EFFECT OF IRRADIATION ON INTERNAL STRUCTURE AND ACTIVE INGREDIENT OF *ECHINACEA PURPUREA*

By

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B.Sc. Agric. Sc. (Horticulture), Ain Shams University, 2004
M.Sc. Agric. Sci. (Agric. Botany), Ain Shams University, 2009

A Thesis Submitted in Partial Fulfillment
Of
The Requirement for the Degree of

DOCTOR OF PHILOSOPHY
in

Agriculture Sciences
(Agricultural Botany)

Department of Agricultural Botany
Faculty of Agriculture
Ain Shams University

2016

Approval Sheet

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ABSTRACT

Mona Shaban Abd EL Aal: Effect of Irradiation on Internal Structure and Active Ingredient of *Echinacea purpurea*. Unpublished Ph.D. Thesis, Department of Agriculture Botany, Faculty of Agriculture, Ain Shams University, 2016.

Series of experiments were carried out successively during the years of 2013 to 2015. The effects of UV-C, UV-B and gamma radiation on the anatomical structure of *Echinacea* seedling and biochemical components of callus and cell suspension were investigated. The results indicated that incubation periods and UV-C treatments were effective on growth parameters and chemical components. It is obvious that growth parameters & guaiacol peroxidase (G-POD) activity in callus and cell suspension cultures in addition to caffeic acid derivatives & phenylalanine ammonia lyase (PAL) in cell suspension and non-enzymatic antioxidant activity in callus culture increased by increasing incubation period and the reverse was true for caffeic acid in callus. On the other hand, incubation period 48 hours was the most influence in total phenols content and PAL activity of callus while, incubation period 72 hours achieved the highest increment in total phenols content and antioxidant activity in cell suspension culture.

All UV-C treatments led to an increment in caffeic acid & antioxidant activity of callus cells and growth parameters, total phenols content & antioxidant activity of cell suspension and the reverse was true for G-POD activity in callus and cell suspension cultures comparing to the control. On the other side, the two exposure times 30 & 60 min led to increasing callus growth and total phenols while exposure time 60 min detected an increment in caffeic acid of cell suspension and PAL activity in the two types of cultures as compared to the control.

UV-B led to an increment of all growth parameters and antioxidant activity in callus and cell suspension and caffeic acid

derivatives in cell suspension by increasing incubation period and the reverse was true for G-POD activity in cell suspension and PAL activity in both types of cultures. While, incubation period 2 weeks was more effective in caffeic acid, total phenols and G-POD activity in callus cells and incubation period one week only for total phenols in cell suspension.

Apart from the effect of second exposure time (4 hours) on fresh weight and growth index of cell suspension, UV-B led to an increment of all growth parameters in the two types of cultures and G-POD activity in callus cells and reverse was true for G-POD activity in cell suspension & PAL activity in callus cells comparing to the control. On the other side, the two exposure times 2 & 4 hours increased antioxidant activity in the two types of cultures. Meanwhile, exposure time 2 hours led to increase caffeic acid and total phenols in callus cells, while, the maximum increase in caffeic acid, total phenols and PAL activity in cell suspension was achieved by 4 hours exposure time. Likewise, using 2 UV-B lamps for 2 hours was the most effective in creating more biochemical components.

Significant increase in fresh weight and growth index of irradiated callus was detected with the doses 40 and 50 Gy of gamma. On the contrary, the dry weight decreased significantly in all gamma treatments against the control, in addition to caffeic acid derivatives, total phenols and antioxidant activity. The reverse was true for enzymes activity G-POD & PAL and irradiated callus with 20 Gy recorded the highest activity values of G-POD and PAL. Also, gamma caused changes in the internal structure of seedling such as harmful of the shoot apex. Also, it affected in the cotyledonary leaf whereas the leaf shape changed and decreased in thickness. The hypocotyl showed decreasing in the cells growth, alteration in the shape and damage or necrosis of its epidermal cells and perception of large size nuclei. Changes in the size and density of the chloroplasts in foliage leaf mesophyll. Decreasing in number of xylem and phloem vessels in the secondary vascular tissues.

Key wards: *Echinacea purpurea,* UV-C, UV-B, gamma, *in vitro,* caffeic acid derivatives, enzymes.

ACKNOWLEDGEMENT

First, I am deeply thankful to GOD, by grace of home, the present work was released.

I would like to express my deep gratitude and everlasting thanks to Prof. Dr. Kawthar Aly Emam Rabie, Prof. Emeritus of Plant Physiology, Faculty of Agriculture, Ain Shams University for her support, supervision, guidance and continuous encouragement, words are not enough to express how grateful I am to her.

I would like also to express my sincere appreciations to Prof., Dr. Osama Khalil Abo El Atta, Prof. Emeritus of Agricultural Botany, Faculty of Agriculture, Ain Shams University for his keen interest, valuable suggestions, and respective contribution during the different stages of this work.

I would like also to express the deepest appreciation to Dr. Hossam Hassan Ahmed Manaf Associate Prof. of Plant Physiology, Faculty of Agriculture, Ain Shams University, for his excellent guidance and facilities in this work.

Thanks are also extended to Dr. Ola Hussein Abd-Elbar Associate Prof. of Agricultural Botany, Faculty of Agriculture, Ain Shams University, for her help in this work.

Thanks should be extended to all faculty members of Agric. Botany Department, Faculty of Agric., Ain Shams University for their continuous encouragement especially Mr. Gamal Shaban and Mr. Aly Mohammed for their help to complete this work.

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INTRODUCTION

Purple coneflower *Echinacea purpurea* (L.) Moench is an herbaceous perennial plant and a member of daisy family Asteraceae (Composite), distributed in North America in the United States and south central Canada in dry prairies and Rocky mountains **(Binns *et al.*, 2004).** In Europe, The major producers are in Germany, Spain, Switzerland, The Netherlands, and Italy **(Galambosi, 2004).** The genus *Echinacea* is represented by 11 taxa. Three species of *Echinacea* are generally used in medicinal plant preparations **(Perry *et al.*, 2001)** *Echinacea purpurea* (L.) Moench (roots and shoots), *Echinacea angustifolia* DC (roots), and *Echinacea pallida* Nutt (roots) *Echinacea purpurea* is the most widespread species **(McGregor, 1968)** and the most widely cultivated medicinal species of the genus **(McKeown, 1999).**

Echinacea species, especially *E. purpurea* contain a variety of chemical compounds (216 different medicinally active compounds) that are structurally diverse, for example, cichoric acid, alkylamides, echinacoside, cinarine, and polysaccharides **(Murch *et al.*, 2006).** Known phenolic compounds in *Echinacea* species include caffeic acid derivatives such as cichoric acid (the main active compound) in *E. purpurea* and *E. pallida*, and echinacoside in *E. angustifolia* **(Harborne and Williams, 2004).** *Echinacea* used as a dietary supplement increased markedly, it has gained international popularity due to claims that it beneficially stimulates the body's immune system (**Bauer and Wagner, 1991; Barrett, 2003).** Extracts from the plant have shown antioxidant activities **(Thygesen *et al.*, 2007),** antibacterial, antiviral and antifungal properties, and are used in the treatment of the common cold, as well as respiratory and urinary diseases **(Barrett, 2003).**

Plant tissue culture techniques have become a powerful tool for studying basic and applied problems in plant biology. Furthermore, these techniques have found wide commercial application in the propagation of

plants, mainly horticulture species. Success in the technology and application of *in vitro* methods is due to a better understanding of the nutritional requirements of cultured cells and tissues **(Murashige and Skoog, 1962).** *Echinacea* species have been increasingly studied for different applications in biotechnology, especially in vitro culture and preparation of natural extracts **(Abbasi *et al.*, 2007).** *Echinacea* species have been regenerated from a range of tissue types varying from *in vitro* seedlings to mature field-grown plants, with a number of studies having described the biomass production by *in vitro* culture of *Echinacea* species **(Lata *et al.*, 2004).** In addition, several studies have focused on the isolation and characterization of different classes of compounds responsible for the multiple activities of *Echinacea* extracts **(Currier and Miller, 2000; Wills and Stuart, 2000; Merali *et al.*, 2003).**

Plant *in vitro* cultures are able to produce and accumulate many medicinally valuable secondary metabolites. Efforts have focused on the stimulation of biosynthetic activities of cultured cells using various strategies such as, improving culture conditions, using selection producer cells, addition precursor and biotransformation, elicitation and stress induced production, transformation hairy roots and never-ending bioreactor (**Matkowski, 2008)** Biotic and abiotic elicitors which are classified on their origin has been considered as effective way to stimulate secondary metabolite due to both plant defense mechanism and metabolite production are interrelated via secondary metabolism**. (DiCosmo and Tallevi, 1985; Eilert, 1987; Barz *et al.*, 1988).**

Ultraviolet-B radiation is one important environmental factor that in many cases induces the production of secondary metabolites. UV-C irradiation is an important factor that can act as a switch, controlling expression of specific genes involved in cell growth and secondary metabolism of plants **(Versari *et al.*, 2001).**