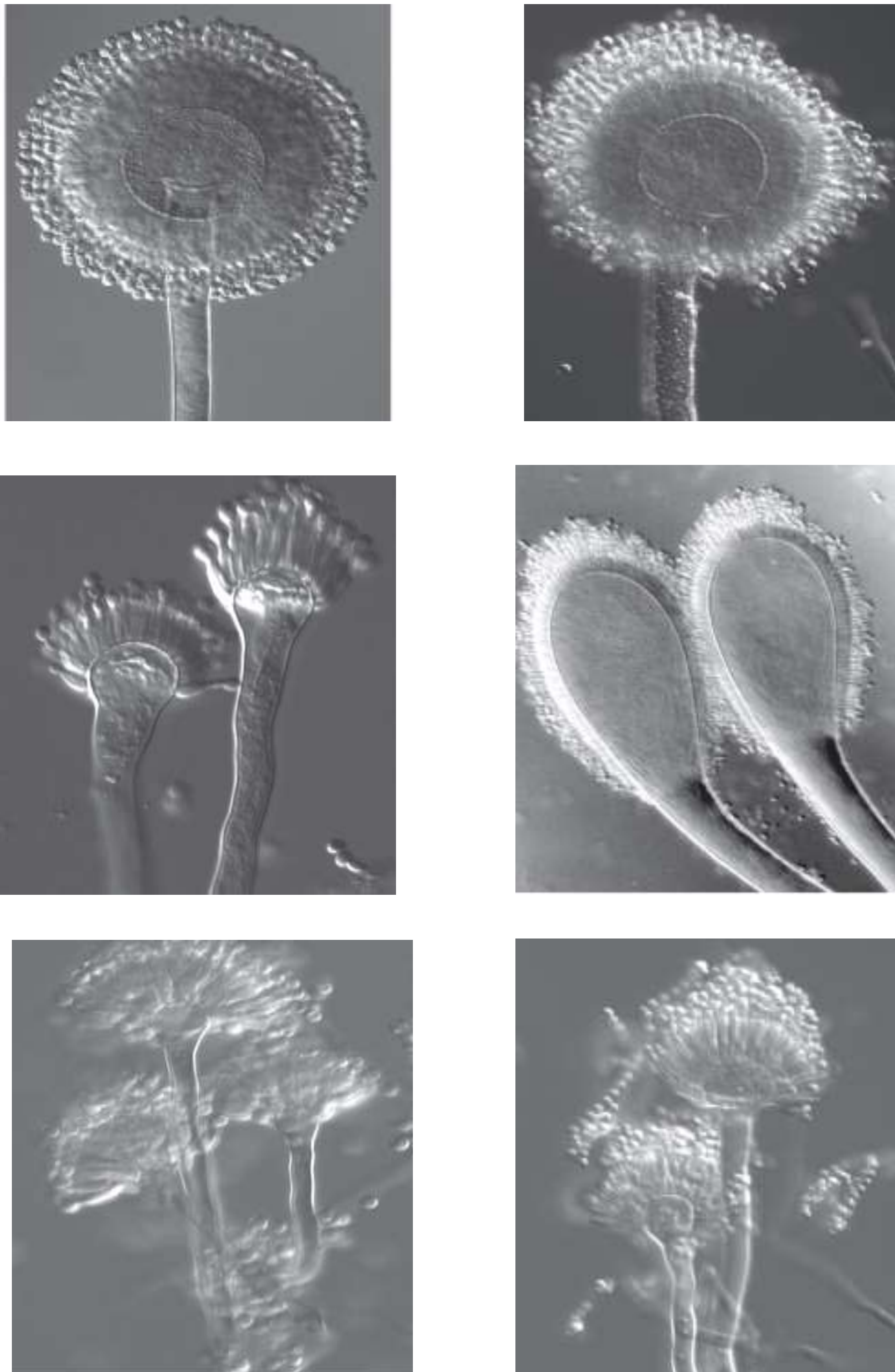

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Figure1.2. Sporing structures of some Aspergillus species: (a) A. carbonarius; (b) A. flavus; (c) A. fumigatus; (d) A. clavatus; (e) A. nidulans; (f) A. terreu

Mycotoxin	Toxicity	Species
Aflatoxin B1 and B2	Acute liver damage, carcinogenic(liver), cirrhosis, immunosuppressive, teratogenic.	*A. flavus, A. parasiticus, A. Nomius*
Aflatoxin G1 and G2	Similar effects to B aflatoxins: G1 toxicity less than B1 but greater than B2	*A. parasiticus,* *A. nomius*
Ochratoxin A	Kidney necrosis(especially pigs), teratogenic, immunosuppressive ,probably carcinogenic	*A. ochraceus* *A. carbonarius,* *A. niger*
Cyclopiazonic acid	Degeneration and necrosis of several organs, low oral toxicity, tremorgenic.	*A. flavus,* *A. tamarii*
Sterigmatocystin	Acute liver and kidney damage, carcinogenic(liver)	*Emericella spp, A. versicolor*
Fumitremorgens	Tremorgenic(rats and mice)	*A. fumigatus*
Territrems	Tremorgenic(rats and mice)	*A. terreus*
TryptoquivalineCytochalasinsEchinulins	TremorgenicCytotoxicFeed refusal (pigs)	*A. clavatus* *E. amstelodami*

Table 1.2. Mycotoxins produced by *Aspergillus* sp. and their toxin effect (Hocking 2001)

CHAPTER 2

TERMINOLOGY

ELECTROMAGNETIC RADIATION

Electromagnetic waves are generally produced by the motion of electrically charged particles. These types of waves are called "electromagnetic radiation" as they radiate from the electrically charged particles.

AFLATOXIN

Aflatoxins are secondary metabolites belonging to a polyketide class which is generally produced by toxigenic strains of *A. parasiticus* and *A. flavus*, and are directly synthesized by the enzymes which is encoded within a large no.of gene cluster. These are also toxic and carcinogenic compound.

THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography is a method for analysis of mixture by separating the compound in the mixture. It can be performed for various purposes generally to help in determining the no. of components in the mixture, the compound’s identity and the purity of compound.

CHAPTER 3

REVIEW OF LITERATURE

Radiofrequency electromagnetic fields of the anthropogenic origin are the most extensively propagating factors that affect the environment. Their lethal effects on biological processes and on human health are of great interest and are discussed intensively. Several theories were proposed that attempt to explain the mechanisms of biological effects of this factor (Grundler *et al*, 1992). Many researchers point out that the use of multi-cellular organisms as a laboratory model in the research leads to problems. The complexity of these organisms is a major problem for evaluating the objective of the biological effects of the electromagnetic fields and understanding the mechanisms that interacts between living matter and physical force. Veelders *et al*, 2010 reported that among different model systems, the *Saccharomyces cerevisiae* baker's yeast is of great importance. This is the most well studied yeast and is widely used organism to study the life process of eukaryotic organisms at different point of view, from molecular and genetically to physiological and even social life.

(Vela and Wu 1979; Woo et al, 2000) demonstrated that in the last few years there have been certain publications in which the lethal effect of microwave treatment on microorganism like bacteria have mentioned. The microwave bands called ultra-high frequency are used in various devices, that include television, microwave ovens, microwave communications, radar technology, medical diathermy and abundance of special equipment that have been designed for specific purpose. The usage of microwave radiation has become advanced nowadays particularly in food and other related industries for the industrial applications.

Several researchers have observed that the complete destruction of microorganisms when applied to a microwave pitch was due to the thermal effects only and the statement reported by Fujikawa *et al* 1992. "According to Olsen, 1965 conidia of *Penicillium* species and *Aspergillus niger* were inactivated by the non-thermal effects of microwave radiation".

Aziz *et al,* 1990 demonstrated that the capability of ionizing radiation for killing the microbes has been determined from the 19th century and the total removal of toxigenic molds in food commodities and coffee beans achieved with doses ranging from 5 to 10 kGy. The fungal sensitivity to gamma radiation has been established by Aziz *et al* in 2002

reported that the effective dose required for the inhibition of fungus in different food products ranges from 4 to 6 kGy. There exist a lot of reports which suggest that the molds are very much sensitive to gamma radiation and in addition after irradiation their mycotoxin production decreased

Lopes *et al*, 2002 established that there are few methods which is used for the determination of cells viability. The cytotoxic test was improved when fluorescent diacetate (FDA) and ethidium bromide (EB) are combined that showed a strong contrast in between the dead and living cells. The main aim of this study was to evaluate the irradiation effect on the viability of *Aspergillus flavus* by (FDA-EB) viability test and the aflatoxins degradation in maize at a frequency of 2, 5 and 10 kGy respectively.

Different fungal species (*Aspergillus, Cladosporium, Acremonium, Fusarium, Penicillium & Trichosporon*), were destructed by CO- 60 irradiation unit with dosing frequency ranges from 14.5 to 25 kGy, and the minimum amount of dose required for complete destruction of these fungi was 16 kGy (Silva *et al*, 2006). It is evident that some fungi developed a efficient mechanisms to protect against the various sources of radiation, while growing in the highly radioactive polluted environments (Strike and Osman, 1993). (Tiryaki, 1990) stated that the sensitivity of storage pathogenic fungi isolated from the pears to gamma rays was as follow: *Botrytis cinerea* and *Alternaria tenuissima* were the most resistant, while *Penicillium expansum* and *Rhizopus stolonfer* were most sensitive. El-khawas *et al*, (1999) reported that 2, 4 and 6 KGy doses of gamma rays reduced the microbial count. Fungi have been successfully inactivated with gamma radiation at a dose ranging from 6 to 15 kGy (McNamara *et al*, 2003).

Brackett, 1987 demonstrated that preventing the contaminated food by the toxic fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, is the most economic approach for avoiding the potential hazards. That's why prevention is not all time possible under the agronomic and storage practices. Thus sanitization has gained importance in the case of recovering food which is already contaminated by toxic fungal metabolites. Detoxification of foods containing aflatoxins is a major problem of current concern and can be accomplished by a variety of methods. Contaminated and non-contaminated segregation of products sometimes may also be carried out by hand sorting. While this approach is effective and economical, where labour costs are not much expensive.

Nakazato *et al*, 1990 founded that four fungal cultures namely *Eurotium herbarioum, Aspergillus niger, Rhizopus* and *Aspergillus flavus* convert aflatoxin B1 to aflatoxicol and may also convert AFL to AFB1.

Over 40 species of *Aspergillus* are listed for the production of toxic metabolites (Cole *et al*, 2003). The *Aspergillus* are having the greatest implication in feeds and foods as aflatoxins (produced by *A. flavus, A. parasiticus* and some of the less common, fewer species that may not play important role in foods), ochratoxin A are generally produced from *A.ochraceus* and some of its related species and from *A. carbonarius* and sometimes sterigmatocystin, *Aspergillus niger*, produced by *A. versicolor* but also by *Emericella* species. (the primary source is *A. flavus* but it is reported to be produced by *Aspergillus tamarii*). Citrinin, penicillic and patulin acid may also be produced by certain *Aspergillus* spp, and tremorgenic toxins produced by *Aspergillus fumigatus* (fumitremorgens), *Aspergillus terreus* (territrems) and *Aspergillus clavatus* (tryptoquivaline) (Hocking, 2001).

Fungal contamination on stored seeds has resulted in major socio-economic problems all over the world. The quality of stored grains can deteriorate due to the infestation both on surface and underneath the seed coat by many fungi (Duan *et al*, 2007; Francisco and Usberti, 2008). The quality of the seed is inversely related to the no. of fungal spores or colonies associated with the seed, especially when these fungi are potential members to produce mycotoxins (Aziz *et al*, 1997; Niessen 2007). Among all the fungi which affect the stored seeds like corn and other grain products, *Aspergillus* is the most notorious (Saleh *et al*, 1988).

Gamma radiation has been shown to inactivate the fungi successfully from different materials, such as paper, woods, stored seed and soil (De Silva *et al*, 2006; Aziz *et al*, 2007). It has been reported in most of the studies that the effect of radiation varying among different organisms (Afifi et *al*, 2003). Meletiadis *et al*, 2001 reported that fungi differ in their interaction with different seeds depending upon their environment, available nutrients and metabolic characteristics of the organism.

Aflatoxins are the most toxin and carcinogenic compound which is produced by the no. of *Aspergillus* species and contains four major types of aflatoxins such as: AFB1, AFB2, AFG1 and AFG2. *A. paraciticus* and *A. flavus* both combine to form the aflatoxin in the agricultural comodities (Nesbitt et al, 1962; Betina, 1989). And according to (Stark and Demain, 1980) AFB1 is the most important and toxic to humans from the public

health point. It is the most toxic and potent teratogen, carcinogen and mutagen to animals and humans (Sweeney and Dobson, 1998; Shahidi, 2004; Seo et al, 2011) causing several damages such as toxic hepatitis, edema, immunosuppression, haemorrhage and hepatic carcinoma (Speijers and Speijers, 2004; Peng and Chen, 2009; Woo et al, 2011). Aflatoxins are contaminating a large variety of foodstuffs and crop commodities such as peanuts, spices, corn, tree nuts, fruit juices, wine, figs, honey, and cotton. It has been estimated that 25% of the world's crops are mainly affected by the mycotoxins, according to the evaluation of the (FAO)Food and Agriculture Organization. The four main aflatoxins frequently encountered are B1, B2, G1 and G2. They are determined by their corresponding colour they fluoresce (blue or green) under the UV light, and the numbers assigned to them are based on the relative distance traveled on a thin layer chromatography (TLC) plate. Aflatoxin B1 is the most toxic amongst the all four toxins. Upon ingestion, aflatoxin B1 may be metabolized into aflatoxin M1, which is expressed in lactating animals milk, and thereby contaminating a large number of dairy products described by Pei.S.C et al., 2009.

Fungi growing on stored food product or living organism and produce harmful secondary metabolites which diffuse into food. Such metabolites are referred as mycotoxins which are produced on different food and feed elements or in some of the agro products before or after crop or else during storage/transportation. Both extrinsic and intrinsic factors (pH, temperature, oxygen tension and humidity) effect the production of mycotoxin on a substrate. The mycotoxins are highly carcinogenic and toxic in nature generally AFB1. Aflatoxin B1 which is widely found in maize, wheat, cereals, nuts, and cottonseeds and for this purpose some of the experimental work has been done on the aflatoxin B1 determination in poultry feed using ELISA (enzyme linked immunosorbent assay).method. The author claimed that the method is much sensitive for the determination of aflatoxins in different nature of samples. And for the sensitivity of ELISA method for the estimation of aflatoxins described by (Maqbool et al., 2004). A microtitration plate method was also optimized by using peroxidase – aflatoxin B1 conjugate and anti- aflatoxin B1 antibody, based on the competitive enzyme immunosorbent assay principle. Standards of concentrations prepared in phosphate-buffered saline of 5, 10, 20, 50, 100, 500 ng/L aflatoxin B1 were used. Standard curves focused that as the concentration of antigen decreased, absorbance increased. Fifty percent inhibition was observed at 37ng/L. The lowest value for the detection limit of aflatoxin B1 was 5ng/L. By using this standardized ELISA technique, aflatoxin B1 was detected in most of the poultry samples and in their components too.

The aflatoxin structure comprises of a coumarin nucleus which is attached to a pentanone or bifuran (AFB1 and AFB2) or a lactone ring of six membered (AFG1 and AFG2). AFB1, AFB2, AFG1, and AFG2 are the four different types of aflatoxins which occurs naturally, among which AFB1 ($C_{17}H_{12}O_6$) is found to be the highly significant in terms of human health risk and animals (Bluma et al., 2008 and Pier, 1992). Aflatoxins are secondary metabolites belonging to a polyketide class which is generally produced by toxigenic strains of *A. parasiticus* and *A. flavus*, and are directly synthesized by the enzymes which is encoded within a large no.of gene cluster (Yu et al., 2004; Yabe and Nakajima 2004).

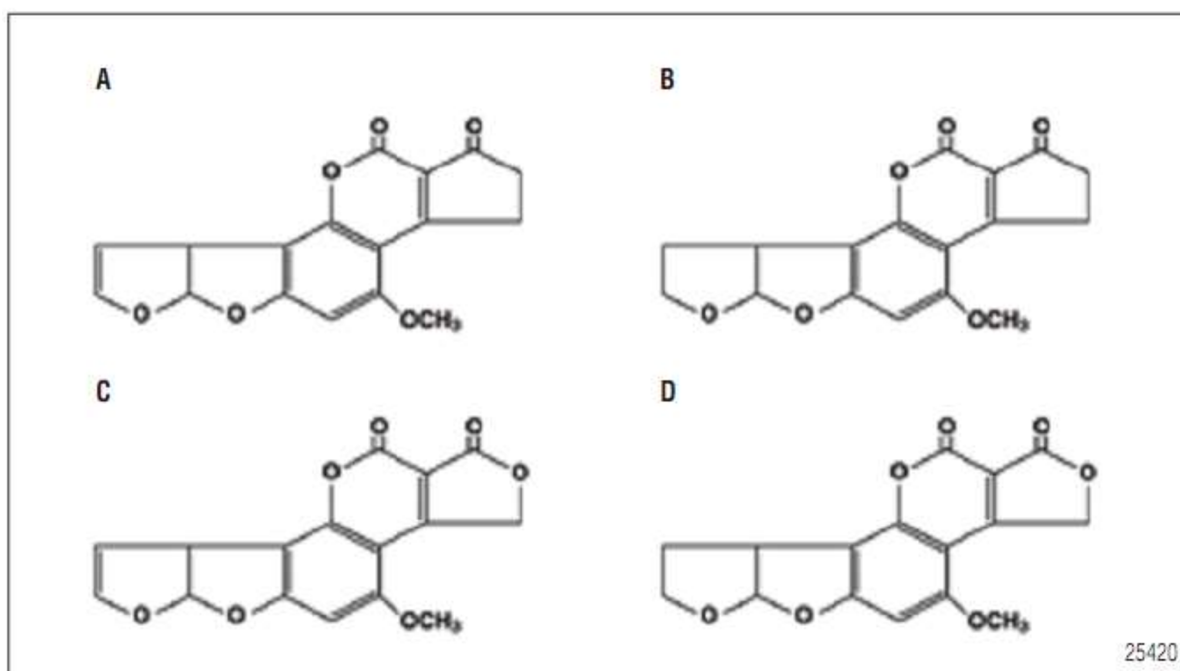


Figure3.1. **Molecular structure of Aflatoxin (1).AFB1 (2).AFB2 (3).AFG1 (4).AFG2**

Different growth inhibitors that may inhibit the biosynthesis of aflatoxin may act at 3 levels: (1) Modulate physiological and environmental factors that affect the biosynthesis of aflatoxin, (2) directly inhibit the enzyme activity or gene expression in the biosynthetic pathway, (3) inhibit signaling circuits upstream of the biosynthetic pathway. “The well-known inhibitory compounds either alter known physiological and environmental modulators of aflatoxin biosynthesis or alter signal transduction pathways in the upstream regulatory network” which was studied by (Holmes et al., 2008). Every step in gene transcription, gene expression, RNA processing, protein processing and translation can be simply inhibited by natural products of plant or other inhibitory agents (Trail et al., 1995).

There exist two techniques for the reduction of aflatoxins levels in various food materials. First method is to prevent or control food impurity in fungi which produces aflatoxins. This approach is so called preharvest approach is much easy and implicated during harvesting and cultivation. Moreover, the postharvest approach which basically deals with the aflatoxin inhibition and aflatoxin producing fungus in the different agricultural commodities exist to be more difficult for the consumption of humans. The major complexity of inhibition of aflatoxin varies depending on the nature and quantity of the food material. The safety of the products which undergoes removal is currently applied for animal feed and their use is not yet recommended for human consumption. Removal or inactivation of aflatoxins in feed and food products can reprocess a large part of the protection for animal use. Physical and chemical treatment is currently major stratagies used at large scale. And the safety of these stratagies can be increased more by using them with phytochemical agents for reducing the level of aflatoxin production in food products. Such agents may not pose a toxic threat to humans when directly or indirectly enter to human food. Hence, it is believed that the replacement of phytochemicals is in the benefit of human safety. And secondly, many of the physical stratagies have been made to suppress, destroy or remove the toxicity of the mycotoxins. These stratagies include physical elimination of different part of the portions of contaminated foodstuffs, treatment with radiation and heat (Park et al., 2007). Many of the physical methods including microwave heating and treatments with ozone (ozonation) have been implicated for the detoxification of aflatoxin contaminated foodstuffs.

It has been shown that the treatment of gamma ray on different types of food products is found to be effective in case of decreased concentration of mycotoxin. It was also stated that by maximizing the doses of gamma ray from 10 to 60 kGy, mycotoxin reduction was significantly increased at a much faster rate; however, at a dose of less than 10 kGy, there was no reduction in mycotoxin level. In other related research, the variable doses of 15, 20, 25 and 30 kGy respectively were used to destroy or kill the aflatoxin B1 in peanuts sample by 55–74% (Prado et al, 2003). AFG2 and AFB2 in all of the treatments showed low reduction when compared with other mycotoxins.

According to (Park et al., 2007) the “self-designed microwave induced argon plasma system is a technique which require minimum exposure time for the degradation of mycotoxin than other methods, such as UV or visible light and gamma rays”. The UV radiation by plasma may be responsible for removing and degrading the mycotoxins. This plasma system has various advantages, relatively high intensity of UV light (75–102

mW/cm2), increased ionization by reactive species, easy operation and low average temperature (75– 130°C). In summary, the AFB1, nivalenol and deoxynivalenol were completely removed after treatment of plasma for 5 seconds.

Reddy et al, 2010 stated that mycotoxins occurring in food products are secondary metabolites of the filamentous fungi. These types of toxins contaminate many varieties of food crops throughout the food chain. According to Gonçales et al, 2008; Rustemeyer et al, 2010; Yassin et al, 2011 mycotoxins are produced by the three main genera *Aspergillus, Penicillium* and *Fusarium* during the crop growth, storage or harvesting. Among these toxins, the aflatoxins are synthesized by filamentous fungi *Aspergillus flavus* and related aspergilla are of concern of many investigators. These funguses affect cereals like corn, wheat, peanuts, and rice. In this context, *Penicillium* and *Aspergillus* were reported as the most dominant genera in Brazilian peanut seeds and Egyptian peanut. And these type of commodities are more liable to the fungal infection particularly *Fusarium, Aspergillus* and *Pencillium* species in tropical region and subtropical regions, which depends on high levels of temperature and moisture (Creepy, 2002).

The stimulation of fungal growth occurs in presence of non-ionic surfactants with 15 or more ethylene oxide units and has reported by Steiner and Watson, 1965. According to (kollmann et al, 2003) due to longer exposure to nonylphenol, the stimulation of spore germination and production in *Fusarium oxysporum* was shown to have resulted. Earlier research has been reported that strains of *A. flavus* and *A. parasiticus* grown in YES media yields a high level of aflatoxin as compared with Czapek based media (Huynh and Lloyd 1984). In YES media, *A. flavus* elaborated 30% more toxin production in comparison with CYA medium which was observed by Gqaleni *et al*, 1997.

Gupta *et al,* 1995 demonstrated that the gamma rays, ultraviolet irradiations or N-methyl, N-nitro-N-nitro so-guanidine (MNNG) techniques which induces mutagenesis are useful for improving the yield of various secondary metabolites by *A. niger*. Alben *et al,* 2004 reported that the filamentous fungi *Aspergillus niger* is the commonly used species for citric acid production. Rohr, 1983 stated that citric acid can be produced by fermentation process using *A. niger,* a fungus commercially used for the first time in 1923. Kapooir *et al,* 1982 reported that the high production of citric acid depends to a extent on the strain used and its response to the composition of the medium can show a great deal of variability.

The mechanism of destructing the microorganisms by microwaves treatment at a temperature lower than the thermal destruction level (Cunningham, 1978) and even significantly increase the growth of *Saccharomyces cerevisiae* on dry media when exposed to a microwave treatment at a frequency of (200-350 GHz) have also been observed that was reported by Hadjiloucas *et al,* 2002. The studies based on the microwaves effects on fungal spores have been very limited and their conclusions are contradictory regarding the influence of such radiation on spore viability (Chipley, 1980).

Vidal, 1973 described the effects of ionic radiation doses of 70 kGy and 45 kGy over many kind of food, this type of radiation was used in order to get the biologically proper food. In such a way, parasites (*Tema, Tnchma),* insects, and pathogen microorganisms were destroyed. Saint-Lebe, 1969 investigated the influence of gamma-rays over technological characteristics of corn starch. In most of the cases, starch radiated with 3 kGy frequency is enabled to get desired quality, usually without altering the technological characteristics of the product.

Adamo *et al*, 2001 stated that the effects of gamma radiation on the viability of microorganisms have a great deal of attention. Gamma rays, electromagnetic waves with high penetrating power, pass through the materials without leaving residue, an advantage comparing to other disinfection treatments. The exposure of fungi to gamma radiation sets of chain of reactions, which give rise to chemical and metabolic or physiological changes, so irradiation represent an additional stress on the cells, which tends to disturb their organization (Lawrence, 1971). For the complete destruction of fungi, gamma radiation was used (Sedlackova, 1992).

The susceptibility of microbial population and their spores exposed to gamma radiation has been established well. The ionizing radiation produces a substrate changes that inactivates microorganisms. Some of the uses of gamma radiation are also decrease the no. of microbial count and eliminating the risks of a poisonous infections and diseases. The DNA molecules of microorganisms are directly effected by the energy of ionizing radiation and thereby, causing the fungal cells damage or bacterial cells. Some of the known indirect effects of radiation is the interaction of energy with water molecules present on food or substrates, producing ions and free radicals that attack the microorganism DNA, killing the microbes (Diehl, 1995; Farkas, 1983).

Maity et al, 2004 and Mironenko et al, 2000 demonstrated that some of the *Alternaria* spp. shows high resistance when exposed to radiation at elevated levels. This genus was tolerant upto doses of 4 kGy because this type of fungus is able to produces melanin, which is accumulated inside the mycelium. Analysis of fungal micro biota in soil samples which were collected around the Chernobyl reactor, revealed a predominance of pigmented fungus, including the species *A. alternata*. "This fact led to the usage of *A. alternata* strains isolated from the radioisotope-contaminated environment around reactor nº 4 of the Chernobyl nuclear power plant as a model for the genetic study of resistance to gamma radiation".

Maize, spices, milk and dried fruits are variety of food commodities on which aflatoxin is produced by *A. flavus* and *A. parasiticus*. Prakash et al, 2011 stated that the basic characteristics inactivation process of aflatoxin is that it may destroy the spores and mycelia of the toxic fungus that must be proliferated under favourable condition and the moisture content and pH content of the food have been reported as the abiotic factors that affect the fungal infestation. In growth and proliferation of molds in the food, substrate profile may also play a major role (Singh et al, 2008).

As the toxicity and cancer-causing possibility of aflatoxin are much higher,due to this most of the emphasis focussed on the control of the fungi and their lethal metabolites in foods. In cultural practices, the collecting time can be effective to a certain extent in preventing pre-harvest aflatoxin contamination. Paster et al, 1995 stated that "some of the fungicides are effective in preventing the growth of *Aspergillus flavus* in storage especially as a fumigant".

The destruction mechanism of micro-organisms via microwave is controversial. Some states that the micro-organisms inactivation by microwave is generally by the process of heat, through the same mechanism as other biophysical processes induced by heat, such as denaturation of nucleic acids, proteins, vital components as well as membrane disruption (Datta and Davidson, 2000; Heddleson and Doores, 1994).

Woo et al, 2000 studied that the "microwave radiation effect on *E. coli* and *Bacillus subtilis* and reported protein and DNA leakage, damage on cell surface and cell wall and dark spots appearance in bacterial cells due to the result of microwave treatment. And indicated that the microwave treated cells were acting as a ghost cells from which intracellular materials had been released into the cell suspension".

Balbani and Montovani, 2008 stated that the "microwaves are non-ionizing radiation varying between 0.3 and 300 GHz in frequencies (i.e., with 1 meter to 1 mm wavelength, respectively). The most important sources of microwave radiation that humans may encounter are the various types of industrial microwave generators that is used for communications, for boosting the chemical reactions or are used for heating (896 or 915 MHz); cellular phones (824–850, 900, 1800 or 1900 MHz); cordless phones (from 46 to 5800 MHz); microwave ovens (915 and 2450 MHz); UHF radios (from 470 to 890 MHz); dish antennas (from 0.8 to 15 GHz); certain diathermy applicators (915 and 2450 MHz); and traffic radar (10.5 and 24 GHz)".

This study is designed to check the effects of EMR on various growth parameters and toxin production by various species of *Aspergillus.*

CHAPTER 4

SCOPE OF STUDY

Nowadays there exist different types of radiations produced from the mobile phones, Wi-Fi, Bluetooth, microwave etc. which have the harmful impacts on the plants, animals, microorganisms and human being and responsible for many serious disorder such as diabetes, asthma, obesity, cancer and brain dysfunction in humans, and on the other hand, the pure culture of different fungi including *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* which are easily survive in the stored food and grains and can be able to produce different type of mycotoxins. So it is essential to observe the effects of EMR on microbes (different species of fungi *Aspergillus*) including its growth, viability or toxin production. And the other scope of the present study is to review the microwave approaches by which aflatoxins can be reduced or eliminated in the food chain without affecting its nutritive value by reducing the toxicity of fungus (mycelia and spores) in comparison with other methods including heat treatment, gamma rays, chemical methods and UV rays may reduce its nutritive value. This technique can be used to eliminate harmful fungal spores from commercial production of various types of grains and their products.

CHAPTER 5

OBJECTIVE

- To determine the effect of Electromagnetic radiation on the various growth parameters of different species of *Aspergillus.*
- To determine the effect of Electromagnetic radiation on the morphology of different sp. of *Aspergillus*.
- To determine the effect of Electromagnetic radiation on the production of Aflatoxin by different sp. of *Aspergillus.*
- To determine the effect of Electromagnetic radiation on its viability of *Aspergillus* spores.

CHAPTER 6

MATERIALS AND METHODS

6.1. EXPERIMENTAL SITE

The present investigation was carried out in the Lovely Professional University, Phagwara, and Punjab. The laboratory experiment was carried out in the Department of Biotechnology and Biosciences at Lovely Professional University, Punjab.

6.2. MATERIALS

6.2.1. INSTRUMENTS

S.No.	Materials	Company
1.	Glass wares	Borosil Glass
2.	Weighing balance	Adventurer, DHAVS
3.	Autoclave	NSW Pvt. Ltd. India
4.	Hot air oven	NSW Pvt. Ltd., India
5.	Incubator	Yorco Incubator Bacteriological
6.	Laminar air flow	Rescholar Equipment
7.	Microwave	INALSA
8.	Microscope	Magnus
9.	Micropipette	P’Fact A
10.	Micro tips	TARSONS
11.	Orbital shaker	REMI
12.	Klystron microwave test bench	Microwave technologies
13.	Refrigerator	LG
14.	Plastic wares	Poly lab
15.	Weighing balance	Adventurer, DHAVS

6.2.2. MEDIA

S.No.	Media/Chemicals/Stain	Company
1.	Czapek dox broth	CDH
2.	Czapek dox agar	CDH
3.	Potato dextrose broth	CDH
4.	Potato dextrose agar	CDH
5.	Ethanol	Changshu Yangyuan Chemical
6.	Chloramphenicol	HI media
7.	Lacto phenol cotton blue	CDH
8.	Toluene	Loba chemie
9.	Acetonitrile	SDFCL
10.	Isopropanol	Qualikems
11.	Methanol	ChangshuYangyuan Chemical
12.	Ninhydrin	Central Drug House Pvt Ltd
13.	Chloroform	Loba chemie

6.2.3. CULTURES

S.No.	Culture	NCIM No.	MTCC No.
1.	*Aspergillus niger*	501	
2.	*Aspergillus flavus*	519	
3.	*Aspergillus fumigatus*		8877

6.3. METHODOLOGY

6.3.1. FUNGAL CULTIVATION

Figure6.3.1. Pure culture of *A.fumigatus* and *A.flavus*

The microbial species selected for the tests: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus.* The pure culture of *Aspergillus niger and Aspergillus* flavus was obtained from National collection of industrial micro-organism (NCIM No.501 and 519) and pure culture of *Aspergillus fumigatus was obtained from MTCC (MTCC No.8877).* The pure culture of *Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus* were grown in 250 ml in the selective media: Czapek dox broth or potato dextrose broth in incubator at their specific time period of 5-7 days at 28 °c. The obtained pure culture used throughout in this research to evaluate the microbial population. The autoclaved media Czapek dox agar (CDA) or potato dextrose agar (PDA) poured in the petriplates for cultivation of fungi, and inoculated with the fungal mycelia from the obtained culture to the petriplates in laminar system and then the plates were incubated at 28 ºC (B.O.D Incubator) for growth.

6.3.2. RADIATION EXPOSURE

For fungal suspension, the inoculum was prepared from 5-7 days old culture. Test cultures in sterile petridish were exposed to microwave treatment (6.41 GHz; 9.48mm) for 2, 4, 5, 7 and 8 mins respectively and the radiation source is klystron microwave test bench. Untreated inoculum was used as control. After radiation the treated samples were incubated for 5-7 days at 28 ℃.

Figure6.3.2. Klystron microwave test bench

6.3.3. MICROSCOPIC STUDY OF FUNGI

The fungal hyphae of different *Aspergillus* culture after the microwave treatment is measured under the 40X and 100X magnification with microscope and the slides were prepared by using a Lacto phenol Cotton Blue stain with the help of tape method in a laminar air flow system.

6.3.3.1. FUNGAL STAINING

1. Diluted Lactophenol cotton blue stain was used to stain the fungal spores and mycelium.
2. The spores and mycelium was taken with the help of cellotape and placed on drop of stain
3. The fungi was observed on 40 X and 100 X and the spore structure and hyphal details were observed.

6.3.4. METHOD OF AFLATOXIN PRODUCTION

Different microbial strains of *Aspergillus* (A. *niger, A. fumigatus* and *A. flavus)* were grown in potato dextrose or czepkdox medium contained in 100ml of conical flask. Incubation of culture for 5-7 days at 30ºC under the static condition. This method was followed by separation of mycelia sheet from the broth. After that mycelium was subjected to dry weight determination after drying at 55ºC till constant weight was achieved. The solvent required for the extraction of aflatoxin is toluene:acetonitrile (9:1) (Nesheim and Stack, 2001), which was mixed with the broth remaining after removal of mycelia, and kept on shaker for 12 h at room temperature. After the mixing of solvent and broth done completely in shaker, solvent layer separated out from the mixture and measurement of aflatoxin at 350 nm was done.

6.3.5. DETECTION OF AFLATOXIN COMPOUNDS BY TLC

Fifty microliter of extracted samples were applied on thin layer chromoplate and the plate were dissolved in a mixture of solvent (toluene: isopropanol:methanol) in a ratio of 90:32:3. After few times when the desired distance travelled by the solute, the plates were removed from the solvent and air dried before observation under a ultra-violet light. Rf values of blue and green fluorescent spots were determined.

6.3.5.1 THIN LAYER CHROMATOGRAPHY

1. Aflatoxins were analyzed on TLC-glass sheets, precoated with silica gel.

2. Each sample extract containing aflatoxins was loaded on the silica gel plates.

3. The plates were developed in a glass jar containing Toluene:isopropanol:methanol in a ratio of (90:32:3, v/v) as developing solvent**.**

4. Aflatoxin was quantified under UV light.

CHAPTER 7

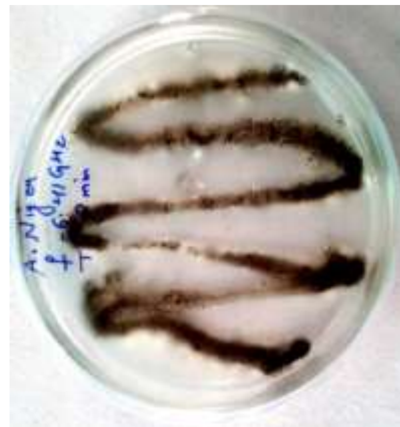
RESULTS AND DISCUSSION

7.1. GROWTH OF MICROBIAL STRAINS

The different fungal strains of *Aspergillus* species (*A.niger, A.flavus and A.fumoigatus*) were obtained from NCIM and MTCC, India. After revival of culture, the fungi were sub cultured on selective growth medium (Czapek dox agar and potato dextrose agar) and then exposed to microwave treatment (Klystron microwave test bench) at a different time period of 2, 4, 5, 7 and 8mins respectively at a frequency of 6.41GHz. The plates were kept for incubation for 5-7 days at 28 ºC.

7.1.1. GROWTH PARAMETERS OF *A.NIGER*

***A.niger* (freq:6.41GHz ; control)**



***A.niger* (freq:6.41GHz ; tim:2min)**

***A.niger* (freq:6.41GHz ; tim:4min)**

***A.niger* (freq:6.41GHz ; tim:5min)**

A.niger **(freq:6.41GHz ; tim:7min)**

A.niger **(freq:6.41GHz ; tim:8min)**

The result indicates that the microwave radiation inhibit the growth rate with increase in the exposure period as shown in figure. The observed variation indicates that the microwave radiation ultimately affects the growth of *Aspergillus niger* at a frequency of 6.41GHz. The gradual decrease was noticed till culture plate of 7 mins but in case of 8min there is no growth of fungi was reported.

7.1.2. GROWTH PARAMETERS OF *A.FLAVUS*

A.flavus **(freq:6.41GHz ; control)**

A.flavus **(freq:6.41GHz ; tim:2min)**

A.flavus **(freq:6.41GHz ; tim:4min)**

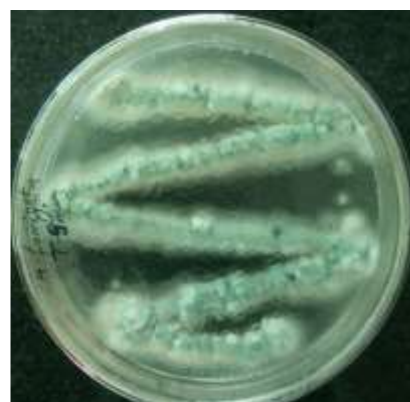
A.flavus **(freq:6.41GHz ; time:5min)**

***A.flavus* (freq:6.41GHz ; tim:7min)** ***A.flavus* (freq:6.41GHz ; time:8min)**

The result indicates that the growth of control plate was less than all treated fungal plates. The mycelial growth was found progressively increasing in case of *Aspergillus flavus.* The plate of 8 min was showing highest mycelial growth but the spore production was not reported that indicated that radiation dose for higher time is suppressive for fungal spores.

7.1.3. GROWTH PARAMETERS OF A.*FUMIGATUS*

***A.fumigatus*(freq:6.41GHz ; control)** ***A.fumigatus*(freq:6.41GHz ; tim:2min)**



***A.fumigatus*(freq:6.41GHz ; tim:4min)** ***A.fumigatus*(freq:6.41GHz ; tim:5min)**

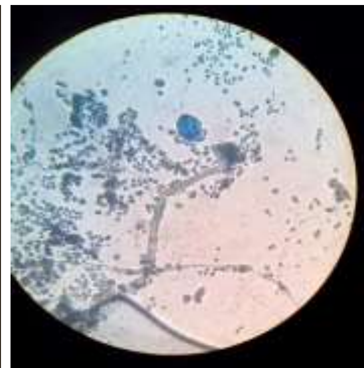
A.fumigatus **(freq:6.41GHz ; tim:7min)** *A.fumigatus* **(freq:6.41GHz; tim:8min)**

The result indicates that mycelial growth and spore formation of *Aspergillus fumigatus* progressively decreasing in all treated samples as compared to control.

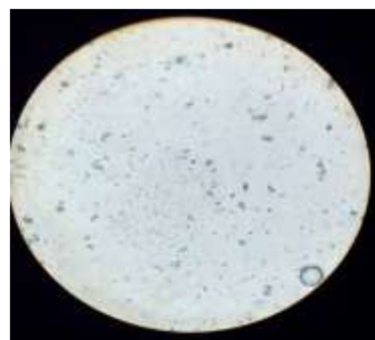
7.2. MICROSCOPIC EXAMINATION OF MICROBIAL STRAINS

The analyses of fungal culture were done via 40X and 100X magnificent lens and the slides were prepared by using Lactophenol cotton blue stain.

7.2.1. Examination of *a.niger*



(freq:6.41GHz ; control) **(freq:6.41GHz ; tim:2min)** **(freq:6.41GHz ; tim:4min)**

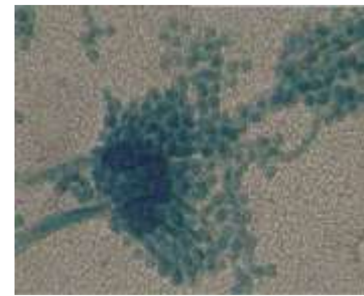


(freq:6.41GHz ; tim:5min) **(freq:6.41GHz ; tim:7min)**

7.2.2. Examination of *A.flavus*

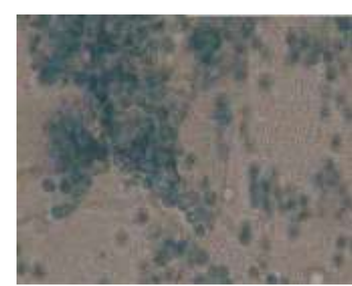
Control

(freq:6.41GHz ; tim:2min)

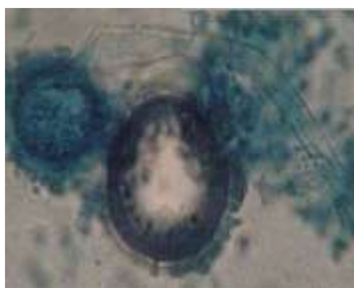

(freq:6.41GHz ; tim:4min)

(freq:6.41GHz ; tim:5min)

(freq:6.41GHz ; tim:7min)


(freq:6.41GHz ; tim:8min)

7.2.3. Examination of *A.fumigatus*


Control

(freq:6.41GHz ; tim:2min)

(freq:6.41GHz ; tim:4min)

(freq:6.41GHz ; tim:5min)

(freq:6.41GHz ; tim:7min)

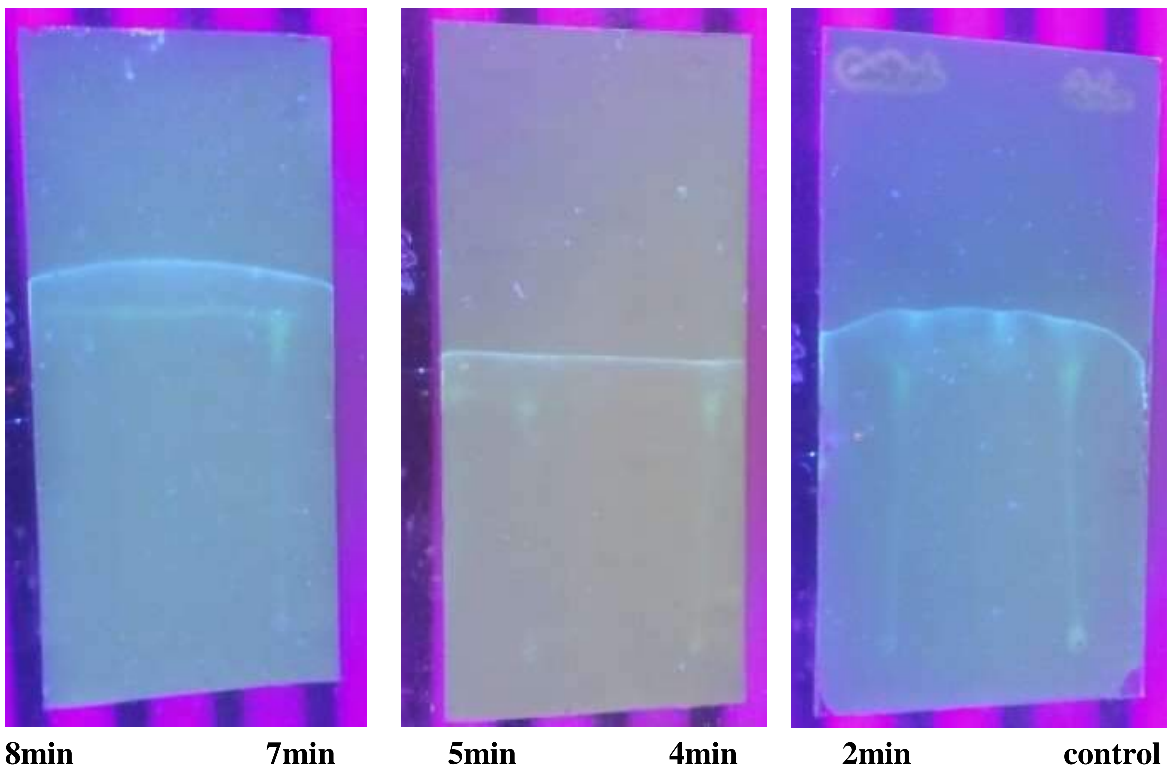
(freq:6.41GHz ; tim:8min)

7.3. EXTRACTION AND ANALYSIS OF AFLATOXIN

7.3.1. Aspergillus niger

8min 7min 5min 4min 2min control

The growth of *A.niger* in liquid media (Czapek dox broth) was showing that moderate growth of mycelium was recorded as the control was not radiated with EMR but the results in other samples were not clearly showing difference in terms of growth approximately. Similar growth was noticed in sample of 2, 4, 5, 7min. The flask of 8min radiated at 6.41GHz was showing only the mycelial growth and no spore production. While all other samples including control are showing prominent spores.



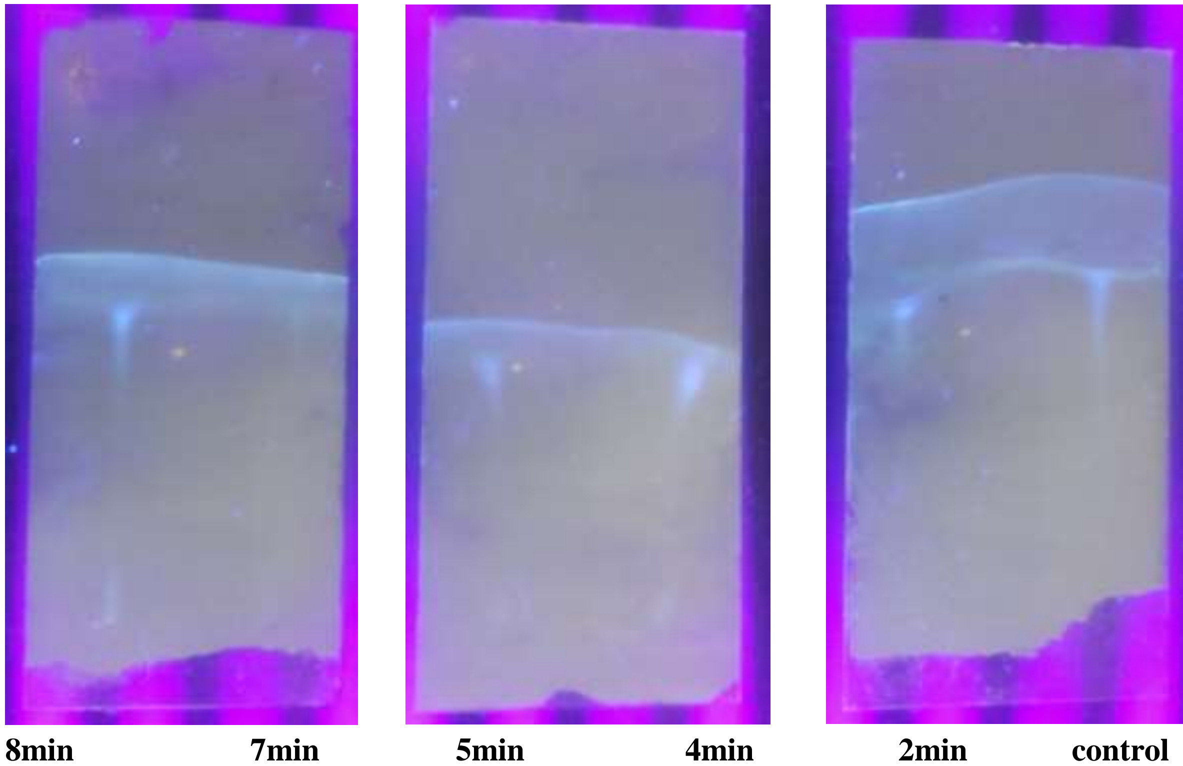
8min 7min 5min 4min 2min control

Thin Layer Chromatography was performed after extracting the aflatoxin compound from the liquid media and the solvent mixture for TLC was Toluene:acetonitrile (9:1). The plates are showing that all the samples are having green florescence spots including control but the sample of 8min was not showing any spots due to no spore formation. And basically it is due to the microwave radiation which inhibits the action of secondary metabolites in the toxic fungi.

7.3.2. Aspergillus fumigatus

Control 2min 4min 5min 7min 8min

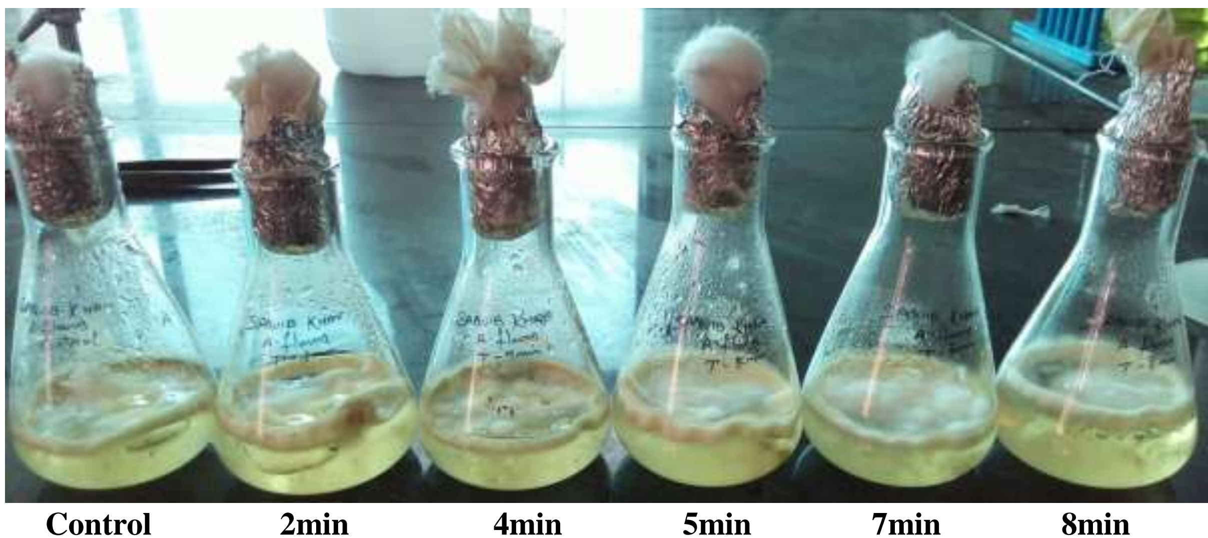
The growth of *A.fumigatus* in potato dextrose broth was recorded less due to the EMR effect as compared to control which was not radiated. But the flask of 7min radiated at 6.41GHz indicating fewer mycelial growth and no spore formation. While the other samples including control are showing less count of spores.



8min 7min 5min 4min 2min control

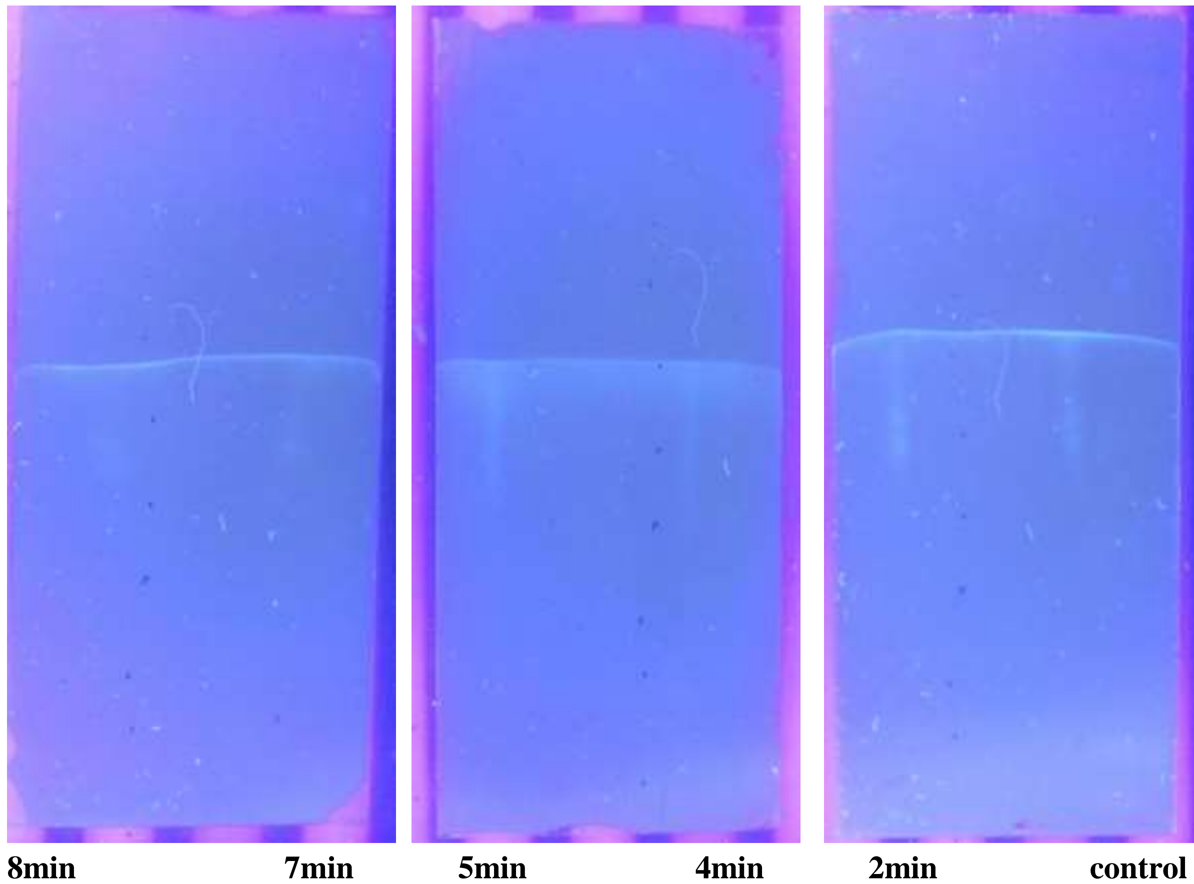
In case of *A. fumigatus* all the samples indicating blue florescence spots including control but in case of 7min sample the toxic compound shows very light floresence spot as shown above due to less mycelial growth and even no spore production.

7.3.3. Aspergillus flavus:



Control 2min 4min 5min 7min 8min

The growth of *A.flavus* in potato dextrose media was showing moderate growth of mycelium in all the samples including control, radiated at a frequency of 6.41GHz but there is no sign of spore production in any of the sample**.**



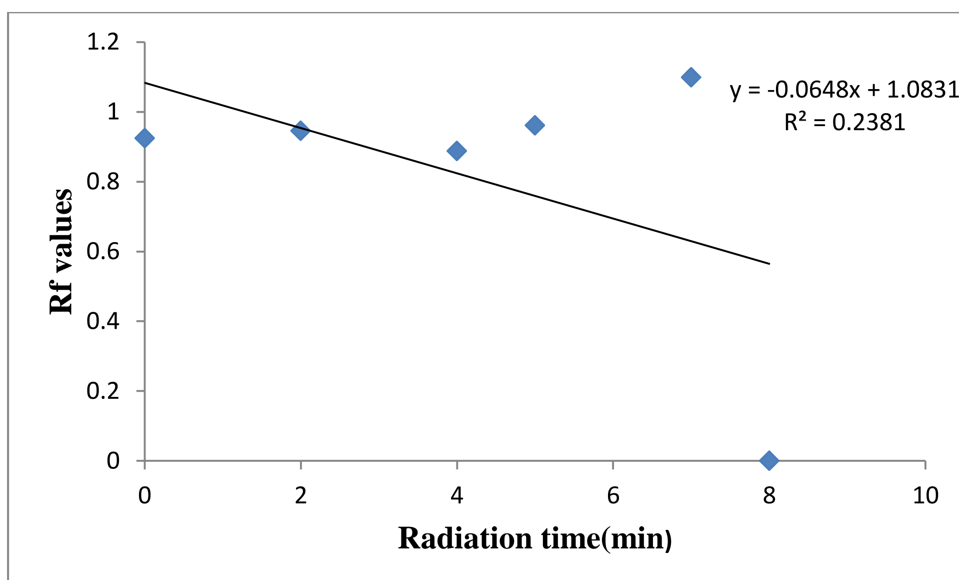
8min 7min 5min 4min 2min control

In case of A.flavus all the samples indicates light blue colour florescence due to less mycelial growth and no spore formation after radiating at a frequency of 6.41GHz.

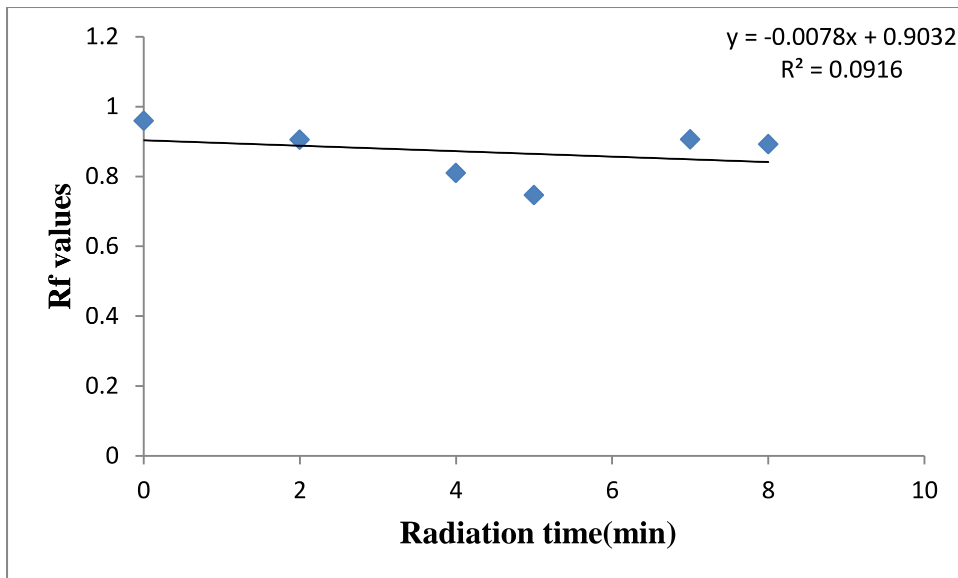
7.4. GRAPHICAL ANALYSIS

Radiation time (min)	Rf values (*Aspergillus niger)*	Rf values (*Aspergillus fumigatus)*	Rf values (*Aspergillus* flavus)
0	0.924	0.959	0.857
2	0.909	0.905	0.887
4	0.887	0.809	0.842
5	0.961	0.746	0.943
7	1.098	0.906	0.945
8	BDL	0.959	0.948

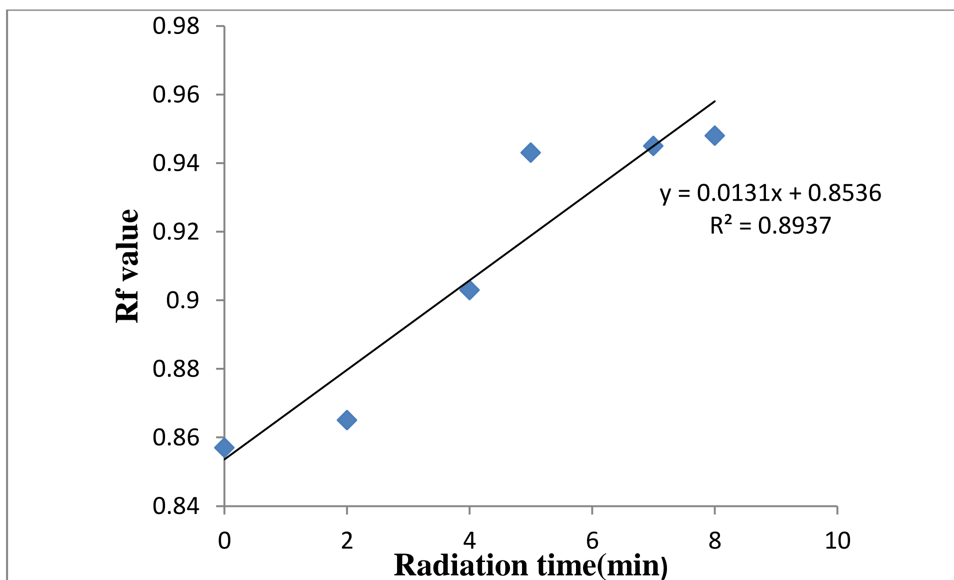
Table7.4.1. Rf values of *Aspergillus* species at different radiation dose



Radiation time Vs Rf values of *Aspergillus niger*



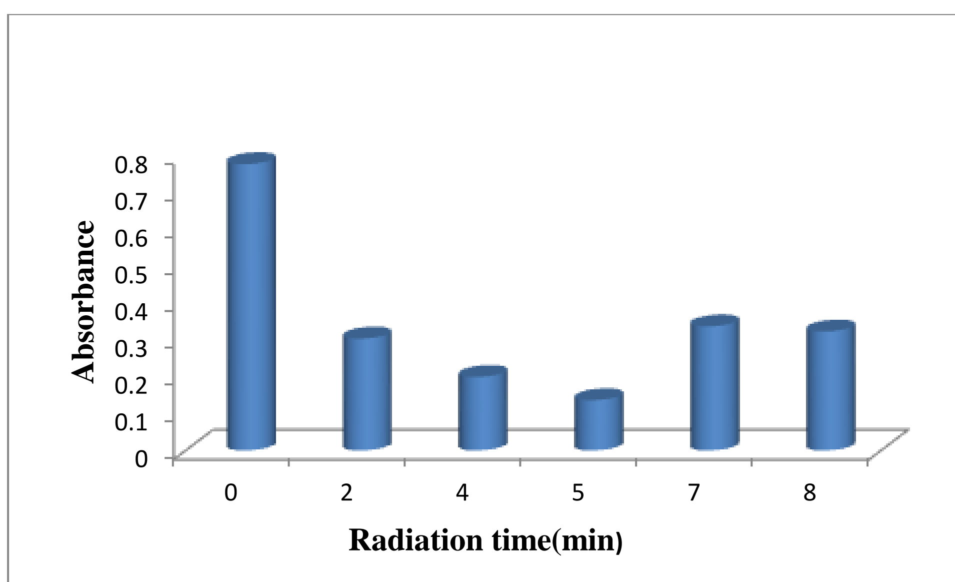
Radiation time Vs Rf values of *Aspergillus fumigatus*



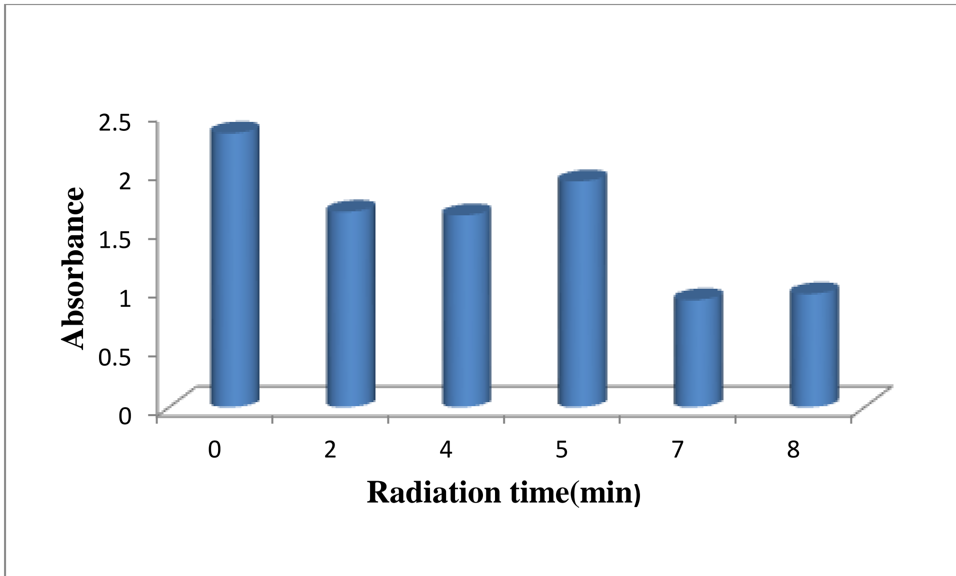
Radiation time Vs Rf values of *Aspergillus flavus*

Radiation time (min)	Absorbance (Aspergillus niger)	Absorbance (Aspergillus fumigatus)	Absorbance (Aspergillus flavus)
0	0.777	2.332	1.075
2	0.304	1.662	0.996
4	0.200	1.631	0.562
5	0.136	1.919	0.680
7	0.337	0.911	0.589
8	0.323	0.962	0.446

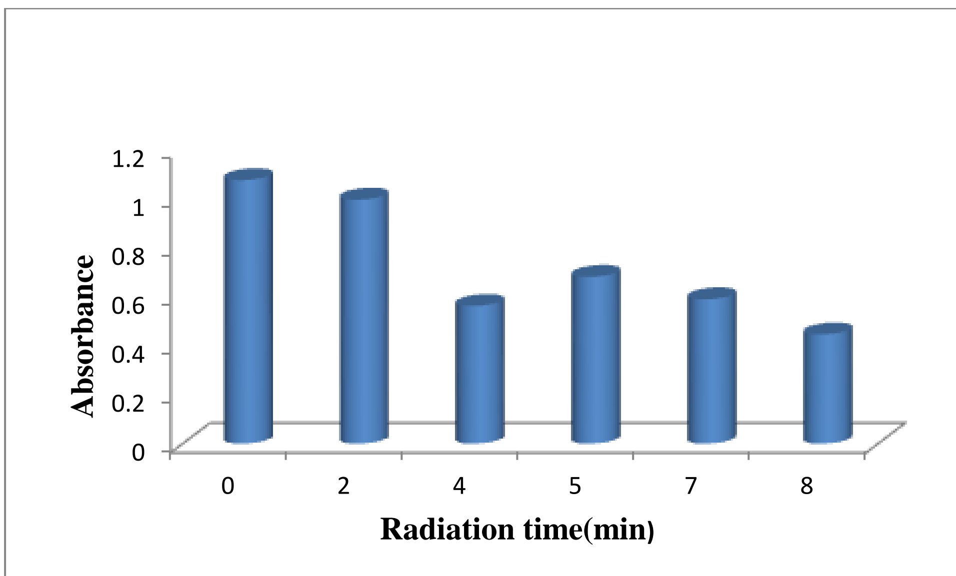
Table7.4.2. Absorbance values of *Aspergillus* species at different radiation dose



Radiation time Vs Absorbance of *Aspergillus niger*



Radiation time Vs Absorbance of *Aspergillus fumigatus*



Radiation time Vs Absorbance of *Aspergillus flavus*

7.5. Effect of MW on aflatoxin production

Duration of MW treatment (min)	*Aspergillus niger*			*Aspergillus fumigatus*			*Aspergillus flavus*		
	Dry weight (gm)	Absorbance	Rf values	Dry weight (gm)	Absorbance	Rf values	Dry weight (gm)	Absorbance	Rf values
0 control	0.732	0.777	0.924	0.187	2.332	0.959	0.563	1.075	0.857
2	0.858	0.304	0.909	0.152	1.662	0.905	0.321	0.996	0.887
4	1.452	0.200	0.887	0.195	1.631	0.809	0.506	0.562	0.842
5	1.635	0.136	0.961	0.155	1.919	0.746	0.719	0.680	0.943
7	1.023	0.337	1.098	0.109	0.911	0.906	0.628	0.589	0.945
8	0.805	0.323	BDL	0.115	0.962	0.892	0.569	0.446	0.948

The above table indicates that the value of dry weight of A.niger control which was not radiated was 0.732gm.The fungal strain was treated with various time periods with EMR of 6.41GHz at 2, 4, 5, 7 and 8min. The results reveals that highest dry weight was obtained on 5min sample and the amount recorded was 1.635.The table shows that giving EMR the biomass of fungi was progressively increases in caser of A.niger.In case of A.fumigatus the dry weght of 4min sample was 0.195gm and found to be the highest among all samples whereas, for A.flavus the highest dry weight evaluation was 0.719gm of 5min radiated sample.The table also indicates that the control sample of A.niger, A.fumigatus and A.flavus was found to 0.777, 2.332 and 1.075 showing the highest absorbance which was not radiated at 6.41GHz. while the other samples are radiated by EMR at a frequency of 6.41GHz at a different time exposure of 2, 4, 5, 7 and 8min respectively. Similarly for Rf values, the 5min sample of A.niger reveals the highest value but in case of A.fumigatus the control was showing the maximum value as compared to others.

Quantification of aflatoxin by Thin Layer Chromatography

Species	**Standard**	**Solvent**	**Floresence under UV light**
Aspergillus niger	Aflatoxin	Toluene:isopropanol:methanol (90:32:3)	Green spots
Aspergillus fumigatus	Aflatoxin	Toluene:isopropanol:methanol (90:32:3)	Blue spots
Aspergillus flavus	Aflatoxin	Toluene:isopropanol:methanol (90:32:3)	Blue spots

DISCUSSIONS

Radiations have different effect on both human being s and microbes and these effects may be positive as well as negative. The purpose of present study was to examine the effects of microwave radiation on the different parameters of *Aspergillus* species. The study concluded that microwave radiations cause changes in the morphology and aflatoxin production of microbes.

The previous research shows that after 6min microwave treatment at a frequency of 2.4 GHz both *A.parasiticus* and *A.flavus* lost their ability for aflatoxin production. However a 2min microwave treatment was found to induce aflatoxin synthesis in A.flavus. The overall growth of *A.parasiticus* was enhanced due to MW treatment, with loss of aflatoxin produation or synthesis, suggesting that growth and aflatoxin production are affected differently by MW exposure. Farag et al. (1996) had reported aflotoxin destruction by MW heating. In their study MW was applied onto pure aflatoxins directly. The rate of aflatoxin destruction was found to increase with the increase of MW power and exposure time. While in present study it has been examined that after irradiating the *A.niger, A.fumigatus* and *A.flavus* at a frequency of 6.41GHz at a various dose of 2, 4, 5, 7 and 8min by MW treatment, the toxicity of the aflatoxin compound was decreased as compared to control.

CHAPTER 8

CONCLUSION

The Thin Layer Chromatography result reveals that when different microbial strains of *Aspergillus niger, Aspergillus fumigatus* and *Aspergillus flavus* are irradiated at a frequency of 6.41GHz at a various time period of 2, 4, 5, 7 and 8 min respectively. The maximum exposure time of 7 and 8 min samples showing no spore count and ultimately affect its toxicity as shown in results, higher dose of radiation on each strains are showing either no florescence spots or very light florescence under UV light as compared to control. In present study the untreated sample was used as control. The study reveals that radiation reduces the aflotoxin production at a higher dose or even helps in reducing the pathogenic effect of fungi, and these EMR can be further used to control the various toxins producing fungus.

Aspergillus species may cause varieties of diseases include (allergic bronchopulmonary aspergillosis, rhinitis, Farmers' lung), superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), and invasive pulmonary and extra pulmonary infection. The species selected for this study is *A. niger, A. flavus* and *A. fumigatus*. Among these *A. niger* being the second most common pathogenic spp. and *A. flavus* and *A. fumigatus* causes Aspergillosis. Some species of *Aspergillus (A.flavus and A.parasiticus*) are able to produce a toxic compound namely aflatoxin which is carcinogenic in nature and these can be easily grown in the laboratory. The mycotoxins occurring in food products are secondary metabolites of the filamentous fungi. These types of toxins contaminate many varieties of food crops throughout the food chain (Reddy et al, 2010). But when it is exposed to electromagnetic radiation (microwave treatment) at a higher dose their toxicity may be inhibited or reduced at significant level, which shows the antifungal activity of EMR on fungi and its toxic metabolites.

CHAPTER 9

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