USE OF TISSUE CULTURE IN THE PRODUCTION OF SECONDARY METABOLITES FROM CHICORY (*Cichorium intybus*) PLANT

By

MARWA OTHMAN MOHAMED SAYED
B.Sc. Agric. Sc. (Plant production), Faculty of Agric., Ain Shams University, 2014

A Thesis Submitted in Partial Fulfillment

Of

The Requirement for the Degree of

MASTER OF SCIENCE
in
Agricultural Sciences
(Ornamental, Medicinal and Aromatic Plants)

Department of Horticulture

Faculty of Agriculture

Ain Shams University

2020

Approval Sheet

USE OF TISSUE CULTURE IN THE PRODUCTION OF SECONDARY METABOLITES FROM CHICORY (*Cichorium intybus*) PLANT

By

MARWA OTHMAN MOHAMED SAYED

B.Sc. Agric. Sc. (Plant production), Faculty of Agric., Ain Shams University, 2014

This Thesis for M.Sc. Degree has been approved by

Dr. Hekmat Yehia Ahmed Massoud **…….....**
Professor Emeritus of Ornamental and Medicinal Plants, Faculty of Agriculture, Mansoura University.

Dr. Asmaa Mohammed Mohammed Abd El-Gyed **…….....**
Associate Professor of Ornamental, Medicinal and Aromatic Plants, Faculty of Agriculture, Ain Shams University.

Dr. Abdelaziz Mohamed Hosni **…….....**
Professor Emeritus of Ornamental, Medicinal and Aromatic Plants, Faculty of Agriculture, Ain Shams University.

Dr. Laila Mohamed Helmi **…….....**
Professor Emeritus of Ornamental, Medicinal and Aromatic Plants, Faculty of Agriculture, Ain Shams University.

Date of Examination: 22/12/2019

USE OF TISSUE CULTURE IN THE PRODUCTION OF SECONDARY METABOLITES FROM CHICORY (*Cichorium intybus*) PLANT

By

MARWA OTHMAN MOHAMED SAYED

B.Sc. Agric. Sc. (Plant production), Faculty of Agric., Ain Shams University, 2014

Under the Supervision of

Dr. Laila Mohamed Helmi

Professor Emeritus of Ornamental, Medicinal and Aromatic Plants, Horticulture Department, Faculty of Agriculture, Ain Shams University. (Principal Supervisor)

Dr. Abdelaziz Mohamed Hosni

Professor Emeritus of Ornamental, Medicinal and Aromatic Plants, Horticulture Department, Faculty of Agriculture, Ain Shams University.

ABSTRACT

Marwa Othman Mohamed Sayed: Use of Tissue Culture in the Production of Secondary Metabolites from Chicory (*Cichorium intybus*) Plant. Unpublished M.Sc. Thesis, Horticulture Department, Faculty of Agriculture, Ain Shams University, 2020.

All experiments of this research study were carried out in the plant tissue culture laboratory of the Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, Shoubra El-kheima, Cairo, Egypt. Experiments on chicory plant were executed for the duration of two consecutive years 2017 and 2018. Chicory (*Cichorium intybus* L.), which belongs to Asteraceae family, is considered as an important medicinal plant due to the presence inside it of many bioactive substances such flavonoids, phenolic compounds, alkaloids, steroids, terpenoids, coumarines, cichoriin, esculetin, inulin, sesquiterpene lactones, chicoric acid, caffeic acid and some vitamins.

In this research, seed sterilization process aseptically was accomplished using three sterilizers namely mercuric chloride, Clorox and hydrogen peroxide in various concentrations and periods (0.1 % for 3-5 min., 10 % for 10 min and 12 % for 20 min), respectively. Clorox showed the best antiseptic effect with the lowest contamination percentages (2.5 % and 3.8 %). *In vitro* germination of chicory seeds used as initial explants were carried out using agar and half-strength Murashige and Skoog (MS) media to grow aseptic seedlings to provide for leaf explants from which callus is induced. Half-strength MS medium was the best in terms of the seedling growth rate (leaf number, width and length).

For friable callus induction, chicory leaf explants were abaxially inoculated on the surface of full-strength MS medium supplemented with different combinations of five plant growth regulators; NAA at 0.5 – 2.0 – 3.0 – 5.0 mg/l, IBA at 0.5 – 2.0 mg/l, 2,4-D at 3.0 – 5.0 mg/l, BA at 0.4 – 0.5 – 0.75 – 1.5 – 2.0 mg/l and IAA at 0.5 – 2.0 mg/l. Only MS medium

supplemented with NAA alone or combined with IBA produced the desirable friable callus and the optimum callus fresh weights were obtained with NAA at 3 mg/l plus IBA at 2 mg/l under total dark incubation condition.

Regarding callus content of phenolics, flavonoids and guaiacol peroxidase activity enzyme, NAA at 2 mg/l was the superior treatment. Meanwhile, reducing sugars, total free amino acids and polyphenol oxidase activity were increased with NAA at 5 mg/l plus IBA at 2 mg/l treatment. Moreover, the superior treatment for total soluble protein content and phenylalanine ammonia lyase activity was NAA at 3 mg/l plus IBA at 2 mg/l under total dark condition.

Keywords: *Cichorium intybus* L., Chicory, Asteraceae, Naphthalene acetic acid, NAA, Indole -3 -butyric acid, IBA, Auxin, Callus, *In vitro* culture, MS medium, Phenolic compounds, Flavonoids, Amino acids, Proteins, Reducing sugars, Enzyme activity, PAL, POD, PPO.

ACKNOWLEDGEMENT

First, praise be to **Allah**, the Lord of the Worlds, and may blessings and peace of **Allah** be upon the most honored of messengers our prophet **Muhammad** and upon all his family members and companions. Also, thanks and gratitude are extended to **Allah**, who guided me to his path and assisted me in all my life.

I would like to express my deep gratitude and everlasting thanks to Dr. **Abdelaziz Mohamed Hosni,** Prof. Emeritus of ornamental, medicinal and aromatic plants, Dept. of Horticulture, Fac. of Agriculture, Ain Shams Univ., who proposed the work plan of this study and provided me encouragement, guidance and continuous support from the start to the final step of my M.Sc. study and in writing and reviewing the manuscript. I would also like to thank him for motivation, which has enabled the production of this thesis. All words and feeling are not enough to express how grateful I am to him.

I wish to express my appreciation and gratitude to Dr. **Laila Mohamed Helmi,** Prof. Emeritus of ornamental, medicinal and aromatic plants, Dept. of Horticulture, Fac. of Agriculture, Ain Shams Univ., for her constructive criticism and helpful suggestions.

I would like to express my sincere appreciation to Mr. **Mohamed Mahmoud Aboul Fotouh Mesalhi,** assistant lecturer of agricultural biochemistry, Dept. of Agricultural Biochemistry, Mr. **Anas Mohammed Mahmoud Mohammed Salama,** assistant lecturer of soil fertility and plant nutrition, Dept. of Soil Sciences and Mr. **Ali Mohammed Mohammed Abd-Elkader,** assistant lecturer of plant physiology, Dept. of Agricultural Botany, Fac. of Agriculture, Ain Shams Univ., each of which provided me with the necessary support to complete the chemical and statistical analyses.

Also, I would never forget Dr. **Sabry Mousa Soliman Youssef Mehana,** Prof. of vegetable crops, Dept. of Horticulture, Fac. of

Agriculture, Ain Shams Univ., for his help, guidance, advice and inspiring words when needed.

I would like to express my gratitude to all the members of the plant tissue culture lab., Agricultural Botany Department, Faculty of Agriculture, Ain Shams University for providing required facilities and instruments for the *in vitro* experiments.

Last but not least, my deepest gratitude is offered to my parents and my grandmother for all the care, kindness, encouragement and support provided by them. This work could not have been concluded with success without their patience and understanding.

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES