IN VITRO STUDIES ON PROPAGATION OF DIFFERENT VARIETIES OF *Rosmarinus officinalis* (L.), PLANT AND EFFECT OF SOME TREATMENTS ON GROWTH AND ACTIVE INGREDIENTS

By

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B.Sc. Agric. Sci. (Ornamental Horticulture), Fac. Agric., Cairo Univ., 2003
M.Sc. Agric. Sci. (Ornamental Horticulture), Fac. Agric., Cairo Univ., 2011

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Ornamental Horticulture)

Department of Ornamental Horticulture
Faculty of Agriculture
Cairo University
EGYPT

2018

Format Reviewer **Vice Dean of Graduate Studies**

APPROVAL SHEET

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SUPPERVISION SHEET

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Title of Thesis: *In Vitro* Studies on Propagation of Different Varieties of *Rosmarinus ofiicinalis* (L.) Plant and Effect of Some Treatments on Growth and Active Ingredients.

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Department: Ornamental Horticulture

Branch: **Approval: / / 2018**

ABSTRACT

This investigation was carried out during the period from 2013 till 2017 at Ornamental Horticulture Department, Fac. of Agric., Cairo Univ. Giza, Egypt. The practical part was done at Plant Tissue Culture Research Lab., Hortic. Inst., Agriculture Research Center, Giza. Egypt.

This study aimed to: studying and recording the morphological and chemical differences between all rosemary cultivars under study *Rosemarinus officinalis* L. C1, *R. officinalis* L. *Pyramidalis*" C2 and *R. officinalis* L. *Angustifolius* C3 by measuring some variability's of all cultivars, determine successful cutting percentage, evaluation essential oil percentage, determine volatile oil composition. it was found that C2 had the highest leave length, leave width and the highest number of axillary shoots also it had the highest percentage of succeed cuttings with 100%. The highest volatile oil was 0.95% for C3 and the main components was verbenon. Study the most suitable protocol of micropropagation for each cultivar by examine the best sterilization method then evaluate the best type of PGR on callus and shoot formation when using alone or in combination then evaluate the best medium type and leave position for production then evaluate using *m*TR on elongation and investigate the best auxins with medium concentration for rooting then evaluate the proper medium for plant adaptation. The most efficient sterilization method was by using Lemon juice and H_2O_2 which resulted in 100% sterilized explants with C2. It was found that, the highest callus weight was obtained from C1 by 7.22 g resulted from 2.0 mg/l CPPU while the highest No. of shoots was obtained from C3 by 12shoot/explants resulted from 2.0mg/l CPPU. Concerning combined PGRs it was found that the highest callus weight was obtained from C3 by 7.91 g at 2.0 K+0.1 2,4-D mg/l while the highest No. of shoots was produced from C3 at 2.0 CPPU + 0. 1 2,4-D mg/l by 12 shoot/explants. For medium type, it was found that the highest callus weight was obtained from C2 by 9.02g from MS medium+1 mg/l CPPU while the highest No. of shoots was obtained from C3 by 18.3shoot/explant from WPM+1 mg/l CPPU. For leave position the highest callus weight for C1 was 6.29 g obtained from terminal leaves explants cultured on MS media supplemented with 2mg/l CPPU while the highest number of shoots produced from C3 by 7.0 shoot/explants from basal leaves cultured on MS medium supplemented with 3.0 mg/l CPPU. For shoot elongation experiment it was found that 0.5 mg**l** *m*TR showed highest rate of growth for C3 by 2.1cm with healthy green shootlets. For rooting experiment, it was found that both C1 and C2 can produce roots without need to auxines while half MS supplemented with 1.0 mg/**l** NAA was suitable to enhance root formation for C3. The best acclimatization medium was sand medium. Determine the salt stress effect on callus formation, shoot formation and volatile oil composition all cultivars, the highest callus weight was 8.55g for C2 at 1000 µM/l, the highest No. of shoot 21.76 for C3 the highest volatiles component was Bornyl acetate for all. Investigate the ability producing polyploidy plant by using Colchicine to improve C3 it was found 0.3 mg/l colchince for 14 days can produce polyploidy plant

Key words: Rosemary, *Rosemarinus officinalis* L, "Upright Rosemary" , "Pine scented", Volatil oil, *In vitro*, Lemon juice, H_2O_2, Nano silver $AgNo_3$, PGRs, CPPU, TDZ.

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my mother spirit (Soumayiah Abd Alrahman Eid) who spent her efforts to brought me up on ethics and to be hard worker Allso my father (Mohammad Mousad Eid) for his support and help all the time, as well as to all my family (Eman Shaeheene, Mohammad Magdy Mohammad, Riham Mohammad, Mohga Mohammad, Karim Mohammad, Omar Mohammad) for all the support they lovely offered along the period of my post graduation.

ACKNOWLEDGEMENT

Before all and always, I thank ***Allah*** *whom give me the ability and power to do all that work which I wish to come bake with benefit on people and humanity*

I wish to express my sincere thanks, deepest gratitude and appreciation to ***Dr. Salwa Salem Sakr*** *Professor of Ornamental Plants, Faculty of Agriculture, Cairo University for suggesting the problem, supervision all over the work, revision and correction the main script of this thesis.*

Thanks due to ***Dr. Ayman Yhia Amin*** *Assistant Professor of Ornamental Plants, Faculty of Agriculture, Cairo University for supervision of this work. Deeper thanks for* ***Dr. El Mewafy Abdo El mewafy*** *Professor of Medicinal and Aromatic Plants, Agriculture Research Center for supervision, continued assistance and their guidance and revision the manuscript of this thesis. Sincere thanks to* ***Dr. Sherif Saeed*** *Researcher at Plant Tissue Culture Laboratory, Agriculture Research Center for his support and help with all tools.*

Grateful appreciation also is extended to ***Dr. Essam Abdel kader*** *the head of the lab and all staff member of Plant Tissue Culture Laboratory, (A.R.C.), and* ***Dr. Mohamed Farag*** *Proffessor of druges science and the head of Natural Products Lab at faculty of Pharmacy, Cairo Univ.*

My special deep appreciation and thanks for my sisters and brothers ***Mohammad Magdy, Reham, Mohaga, Kareem and Omar,*** *my step mother* ***Eman shahin***

I present my deepest grateful thanks and love to my late lovely ***Mam*** *and my lovely* ***Dad****.*

ABREVIATIONS

$AgNo_3$	Silver nitrate
C1	Cultivar No1 *Rosmarinus officinalis* (L.)
C2	Cultivar No 2 *Rosmarinus officinalis* (L.) *Pyramidalis* " Upright Rosemary"
C3	Cultivar No 2 *Rosmarinus officinalis* (L.) *Angustifolius* "Pine scented"
CPPU	Forchlorfenuron
BAP	6-Benzylaminopurine or benzyl adenine
2,4-D	2,4-dichlorophenoxyacetic acid
DW	Dry weight
ETOH	Ethanol alcohol
FW	Fresh weight
GA_3	Gibberellic acid
GC-MS	Gas chromatography–mass spectrometry
$HgCl_2$	Mercury (II) chloride or mercuric chloride
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
IAA	Indol acetic acid
IBA	Indole-3-butyric acid
2iP	Isopentenyl-adenine
K	Kinetin
KOH	Potassium hydroxide
MS	Murashige and Skoog media 1962
MSMO	(Murashige and Skoog Minima Organics)
*m*T	*meta*-Toplin

*m*TR	*meta*-Toplin riboside
NAA	α-Naphthalene acetic acid
NaOCl	Sodium hypochlorite
NaCl	Sodium chloride
NS	Nano Silver
PVP	Polyvinylpyrrolidone
PGRs	Plant growth regulators
Sec.	Second
TDZ	Thidiazuron
WPM	McCown Woody plant medium (1980)