



Botany Department
Faculty of Science
University of Cairo
Egypt

EFFECT OF RADIATION ON ANTIMYCOTIC ACTIVITY OF SOME DRUGS AND NATURAL PRODUCTS ON *CANDIDA* *ALBICANS*

THESIS
Submitted in Partial fulfillment
Of requirements for award
of the degree of M.Sc.
IN
(Microbiology)

BY
Bassant Mohammed Ibrahim Hashem
(B.Sc.)

Supervisors

Dr. Tarek A. A. Moussa
Associate Professor of Microbiology
Botany Department
Faculty of Science
Cairo University

Prof.Dr. Samir Mostafa Abd El-Aziz
Professor of Radio-Biochemistry
General director of Middle Eastern Regional
Radioisotope Center for the Arab Countries

Dr. Thoraya Mohamed Abdel Hamid
Assistant Professor of Medical Oncology
NCI- Cairo

**Department of Botany
Faculty of Science
University of Cairo
Egypt**

2009

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قالوا سبحانك لا علم لنا الا ما علمتنا انك انت العليم الحكيم))

صدق الله العظيم

Approval sheet

TITLE OF THE M.SC. THESIS:

**EFFECT OF RADIATION ON ANTIMYCOTIC ACTIVITY OF SOME
DRUGS AND NATURAL PRODUCTS ON *CANDIDA ALBICANS***

Name of Candidate:

Bassant Mohammed Ibrahim Hashem

Submitted to:

Botany Department, Faculty of Science, Cairo University

Supervision Committee:

Dr. Tarek A. A. Moussa
Associate Professor of Microbiology
Botany Department
Faculty of Science, Cairo University

Prof.Dr. Samir Mostafa aAbdel El-ziz
Professor of Radio-Biochemistry
General director of Middle Eastern Regional

Dr. Thoraya Mohamed Abdel Hamid
Assistant Professor of Medical Oncology
NCI- Cairo

Prof.Dr. Effat Fahmy Shabana
Head of Department of Botany
Faculty of Science, Cairo University

DECLARATION

**THIS THESIS HAS NOT BEEN PREVIOUSLY
SUBMITTED FOR A DEGREE AT THIS OR AT ANY
OTHER UNIVERSITY AND IS THE ORIGINAL WORK
OF THE WRITER.**

Bassant Mohammed Ibrahim Hashem

DEDICATION

I would like to dedicate this work to my parents and my sisters for their encouragement, putting up with me and supporting me through all this work.

Many thanks to all of them

Bassant mohammed

ACKNOWLEDGEMENT

I am deeply thankful to ALLAH for showing me the right path and helping me to complete this work.

My profound gratitude and appreciation to **Dr. Tarek A. A. Moussa**, Associate Professor of Microbiology, Botany Department, Faculty of Science, Cairo University for suggesting the topics and assessing the work giving much of his time and effort in constructing the data, the practical part of this thesis and revising this work.

I would like to express my appreciation to **Prof.Dr. Samir Mostafa Abd El-Aziz**, Professor of Radio-Biochemistry, General director of Middle Eastern Regional Radioisotope Center for the Arab Countries, for his kind supervision and advices offered to accomplish this work.

I'm thankful to **Dr. Thoraya Mohamed Abdel Hamid**, Assistant Professor of Medical Oncology, NCI- Cairo for her interest in the work presented here many valuable efforts in fulfillment of the medical part of the work.

I would like to express my appreciation to **Prof. Dr. Tahany M. A. Abdel- Rahman**, Professor of Microbiology, Botany Department, Faculty of Science, Cairo University for suggesting the topics, her advices and reading the manuscript.

Finally, my great respect and gratitude to every body who participated in completion of this work.

Bassant mohammed

CONTENTS

	Page
LIST OF TABLES.....	V
LIST OF FIGURES.....	VI
LIST OF APPREVIATIONS.....	VII
ABSTRACT.....	VIII
INTRODUCTION AND AIM OF WORK.....	1
I- REVIEW OF LITERATURE.....	3
II- MATERIALS AND METHODS.....	34
2.1. Patient and lab characteristic.....	34
2.2. Sample collection.....	36
2.3. Isolation and Purification of yeast fungi.....	36
2.4. Identification of isolated yeasts.....	37
2.4.1. Morphological characterization.....	37
2.4.1.1. Morphological characterization on corn meal.....	37
2.4.1.2. Germ tube test.....	37
2.4.1.3. Cycloheximide sensitivity test.....	38
2.4.2. Biochemical characterization.....	38
2.4.2.1. API <i>Candida</i> kit.....	38
2.4.2.1.1. Selection of colonies.....	38
2.4.2.1.2. Preparation of the strip.....	39
2.4.2.1.3. Preparation of the inoculum.....	39
2.4.2.1.4 Inoculation of the strip.....	39
2.4.2.1.5 Reading of the strip.....	39
2.4.2.2 API 20 C AUX.....	40
2.4.2.2.1. Selection of colonies.....	40
2.4.2.2.2 Preparation of the strip.....	40
2.4.2.2.3. Preparation of the inoculum.....	40
2.4.2.2.4. Inoculation of the strip.....	40
2.4.2.2.5. Morphology test.....	40
2.4.2.2.6. Reading the strip.....	41
2.5. Gamma radiation studies.....	41
2.5.1. Source and doses of radiation.....	41
2.5.2. Determination of D ₁₀ -values for <i>Candida albicans</i>	41
2.5.3. γ -Irradiation procedures.....	42

2.5.3.1. Effect of gamma radiation on enzyme activity.....	42
2.5.3.1.1. Production and quantitative assay of proteolytic enzymes activity.....	43
2.5.3.1.2. Production and assay of keratinolytic enzyme activity.....	43
2.5.3.1.3. Production and qualitative assay of lipase enzyme.....	44
2.5.3.2. Effect of γ -radiation on antimycotic activity of drug and natural product.....	44
2.5.3.3 Electron microscope.....	45
2.6. Solar radiation studies.....	46
2.6.1. Effect of solar radiation on growth and enzyme activity.....	46
2.6.2. Effect of solar radiation on antimycotic activity of drug and natural product.....	46
2.6.3. Effect of darkness on growth and enzyme activity.....	46
2.6.4. Effect of darkness on antimycotic activity of drug and natural product.....	47
2.7. Statistical analyses.....	47

III- EXPERIMENTAL RESULTS

1- Patients and lab characteristic.....	48
2-Collection of sample from patients subjected to chemotherapy and radiotherapy.....	50

Identification of isolated yeasts

1- Morphological characterization of isolated yeast.....	54
2- Biochemical characterization.....	69
3- Percentage of pathogenic microorganisms isolated from patients subjected to chemotherapy and radiotherapy.....	73

I- Gamma irradiation process

A- Effect of different gamma radiation doses on growth viability of <i>C. albicans</i>	73
B- Determination of the D ₁₀ -values of <i>C. albicans</i>	75
C- Effect of different gamma irradiation doses on proteolytic and keratinolytic activities of <i>C. albicans</i>	75
D- Effect of different gamma radiation on lipolytic activity in <i>C. albicans</i>	76
E- Effect of different doses of gamma radiation on antimycotic activity of drug and natural product.....	77
D- Effect of gamma radiation on cell structure of <i>C. albicans</i>	80

II- Solar radiation studies

A- Effect of different solar radiation exposure time on cell growth viability of <i>C. albicans</i>	81
B- Effect of solar radiation on proteolytic and keratinolytic activities in <i>C. albicans</i>	82
C- Effect of different solar radiation on lipolytic activity in <i>C. albicans</i>	82

D- Effect of different solar radiation doses on antimycotic activity of drug and natural product.....	83
---	----

III-Effect of darkness

A- Effect of dark periods on cell growth of <i>C. albicans</i>	85
B- Effect of dark periods on proteolytic and keratinolytic activities in <i>C. albicans</i>	86
C- Effect of dark periods on lipolytic activity in <i>C. albicans</i>	87
D- Effect of dark periods on antimycotic activity of drug and natural product.....	88

V- DISCUSSION.....	90
---------------------------	-----------

SUMMARY AND CONCLUSIONS.....	106
-------------------------------------	------------

REFERENCES.....	110
------------------------	------------

ARABIC SUMMARY.....	
----------------------------	--

LIST OF TABLES

	Page
Table 1 : Biochemical reactions of API <i>Candida</i>	38
Table 2 : Numerical table of <i>Candida</i> spp. using API <i>Candida</i>	40
Table 3 : Numerical table of <i>Candida</i> spp. using API 20 AUX.....	41
Table 4 : Patients and lab characteristic.....	48
Table 5 : Number of clinical specimens collected from patients subjected to chemotherapy and radiotherapy.....	51
Table 6 : Number and frequency of positive and negative specimens for microbial growth from different clinical specimens collected from patients subjected to chemotherapy.....	53
Table 7 : Number and frequency of positive and negative specimens for microbial growth from different clinical specimens collected from patients subjected to radiotherapy.....	54
Table 8-A : Morphological characterizations of pathogenic yeasts isolated from patients subjected to chemotherapy.....	55
Table 8-B : Identification of pathogenic yeasts isolated from patient subjected to radiotherapy.....	66
Table 9 : Morphological characterization of isolated yeast organisms.....	69
Table 10-A : API <i>Candida</i> reactions- Numerical profiles of the six isolates.....	70
Table 10-B : API 20 C AUX reactions-Numerical profiles of the six isolates.....	70
Table 10-C : Numerical profiles of API <i>Candida</i> - API 20 C AUX of the six isolates.....	71
Table 11 : List no. of organism and percentage of isolates from patients subjected to chemotherapy and radiotherapy.....	73
Table 12 : Effect of different gamma irradiation doses on cell growth of <i>C. albicans</i> and D ₁₀ determination.....	74
Table 13 : Effect of different doses from gamma radiation on proteolytic and keratinolytic activities in <i>C. albicans</i>	76
Table 14 : Effect of gamma radiation dose on lipolytic activity in <i>C. albicans</i>	77

Table 15	: Effect of different gamma radiation doses on antimycotic activity of drug and natural products and combination of them.....	78
Table 16	: Effect of solar radiation doses on cell growth of <i>C. albicans</i>	81
Table 17	: Effect of solar radiation on proteolytic activity in <i>C. albicans</i>	82
Table 18	: Effect of different solar radiation doses on lipolytic activity in <i>C. albicans</i>	83
Table 19	: Effect of different solar radiation doses on antimycotic activity of drug and natural products and combination of them.....	84
Table 20	: Effect of dark period on cell growth of <i>C. albicans</i>	86
Table 21	: Effect of dark period on proteolytic& keratinolytic activities in <i>C.albicans</i>	87
Table 22	: Effect of dark periods on lipolytic activity in <i>C. albicans</i>	87
Table 23	: Effect of dark periods on antimycotic activity of drug and natural products and combination between them.....	88

LIST OF FIGURES

	Page
Figure 1 : Chronic oral candidiasis of the tongue characteristic with white Pseudomembrane.....	49
Figure 2 : An extensive yeast infection around the lip, with red bumps.....	49
Figure 3 : Chronic oral candidiasis of the tongue and angular- intertrigo and fissuring caused by maceration of the corners of the mouth.....	50
Figure 4 : Percentage of clinical specimens from patients subjected to chemotherapy.....	50
Figure 5 : Percentage of clinical specimens from patients subjected to radiotherapy.....	51
Figure 6 : Percentage of positive and negative specimens for microbial growth of different clinical specimens isolated from patients subjected to chemotherapy.....	52
Figure 7 : Percentage of positive and negative specimens for microbial growth of different clinical specimens isolated from patients subjected to radiotherapy.....	53
Figure 8 : Numerical profiles of API <i>Candida</i> of the six isolates. (A); <i>Candida albicans</i> (7512), (B); <i>Candida tropicalis</i> (7510), (C); <i>Candida parapsilosis</i> (7000), (D); <i>Candida krusei</i> (1000), (E); <i>Candida norvogenesis</i> (3000), (F); <i>Saccharomyces cerevisiae</i> (7310).....	72
Figure 9 : Inhibition zone diameter of diflucan and clove oil after exposure to gamma radiation. (A); non irradiated diflucan and clove oil as control (B); diflucan and clove oil after 0.5 KGy (C); diflucan and clove oil after 1 KGy (D); diflucan and clove oil after 2 KGy (E); Combination between Diflucan and clove oil (control, 0.5, 1 and 2 KGy).....	79
Figure 10 : Figure 10A. represent the untreated cells of <i>Candida albicans</i> Figure 10B. represent the effect of lethal dose on <i>Candida albicans</i>	80
Figure 11 : Inhibition zone of diflucan, colve oil and combination between them after exposed to solar radiation. (A); diflucan and colve oil after 2 solar days, (B); diflucan and colve oil after 4 & 8 solar days, (C); diflucan and colve oil after 12 solar days, (D); combination between diflucan and colve oil after each solar days.....	85
Figure 12 : Effect of dark periods on antimycotic activity of diflucan , clove oil and combination between them.....	89

LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ALL	Acute Lymphatic Leukemia
AML	Acute Myloid Leukemia
Amp B	Amphotericin B
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
BSA	Bovine serum albumin
CDC	Chronic disseminated candidiasis
CFU	Cell Forming Unit
CG	Centi -Gray
CLSI	The Clinical and Laboratory Standard Institute
CM	Cell membrane
CMC	Chronic mucocutaneous candidiasis
CR	Complete remission
CW	Cell wall
DM	Diabetes mellitus
ELBW	Extremely low-birth weight
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSE	Grape seed extract
Gy	Gray
HAART	The pre-highly active antiretroviral therapy
HAM regimen	High dose cytarabine plus mitoxantron
HIV	Human immunodeficiency virus
HSV	herpes simplex virus

ICU	Intensive care unit
m	Mitochondria
MSC	Multiple-species candidemia
n	Nucleus
NCI	National Cancer institute
nu	Nucleolus
OPC	Oral pseudomembranous candidosis
PL	Phospholipase
PL A₂	Phospholipase A ₂
RAT	Rice Agar Tween medium
RHE	Reconstituted human oral epithelium
Sap	Secretes aspartic proteinase
Saps	Secretes aspartic proteinases
SBA	Sabouraud dextrose agar medium
TLC	Total leukocytic count
UTIs	Urinary tract infections
Vac	Vacuole
VLBW	Very low-birth weight
VVC	Vulvovaginal candidiasis
YED	Yeast extract dextrose medium

Abstract

A total of 133 clinical specimen were collected from 108 patients subjected to chemotherapy and 17 patients subjected to radiotherapy. 81 Tongue specimens (77 from chemotherapy and 4 from radiotherapy with ratio 66.4%, 23.5% respectively) 11 Buccal mucosa specimens (9 from chemotherapy, 2 from radiotherapy with ratio 7.8%, 11.8 % respectively) specimens of labial, Sputum and Gum were collected only from chemotherapy with number 10, 17 and 3 specimens and ratio 8.6%, 14.6% and 2.6% respectively, and the specimens of throat and hard palate were collected only from radiotherapy with number 9 and 2 with ratio 52.9%, 11.7% respectively. The identification of isolates was done by morphological characterization, Biochemical characterization using API *Candida* kit. The selected isolate for further study was *Candida albicans* because it was the most permanent isolate. The lethal dose was 10 kGy and the calculated D₁₀-value obtained was 1.5 kGy.

Electron microscopy was made to the cells of *C. albicans* subjected to γ -radiation. The cells of *C. albicans* showed deformation in shape and the cell wall of many cells was ruptured and their internal contents showed complete lyses. Growth ceased at 12-days exposure while dark periods had a clear positive effect on cell growth of *C. albicans* causing the cell growth increased with increasing period of incubation in dark reaching maximum growth at 12 days compared with that control.

Proteolytic, keratinolytic and lipolytic activity of *C. albicans* were inhibited by γ - doses up to 8 kGy. While they had been decreased with increase in solar doses, till it ceased at 12- day solarization. But this activities stimulated by incubation in dark up to 12 days.

Diffucan and clove oil as natural product were exposed to different doses of γ -radiation as follow (0.5, 1 and 2 KGy). The inhibition zones were recorded for *C. albicans* with each drugs treatment when using alone and in combination with each other. γ -irradiation had an inhibitory effect on diflucan alone but there was not effect on clove oil. On other hand, the combination between diflucan and clove oil showed a decrease in inhibition zone. Diflucan activity highly decreased to reach a minimum value after 12 days but a clove oil was slightly decreased reaching minimum value after 12 days solar radiation. On other hand the combination between irradiated diflucan and clove oil showed slightly high activity than control after 4 days exposure.

The antimicrobial activity of diflucan, colve oil and combination between them against *C. albicans* were not affected by storage each of them in dark for 24 days.

Key words: *Candida albicans*, gamma and solar radiation, dark condition, keratinolytic activity, proteolytic activity, lipolytic activity, electron microscope, diflucan, clove oil, drug combination