

# PHYSIOLOGICAL STUDIES ON THE EFFECTS OF BRASSINOLIDE ON NIGELLA SATIVA L.

THESIS SUBMITTED FOR THE MASTER DEGREE IN SCIENCE TEACHER'S PREPARATION (BOTANY)

BY

## **HEBA EBRAHIM MOHAMED**

General Diploma in Preparation of Science Teacher (Botany-2001) Special Diploma in Preparation of Science Teacher (Botany-2002)

#### SUPERVISED BY

Dr. Amin Erfan Dowidar Professor of plant physiology Faculty of Education Ain Shams University

Dr. Ali Abdel-Aziz. Abdallah Assoc. Prof. of plant physiology Faculty of Education Ain Shams University Dr. Salwa Mohamed Abbas Lecture of plant physiology Faculty of Education Ain Shams University

2005



#### APPROVAL SHEET

Name: Heba Ebrahim Mohamed Ebrahim

Title: PHYSIOLOGICAL STUDIES ON THE EFFECTS OF BRASSINOLIDE ON NIGELLA SATIVA L.

## **Supervisors:**

### Dr. Amin Erfan Dowidar

Professor of plant physiology Department of Biological and Geological Sciences Faculty of Education Ain Shams University

## Dr. Ali Abdel-Aziz Abdallah

Assoc. Prof. of plant physiology Department of Biological and Geological Sciences Faculty of Education Ain Shams University

## Dr. Salwa Mohamed Abbas

Lecturer of plant physiology Department of Biological and Geological Sciences Faculty of Education Ain Shams University



#### ACKNOWLEDGEMENT

First of all thanks to God for giving me the strength to fulfill this hard mission.

I wish to express my sincere appreciation and deep gratitude to **Dr. Amin Erfan Dowidar**, Professor of Plant Physiology, Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University for his kind supervision, suggesting the problem, fruitful help, patriotic patience, energetic guidance and conclusive instructions throughout the course of this investigation.

Great thanks are due to **Dr. Ali Abdel- Aziz Abdallah**, Associate Professor of Plant Physiology, Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University for his kind supervision, suggesting the problem, unlimited support on both scientific and personal levels and for his sincere guidance from the very beginning and up to writing the manuscript.

Abbas, Lecturer of Plant Physiology, Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University for her kind supervision, suggesting the problem, continuous material and moral support, valuable advices and being a very effective factor in driving over this work successfully.

Thanks are extended to **Dr. Ehab Abdel Razik Kamel**, Associate Professor of Genetics,
Department of Biological and Geological Sciences, Faculty
of Education, Ain Shams University for kindly offering all
the facilities of his laboratory for the biochemical genetic
studies continous encouragement and valuable remarks.

I am greatly indebted to **Dr. Tahani Hathoot**, Professor of Plant Physiology, Botany Department, Faculty of Grils, Ain Shams University for doing her best in solving the problem of the chemical used (brassinolide) in this investigation.

I would like to express my deep thanks and gratitude to all the members of Biological and Geological Science for their valuable help during the course of this investigation especially **Prof Dr. Mohamed Abdel Hameed Shahin**, Chairman of Biological and Geological Sciences, Faculty of Education, Ain Shams University for providing facilities during the practical work.

#### ABSTRACT

In the present investigation, Nigella sativa seeds cv. Egyptian and Syrian were presoaked in the different concentrations of brassinolide (0.0025, 0.05 and 1.0 ppm). The effect of brassinolide on growth, certain metabolic activities, rate of respiration, protein pattern, cytological criteria and the cell division of the produced seedlings of the two cultivars after 10 days from sowing were studied. The results revealed that, the low concentrations of brassinolide (0.0025 and 0.05 ppm) caused high significant increases in the growth criteria, total carbohydrates, total nitrogen, total protein, nucleic acid contents (DNA & RNA), the respiratory rate and the rate of cell division (Mitotic index). While the high concentration of brassinolide caused high significant reduction in these parameters. The percentage of unsaturated fatty acids, oil contents and iodine value were increased when the low concentrations of brassinolide were used, while a reverse pattern was observed in seedlings by using the high concentration of brassinolide.

Also, in this investigation, the effect of brassinolide on the two cultivars of *Nigella sativa* plants produced from the seed previously presoaked in the different concentrations of brassinolide or treated with foliar spraying were studied. The results revealed that, the low concentrations of brassinolide caused high significant increases in the morphological criteria, yield components, photosynthetic pigments, total carbohydrates, total nitrogen, total protein and nucleic acid contents of the two cultivars of *Nigella sativa* plants at all stages of plant growth and development. While the highest concentration (1.0 ppm) of brassinolide resulted in a reduction in these parameters.

Key words: Nigella sativa, Brassinolide, growth, yield, metabolism

## CONTENTS

Title	Page
• LIST OF TABLES.	I
• LIST OF FIGURES.	V
• LIST OF PLATES.	IX
• INTRODUCTION.	1
* Effect on Growth and Yield Components.	4
I-Growth Parameters.	4
II-Yield Components.	8
* Effect on Photosynthetic Pigments.	10
* Effect on Carbohydrate Contents.	13
* Effect on Nitrogen Contents.	14
* Effect on Protein.	15
* Effect on Nucleic Acid Contents.	17
* Effect on Seedlings Protein Electrophoresis.	17
* Effect on Fatty Acids.	18
* Effect on Respiration.	18
* Aim of the Present Work.	19
• MATERIALS AND METHODS.	20
• MATERIALS.	20
• TIME COURSE EXPERIMENTS.	20
• GERMINATION EXPERIMENT	20
POT EXPERIMENT	21
• METHODS.	22
* Estimation of Photosynthetic Pigments.	22
* Determination of Total Carbohydrate.	23
* Determination of Total Nitrogen and Protein	25
- Estimation of Total Nitrogen.	25
* Quantitative Estimation of Nucleic Acids.	28
- Procedure.	28
- Ribonucleic acid content.	29
<ul> <li>Deoxyribonucleic acid content.</li> </ul>	29

Title	Page
* Protein Electrophoresis.	32
- Buffers and Solution.	32
- Protein Extraction.	34
- Gel preparation.	34
- Electrophoretic Procedure.	35
- Recording Results.	35
* Determination of Oil Content.	36
* Determination of Fatty Acids by GLS.	36
* Iodine Value.	37
* . Determination of Respiratory Quotient.	38
* Cytology.	39
- Reagents.	39
- Cytological Procedures.	40
- Karyotype Technique.	42
• EXPERIMENTAL RESULTS:	45
Changes in Growth Criteria, Metabolites,	45
Respiration and Protein Electrophoresis of the	
seedlings produced from Germination of Nigella	
sativa cv. Egyptian and Syrian seeds in response	
to Their Pre-emergence treatment with the	
different concentrations of brassinolide.	
* Changes in Growth Criteria.	45
* Changes in some metabolites.	47
- Total Carbohydrates.	47
- Total Nitrogen.	47
- Total Protein.	47
- Nucleic Acid Contents.	50
- Electrophoretic Pattern of the Seedlings Protein.	50
- Fatty Acids Composition.	57
- Iodine Value:	58

Title	Page
- Seed Oil %.	62
- Changes in Respiration.	62
* Cytological studies:	66
a-Frequency of Mitotic Phases.	66
b-Effect on Mitotic Index (M.I.).	67
c-Frequency and Types of Abnormal Mitotic Phases.	69
d- Karyotype.	79
• Effect of Soaking The Seeds of <i>Nigella sativa</i> In	87
Brassinolide On Growth and Some Metabolic	07
Activities.	
* Changes in Morphological Criteria.	87
* Changes in Some Metabolites:	91
- Photosynthetic Pigments.	91
- Carbohydrate Contents.	95
- Nitrogen Contents.	96
- Protein Contents:	96
- Nucleic Acid Contents.	97
• Effect of Spraying Nigella sativa Plants With	102
Brassinolide On.Growth and Some Metabolic	
Activities.	
* Changes in Morphological Criteria.	102
* Changes in Some Metabolites:	106
- Photosynthetic Pigments.	106
- Carbohydrate Contents.	110
- Nitrogen Contents.	111
- Protein Contents.	111
- Nucleic Acid Contents.	111
• ANALYSIS OF HARVESTED SEEDS	116
• DISCUSSION AND CONCLUSION.	120
• ENGLISH SUMMARY.	140

## **CONTENTS**

• REFFERENCES.	144
• ARABIC SUMMARY.	1

## **ABBREVIATIONS**

Abbreviations	Meaning
A.O.A.C.	Association of Official Analytical Chemistis
A∖Bis	Acrylamide\bisacrylamide
Aps	Ammonium persulphate
BDH	British Drug House
BRs	Brassinosteroids
cv.	Cultivar
DEGS	Di ethylglycol succsinate
DNA	Deoxyribonucleic acid
DPA	Diphenylamine
GLS	Gas Liquid Chromatography
KDa	Kilodalton
L.S.D.	Least significant difference
M.I	Mitotic index
M.S.I.	Mitotic index stage
M.Wt	Molecular weight
MAR	Mean Arm Ratio
ppm	Part per million
RM	Mobility rate
RNA	Ribonucleic acid
SDS-PAGE	Sodium dodecyl sulphate polyacrelamide gel electrophoresis
TAR	Total Arm Ratio
TCA	Trichloroacetic acid
TCL	Total Chromosome Length
TEMED	Tetramethylethylene diamine
Tris\Hcl	Tris hydroxyl methylamine hydrochloride

# LIST OF FIGURES

No.	Title	Page
1	Standard curve of glucose.	24
2	Standard curve for the determination of total nitrogen.	27
3	Standard curve of RNA.	30
4	Standard curve of DNA.	31
5	Effect of pre-soaking seeds in the different concentrations of brassinolide on the total carbohydrate, nitrogen, protein and nucleic acid contents of Nigella sativa cv. Egyptian and Syrian seedlings.	49
6	Electrophoretic banding profiles of the protein extracted in Tris HCl buffer produced from the germinated seeds of Nigella sativa cv. Egyptian in response to their pre-emergence treatment with the different concentrations of brassinolide.	53
7	The scanning profile of the proteins of the seedlings produced from the germination of Nigella sativa cv. Egyptian in response to their pre-emergence treatment with the different concentrations of brassinolide.	54
8	Electrophoretic banding profiles of the protein extracted in Tris HCl buffer produced from the germinated seeds of Nigella sativa cv. Syrian in response to their pre-emergence treatment with the different concentrations of brassinolide.	55

## LIST OF FIGURES

No.	Title	Page
9	The scanning profile of the proteins of the seedlings produced from the germination of Nigella sativa cv. Syrian in response to their preemergence treatment with the different concentrations of brassinolide.	56
10	Effect of different concentrations of brassinolide on Fatty acids composition of Nigella sativa seeds cv. Egyptian after 10 days of germination.	60
11	Effect of different concentrations of brassinolide on Fatty acids composition of Nigella sativa seeds cv. Syrian after 10 days of germination	61
12	Effect of different concentrations of brassinolide on the rate of respiration of Nigella sativa cv. Egyptian and Syrian during germination and seedlings growth.	65
13	Effect of pre-soaking seeds in the different concentrations of brassinolide on the photosynthetic pigments of leaves of Nigella sativa cv. Egyptian plants at three stages of growth.	93
14	Effect of pre-soaking seeds in the different concentrations of brassinolide on the photosynthetic pigments of leaves of Nigella sativa cv. Syrian plants at three stages of growth.	94

## LIST OF FIGURES

No.	Title	Page
15	Effect of pre-soaking seeds in the different concentrations of brassinolide on the carbohydrate, nitrogen and protein contents in shoots of Nigella sativa cv. Egyptian and Syrian plants at three stages of growth.	100
16	Effect of pre-soaking seeds in the different concentrations of brassinolide on the nucleic acids contents in shoots of Nigella sativa cv. Egyptian and Syrian plants at three stages of growth.	101
17	Effect of foliar spray with the different concentrations of brassinolide on the photosynthetic pigments of leaves of Nigella sativa cv. Egyptian plants at three stages of growth.	108
18	Effect of foliar spray with the different concentrations of brassinolide on the photosynthetic pigments of leaves of Nigella sativa cv. Syrian plants at three stages of growth.	109
19	Effect of foliar spray with the different concentrations of brassinolide on the carbohydrate, nitrogen and protein contents in shoots of Nigella sativa cv. Egyptian and Syrian plants at three stages of growth.	114