# PHYSIOLOGICAL, PHYTOCHEMICAL AND BIOTECHNOLOGICAL STUDIES ON ANISE (*Pimpinella anisum* L.) PLANT

**By**

**DALIA ABDEL HALIM MOHAMED ABDEL HALIM**
**B.Sc. Agric. Sci. (Biotechnology), Fac. Agric.,Cairo Univ., Egypt, 2005**
**M.Sc. Agric. Sci. (Plant physiology), Fac. Agric.,Cairo Univ.,Egypt, 2011**

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## APPROVAL SHEET

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APPROVAL COMMITTEE

**Dr. MOHAMED NASR EL-DEIN HELALY** ……
**Professor of Agricultural Botany, Fac. Agric., Mansoura University**

**Dr. EGLAL MOHAMED ZAKI HARB**……..……
**Professor of Plant Physiology, Fac. Agric., Cairo University**

**Dr. MOHAMED KHALIL KHALIL** ……..……
**Professor of Plant Physiology, Fac. Agric., Cairo University**

**Date: 23 /3/2017**

SUPERVISION SHEET

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SUPERVISION COMMITTEE

**Dr. MOHAMED KHALIL KHALIL**
**Professor of Plant Physiology, Fac. Agric., Cairo University**

**Dr. ABEER ABDEL-RAHMAN MAHMOUD**
**Assistant Professor of Plant Physiology, Fac. Agric., Cairo University**

**Dr. GAMAL MAHMOUD EL-SAID GHAZAL**
**Assistant Professor of medicinal plants and natural products, (NODCAR), Ministry of Health**

**Name of Candidate:** Dalia Abdel Halim Mohamed Abdel Halim **Degree:** Ph.D.
**Title of Thesis:** Physiological, phytochemical and biotechnological studies on anise (*Pimpinella anisum* L.) plant
**Supervisors:** Dr. Mohamed Khalil Khalil
Dr. Abeer abdel-Rahman Mahmoud
Dr. Gamal Mahmoud El-Said Ghazal
**Department:** Agricultural Botany
**Branch:** Plant Physiology **Approval:**23/3/ 2017

## ABSTRACT

The present work aimed to study the effect of inoculation *Pimpinella anisum* L. seeds with biofertilizer [arbuscular mycorrhizal fungi (my) and/or microbein (mi) ] and/or plants were spray with Thidiazuron (TDZ) combined with chemical fertilizer at half or full dose of NPK on number of spores Am fungi per kg soil, AM fungi colonization , enzymatic activities (dehydrogenase & Nitrogenase), growth parameters , chemical composition and active constituents . Also, some molecular studies (protein electrophoresis) were studied.

This work was carried out during two successive seasons 2012-2013 and 2013-2014 at Biotechnology Department, Phytochemistry Department and Farm of Applied Research Center of Medicinal Plants (ARCMP) affiliated to the National Organization for Drug Control And Research (NODCAR), ministry of health, Egypt. The most important results could be summarized as follow:

The results in both seasons showed that, the highest values of number of AM fungi spores per kg soil in anise (*Pimpinella anisum* L.) roots, AM fungi colonization %, enzymatic activities, growth parameters**,** chemical composition and active constituent obtained at inoculated seeds with mixture of mycorrhizal spores and mycrobein at full dose of NPK.

The profile of SDS-PAGE showed appearance and disappearance of some protein bands occurred after chemical , bio fertilizers and foliar sprayed by TDZ of anise plant compared to the control. The highest number of bands (up to 19 bands) were observed with the inoculated anise (*Pimpinella anisum* L.) seeds with mixture of mycorrhizal and microbein.

**Key words**. Anise, *Pimpinella anisum* L., chemical fertilizer, biofertilizer, mycorrhizae, microbein, TDZ.

# *DEDICATION*

*I dedicate this work to whom my heartfelt thanks; to my father Dr. Abdel Halim Sallam , my mother, my husband Amr Basha , my son Malek , my brothers Amr and Walid and my friends Samir Sabry and Nora Ahmed for all the support they lovely offered along the period of my post graduation.*

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| | |
|---|---|
| a.i. | Active ingredient |
| AM | Arbscular Mycorrhizae |
| ARCMP | Applied Research Center of Medicinal Plants |
| Caro. | Carotenoides |
| Chl. A | Chlorophyll a |
| Chl. B | Chlorophyll b |
| Cm | Centimeter |
| DAP | Day after planting |
| dd | distilled deionized |
| DPPH | 2,2'-diphenyl,1-picryl hydrazyl |
| Fad | Fadden |
| FM or FYM | Farmyard manure |
| FRAP | Ferric reducing ability of plasma |
| F. W. | Fresh weight |
| g | Gram |
| $GA_3$ | Gibberellic acid |
| GAE | Gallic acid equivalents |
| Ha | Hectare |
| IAA | Indole acetic acid |
| IBA | Indole butyric acid |
| K | Potassium |
| KDa | Kilo dalton |

| | |
|---|---|
| Kin. | Kintein |
| ml | Milli liter |
| mM | Milli Mole |
| M. W. | Molecular weight |
| N | Nitrogen |
| NODCAR | National Organization for Drug Control And Research |
| PGPR | Plants growth promoting rhizobacteria |
| P | Phosphorus |
| Ppb | Part per billion |
| Ppm | Part per milllion |
| PSB | Phosphate solublizing bacteria |
| PTZ | Pentylenentetrazol |
| RBC | Red blood cells |
| TDZ | Thiodiazuran |
| TPF | Triphenyl formazon |
| TTC | Triphenyltetrazolium |
| VAM | Vesicular arbscular mycorrhizae |

# INTRODUCTION

*Pimpinella anisum* L. (Apiaceae)is an annual herb, grassy with white flowers and small green to yellow seeds, which grows in the Mediterranean region, India and many other worm regions in the world. *Pimpinella anisum* L. is primarily grown for its fruits (seeds) that are currently used for flavoring and for different purposes (Rajeshwari *et al*., 2011[b]). The essential oil from *Pimpinella anisum* L. seeds is used in food preparation, traditional medicine and perfumery industry. Shojaii and Fard, (2012) reported that anise seeds has several therapeutic effects on several conditions such as digestive, neurologic, cough (Haggag *et al*., 2003) and respiratory disorders. The essential oil is obtained by steam distillation of seeds and varies between 1.5% - 6% v/w and contains mainly trans-anethole (80-95%), also contains much smaller amounts of estragole, cis-anethole, p-anisaldehyde and pseudoisoeugenyl-2-methylbutyrate (Hänsel *et al.*, 1994).

Anise seeds are used in middle east as appetizer and is specially known for its digestive properties (Amad *et al*., 2011). Anise seeds extract of water when consumed after meals helps in the process of digestion. Among the reported pharmacological effects we can find anise extracts active as antiulcer (Al Mofleh *et al*., 2007 and Robles *et al*., 2011), Antispasmodic (Tirapelli *et al*., 2007) , Antibiotic (Al-Bayati, 2008) , Performance enhancement (immunomodulation) (Durrani *et al*., 2007), Insecticidal (Burgess *et al*., 2010).

A biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surface, or soil, colonizes the rhizosphere or the anterior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Gaur, 2010).

Biofertilizers are supposed to be safe alternative to chemical fertilizers to minimize the ecological disturbance. Biofertilizers are cost effective, eco-friendly and when they are required in bulk, can be generated at the farm itself. They increase crop yield by 10-40% and fix nitrogen up to 40-50%. They improve soil texture, pH, and other properties of soil (Anonymous, 2012).

Benefit of biofertilizers were cheap source of nutrients , suppliers of microelements, suppliers of micro nutrients, Suppliers of organic matter , counteracting negative impact of chemical fertilizers and secretion of growth hormones (Gaur, 2010).

Thiodiazuran was classified as a type of cytokinin that induces many responses that were similar to the responses induced by natural cytokinins. TDZ, unlike traditional phytohormones, individual fulfilled the requirements of various regenerative responses of many different plant species. The morpho-regulatory potential of TDZ has led to its application in plant tissue culture for the development of feasible morphogenetic systems. TDZ emerged as an effective bioregulant in cell and tissue cultures in wide array of plant species (Li *et al*., 2000; Hosseini and Rashid, 2000; Svetla *et al*., 2003 and Matand and Prakash, 2007).