BANAT'S UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE "KING MICHAEL I OF ROMANIA "REGELE MIHAI I AL ROMÂNIEI„ OF TIMIŞOARA

FACULTY OF AGRICULTURE

CRISTIAN FLORIN RUS

DOCTORAL THESIS

SUMMARY

A STUDY OF ACTIVE PRINCIPLES OBTAINED FROM MEDICINAL PLANTS FROM THE FAMILY *LAMIACEAE* ON CEREAL FUNGI

CONDUCĂTOR ŞTIINŢIFIC :

PROF. UNIV. DR. POP GEORGETA

2016

ABSTRACT

Structure of the Thesis

This doctoral thesis has two parts: Part I – Synthesis of literature data, covering the chapters I: General aspects of the studied medicinal plants, II: General considerations on essential oils; and Part II: Personal contributions, covering the chapters III: Objectives. Justification of the theme. Material and method, IV: Results and discussion; and a final chapter of Conclusions followed by Annexes and References.

Keywords: Medicinal plants, essential oils, minimum inhibitory concentration, Minimum fungal concentration, Chemical composition, Essential oil yield, Drying yield

Hypothesis

This doctoral thesis approaches one of the main issues for agriculturists: controlling fungi to reduce cereal loss from storehouses.

The concern for the quality of raw vegetal products is increasing nowadays. Avoiding the use of chemical compounds in phyto-sanitary treatments is the main way of ensuring products not contaminated by pesticide residues. To do so, they are looking for alternative solutions that ensure the protection of biological crops. Together with other phyto-compounds, essential oils are among the most promising solutions for the replacement of synthesis antifungal and antimicrobial substances used at present.

Volatile oils are mainly liquids volatile at environmental temperature, colourless, yellow and more rarely coloured (blue as those of chamomile and yarrow), with subunit density (except for the oil of cloves and cinnamon); they boil at 150-300^{0}C and can be removed with water vapours. They act as condiments and have an antiseptic, antispasmodic and stomachic role. Chemically, volatile oils are mixtures of substances made up of terpene hydrocarbons, aromatic hydrocarbons, alcohols, aldehydes, ketones, organic acids, pigments, ethers, and esters. [81]

Verticillium dahliae and *Penicillium aurantiogriseum* are fungi specific to cereals and are hard to control even with special synthesis antifungal substances. These two fungi species cause considerable cereal losses in storehouses. This is why it is important to test the antifungal action of essential oils from these medicinal plants on these types of fungi.

In this context, we believe that the theme of this doctoral thesis – "A Study of Active Principles Obtained from Medicinal Plants from the Family Lamiaceae on Cereal Fungi" – focuses on the identification and confirmation of ecological variants for the protection, proper storage and reduction of cereal loss from storehouses.

Objective of the Thesis

This thesis presents an in vitro (controlled conditions in a laboratory) study of the antifungal potential of essential oils from medicinal plant species such as *Thymus vulgaris* L., *Thymus serpyllum* L., *Satureja montana* L., *Origanum majorana* L., *Origanum vulgare* L. and *Salvia officinalis* L. from the Family Lamiaceae.

***Main Goals*:**

- Determination of chemical composition of the studied essential oils;
- Determination of minimum inhibitory concentration of the studied essential oils on *Verticillium dahliae* and *Penicillium aurantiogriseum* fungi;
- Determination of minimum fungicidal concentration of the studied essential oils on the fungi *Verticillium dahliae* and *Penicillium aurantiogriseum*.

***Secondary Goals*:**

- Determination of the chemical primary composition and the micro- and macro-element content in the vegetal material;
- Determining the drying yield;
- Determining the essential oil yield.

Materials and Method

Studied medicinal plant species: *Thymus vulgaris* L., *Thymus serpyllum* L., *Satureja montana* L., *Origanum majorana* L., *Origanum vulgare* L. and *Salvia officinalis* L.

Tested fungi: *Verticillium dahliae* and *Penicillium aurantiogriseum.*

The work method consisted of harvesting the vegetal material, determining the primary chemical composition and the micro- and macro-nutrients in the fresh vegetal material, drying the vegetal material, extracting the essential oil, determining the chemical composition of essential oils, preparing culture media with essential oils, inoculating fungi on culture media with essential oils of different concentrations, evaluating fungi growth after nine days, and re-inoculating undeveloped fungi on fresh medium without essential oils to determine fungal concentration.

The studied medicinal plants (*Salvia officinalis* L., *Origanum vulgare* L., *Origanum majorana* L., *Thymus vulgaris* L., *Thymus serpyllum* L. and *Satureja montana* L.) were planted in the field on May 1, 2011. Harvesting for extracting essential oils was done in 2014 upon the blooming of 50% of the flowers.

Determining the content of lipids, proteins and carbohydrates was done using the official methods AOAC (AOAC, 1997): protein (method 954.01), lipid (method 920.39),

ashes (method 923.03) and moisture (method 925.09). Carbohydrate content was determined through the difference to other macronutrients. Each result is the mean value of three essays.

Essential oils were obtained through continuous distilling (Soxhlet extraction).

Components: boiling balloon, balloon for the storage of vegetal material, cooling column and separator. The boiling balloon and the storage balloon have a capacity of 2 l.

The chemical composition of essential oils was determined with a mass spectrometer gas chromatograph (GC/MS). We used the chromatograph Agilent Technology 7820A (Agilent Scientific, USA) coupled with a mass spectrometer MSD 5975 equipped with a capillary column with the characteristics: DB 5 (30 m X 250 μm X 0.25 μm, Agilent, USA).

The method used to point out the inhibition of the mycelium was that of poisoning medium. Thus, for each type of oil we prepared the following concentrations: 0.25 $mg \cdot L^{-1}$, 0.5 $mg \cdot L^{-}1$, 1 $mg \cdot L^{-1}$, 5 $mg \cdot L^{-1}$, 10 $mg \cdot L^{-1}$, 15 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$. We prepared a single sample with the medium CYGA 100% and one sample with *thiophanate-methyl* (a synthesis fungicide used in agriculture) for control. Measurements were made in the ninth day.

Using the technique of re-inoculation from poisoning medium on fresh medium, we determined fungal concentration. We used round discs that did not grow in nine days of inoculation on essential oil medium. These round discs were re-inoculated on fresh CYGA medium on Petri plates that were later sealed with para-film and incubated in dark conditions at $22 \pm 2^{0}C$. Observations were made in the fifth day.

Conclusions

- Determining the chemical composition of the vegetal material shows that medicinal plants from the Family *Lamiaceae* have a significant content of macro-elements, particularly Ca, Mg, Fe and Zn.
- After determining the chemical composition of volatile oils, we could see that the studied essential oils have a different chemical composition.
- The main chemical compounds identified in the studied essential oils are:
  - *thymi herba*: *p-cimen* – 32.92%, *Thymol* – 19.98%, *γ-terpinen* – 14.31%;
  - *serpylli herba*: *γ-terpinen* – 23.85%, *Thymol* – 22.22%; *p-cimen* – 16.16%;
  - *satureja herba*: *Thymol* – 60.48%, *p-cimen* – 12.68%;
  - *salvia herba*: *Camphor* – 20.645%, *Eucaliptol* – 11.751%, *Camfen* – 11.587%, α *–pinen* – 9.597, *cis-Tuionă* – 8.644%, *Borneol 1* – 8.803%;
  - *origami vulgare herba*: *Trans-Cariofilen* – 30.729%, *Sabinen* – 18.16%, *Germacren-D* – 8.159%;

- *majorana herba*: *Linalil acetat* – 17.407%, *γ-terpinen* – 14.168%, *p-cimen* – 13.338%, *Germacren-D* – 9.207%.

- The different antifungal effect of the tested essential oils can be explained by the different ratio between antifungal constituents as shown by other studies. Previous research shows that antifungal effect is not the result of one single main compound: it can be the result of the synergism of all compounds.
- Minimum inhibitory concentrations, nine days after inoculation of fungi on culture media were:
  - Essential oil from *thymi herba*: *Verticillium dahliae* 0.5 $mg \cdot L^{-1}$, *Penicillium aurantiogriseum* 0.5 $mg \cdot L^{-1}$;
  - Essential oil from *serpylli herba: Verticillium dahliae* 1 $mg \cdot L^{-1}$, *Penicillium aurantiogriseum* 1 $mg \cdot L^{-1}$;
  - Essential oil from *satureja herba*: *Verticillium dahliae* 0.5 $mg \cdot L^{-1}$, *Penicillium aurantiogriseum* 0.5 $mg \cdot L^{-1}$;
  - Essential oil from *salvia herba: Verticillium dahliae* 10 $mg \cdot L^{-1}$, *Penicillium aurantiogriseum* 10 $mg \cdot L^{-1}$;
  - Essential oil from *origani vulgare herba: Verticillium dahliae* 0.5 $mg \cdot L^{-1}$, *Penicillium aurantiogriseum* 0.5 $mg \cdot L^{-1}$;
  - Essential oil from *majorana herba*: *Verticillium dahliae* 1 $mg \cdot L^{-1}$, *Penicillium aurantiogriseum* 0.5 $mg \cdot L^{-1}$.
- The highest fungal resistance was to the essential oil from *salvia herba.*
- The tested essential oils have a strong antifungal activity: they inhibit fungal growth at low concentrations (0.25 $mg \cdot L^{-1}$).
- Antifungal activity can be attributed to the aromatic nucleus that contains a polar phenol group from the structure of main chemotypes of the analysed species.
- Fungal sensitivity to the action of essential oils is different.
- Minimum fungal concentration when testing the fungus *Verticillium dahliae* was:
  - 1 $mg \cdot L^{-1}$, 5 $mg \cdot L^{-1}$**,** 10 $mg \cdot L^{-1}$, 15 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$ oil from *thymi herba*;
  - 1 $mg \cdot L^{-1}$, 5 $mg \cdot L^{-1}$**,** 10 $mg \cdot L^{-1}$, 15 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$ oil from *satureja herba*;
  - 10 $mg \cdot L^{-1}$, 15 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$ oil from *serpylli herba*;
  - 1 $mg \cdot L^{-1}$, 5 $mg \cdot L^{-1}$**,** 10 $mg \cdot L^{-1}$, 15 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$ oil from *origani vulgare herba*;
  - In the case of the essential oils from *salvia herba* and *majorana herba*, the tested concentrations did not control the fungus *Verticillium dahliae*.

- Minimum fungicide concentration when testing the fungus *Penicillium aurantiogriseum* was:
  - 1 mg·L^{-1}, 5 mg·L^{-1}**,** 10 mg·L^{-1}, 15 mg·L^{-1}, 20 mg·L^{-1} oil from *thymi herba*;
  - 1 mg·L^{-1}, 5 mg·L^{-1}**,** 10 mg·L^{-1}, 15 mg·L^{-1}, 20 mg·L^{-1} oil from *satureja herba*;
  - 10 mg·L^{-1}, 15 mg·L^{-1}, 20 mg·L^{-1} oil from *serpylli herba*;
  - 5 mg·L^{-1}**,** 10 mg·L^{-1}, 15 mg·L^{-1}, 20 mg·L^{-1} oil from *origani vulgare herba*;
  - In the case of the essential oils from *salvia herba* and *majorana herba* the tested concentrations did not control the fungus *Penicillium aurantiogriseum*
- Minimum fungicide concentration of the essential oil from *origanum vulgare herba* was lower in the case of the fungus *Verticillium dahliae* (1 mg·L^{-1}) compared to the minimum inhibition concentration in the case of the fungus *Penicillium aurantiogriseum* (5 mg·L^{-1}).
- All the tested essential oils show strong antifungal activity but the strongest effect was of the essential oils from *Thymus vulgaris* L., *Satureja montana* L. and *Origanum vulgare* L.
- The best antifungal effect among essential oils compounds are *p-cimen* and *thymol.* There is high sensitivity of the fungi towards these compounds.
- The lowest antifungal effect among essential oils is camphor.
- We need to continue research on the optimum combination of chemical compounds separated from essential oils that inhibit the growth of fungi specific to cereal storehouses.
- Results of this research can be a starting point in controlling fungi that cause losses in fruit and vegetable storehouses.
- A more modern and efficient technology could be the development of stable nano-emulsions that can guarantee antifungal protection for at least 14 days due to the diminution of essential oil volatility.
- We need to test in vivo and standardise a testing method of antifungal capacity.

The efficiency of this thesis consists in the solutions and conclusions presented, capable of leading to the improvement of the phenomenon and of the studied economic process. If our suggestions can be implemented in the economic practice, then this doctoral thesis is efficient.

Data presented here are a novelty: they are related to the minimum inhibition concentrations, particularly on the fungus *Penicillium aurantiogriseum.*

This research can be the starting point in the development of fungal and fungi static products containing essential oils for industrial use starting from minimum inhibition concentrations and minimum fungicide concentrations as determined in this study. We could also consider them for the development of ecological fungicide agents.