EFFECT OF SOME ESSENTIAL OILS TREATMENTS ON THE GERMINATION PROCESS OF MAIZE (*ZEA MAYS* L.) SEEDS

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Abstract: *The present scientific work presents the results of the effects of lavandin (Lavandula x intermedia) and marjoram (Origanum majorana) essential oil treatments at different concentrations (25 and 50 µl/ml, respectively) on the germination of maize seeds, considering the antifungal and antibacterial properties of the essential oils mentioned above. The essential oils were obtained by hydrodistillation of the aerial of the plants. To evaluate the phytotoxicity of lavandin and marjoram essential oils, on the germination process of maize seeds, an in vitro experiment was performed. The experiment showed that the application of lavandin essential oil at a concentration of 25 µl/ml has a beneficial effect on the germination process of maize seeds, causing stimulation of the development of vegetative organs. On the other hand, increasing the concentration from 25 µl/ml to 50 µl/ml, produced an inhibition of corn pore germination, thus obtaining a germination percentage of only 70%, compared to the control group where a percentage of 90% was obtained. Based on the results obtained, and taking into account the proven antimicrobial properties of lavandin oil, it can be stated that this oil, in the optimal concentration, can be considered as an ecofriendly fungicide in grain warehouses. Marjoram oil proved an inhibitory effect regarding the germination of maize seeds, for all tested concentrations.*

Keywords*: Lavandula x intemedia, Origanum majorana, phytotoxicity, ecofriendly fungicides*

INTRODUCTION

Synthetic chemicals (based on: carbamates and dithiocarbamates, benzimidazoles, imidazoles and triazoles, morpholines, etc.) are currently used in agriculture to control pathogenic fungi, insect pests, weeds, etc., due to their effectiveness and ease of application. They have negative effects on the environment (soil, water and air pollution) and on consumers, due to the chemical residues resulting from their application; chemical residues that will accumulate in the soil, water and living organisms (organs, muscle tissue, fat, milk) and cause toxic, carcinogenic, allergenic effects, negative effects on the endocrine, reproductive, gastrointestinal, respiratory and neurological systems of consumers (AIMAD *et al*., 2022; AKTAR *et al.*, 2009; LI *et al.*, 2022; NICOLOPOULOU-STAMATI *et al.*., 2016; VARONA *et al.*, 2013).

Around two million tonnes of pesticides are used worldwide every year. Of the two million tonnes of pesticides, 17.5% are fungicides. China is the largest contributor, followed by the US, Argentina, Thailand, Brazil, Italy, France, Canada, Japan and India. However, global pesticide use is estimated to increase by up to 3.5 million tonnes a year (SHARMA *et al.,* 2019; KUMAR *et al.,* 2021).

There is an urgent need to reduce reliance on pesticides and antimicrobials, reduce over-fertilisation, increase organic farming and reverse the loss of biodiversity in light of expert estimates of increasing amounts of synthetic substances used in agriculture worldwide, which is why the European Commission's plan is to ensure that the global use and risk of chemical pesticides is reduced by 50% by 2030 (CHRAPAČIENĖ *et al.,* 2021).

Natural substances, such as essential oils, are today an important ecological reservoir. The use of volatile oils as biopesticides in agriculture, especially in post-harvest practices, can be a more sustainable solution to protect crops, the environment and not least to protect human and animal/consumer health. Many studies highlight the antibacterial, antifungal and antimycotoxigenic properties of the main bioactive compounds of essential oils. Biopesticides, such as volatile oils, are very effective in small quantities and decompose rapidly without leaving potentially toxic residues in the environment (ACHIMÓN *et al.,* 2021; AGRIOPOULOU *et al.,* 2020; EL KHOURY *et al.,* 2016; ESPER *et al.,* 2014; GWIAZDOWSKA *et al.,* 2022; HAN *et al.,*2022; KALAGATUR *et al.,* 2020; KUMAR *et al.,* 2021; OLIVEIRA *et al.,* 2020; OZCAKMAK *et al.,* 2017; PERCZAK *et al*., 2019; WANG *et al.,* 2018). Recent research confirms the biocidal action of some essential oils. These essential oils exhibit contact insecticidal and fumigant actions against a large number of economically important pests (insects and mites) as well as plant pathogens - bacteria, fungi (POKAJEWICZ, *et al.*, 2023; VARONA *et al.*, 2013).

The objective of the present study was to evaluate the influence of the application of essential oils of *Lavandula x intermedia* and *Origanum majoranum*, of different concentrations, on the germination process of maize seeds, taking into account the biological properties of the essential oils mentioned above. The motivation was to identify sustainable and ecofriendly solutions in the control of crop pests or grain storage pests, given the urgent need to reduce dependence on synthetic pesticides and antimicrobials used in agriculture today.

MATERIAL AND METHODS

The research focused on following the effects of treatments of two different concentrations of essential oils (25 and 50 µl/ml respectively) on the germination process of maize seeds. Two essential oils obtained by steam hydrodistillation of the aerial parts of the *Lavandula x intermedia* (lavandin, a hybrid between two species lavender - *Lavandula angustifolia* and spike lavender - *Lavandula latifolia)* and *Origanum majorana* (marjoram), were tested.

From the literature review we found that most of the experiments evaluating the influence of essential oils on the germination degree and germination rate of different seeds are performed in vitro in Petri boxes. Therefore, in order to evaluate the effects of the two essential oils mentioned above, at two different concentrations, on the influence of the germination rate of maize seeds, an in vitro experiment was carried out in Petri boxes.

Sterile Petri boxes, 90 x15 mm in size, were used for the *in vitro* experiment. It was also necessary to place a filter paper inside each sterile Petri boxes.

Five experimental lots were set up, so each experimental lot was divided into Petri boxes which were scored as follows:

Control lot: containing 10 selected maize seeds, intact and of the same size;

- Lot L1: contains 10 selected maize seeds, intact and of the same size, which were subjected to the vapour of essential oil of lavandin of concentration of 25 µl/ml;
- Lot L2: contains 10 selected maize seeds, intact and of the same size, which have been subjected to the vapour of lavandin essential oil at a concentration of 50 µl/ml;
- Lot O1: contains 10 selected maize seeds, intact and of the same size, which have been subjected to the vapour of marjoram essential oil at a concentration of 25 µl/ml;
- Lot O2: contains 10 selected maize seeds, intact and of the same size, which have been subjected to the vapour of marjoram essential oil at a concentration of 50 µl/ml.

After micropipetting each essential oil at concentrations of 25 µl/ml and 50 µl/ml, Petri boxes were sealed, all around with adhesive tape to prevent evaporation of volatile compounds.

Corn seeds were subjected to *Lavandula x intermedia* and *Origanum majorana* essential oil vapour treatments for 8 days. After the 8 days of fumigation, the seeds were moved to clean Petri boxes, over which 10 ml of distilled water was added, and then germinated at 25±2°C.

The appearance of radicles was considered an index of germination.

The germination percentage was calculated and expressed as a percentage for each batch.

After germination, a series of measurements were made for each sample belonging to the experimental and control lots.

The following parameters were determined for each sample: coleoptile length, embryonic root length and adventitious root length (mean), and the data obtained were statistically analysed.

RESULTS AND DISCUSSIONS

The chemical composition of lavender and marjoram essential oil

The principal chemical compounds identified in lavandin (*Lavandula x intermedia*) essential oil, obtained by steam hydrodistillation of the aerial parts of the plant by GC-MS analysis are: linalool 35.86%, eucalyptol 17.84%, endo-borneol 6.8%, accounted for 60.5% of the total chemical compounds identified by chemical analysis (GURIȚĂ, 2019; GURIȚĂ *et al.*, 2019).

According to the standards, ISO 3515:2002 and ISO 8902:2009, regarding the percentage of linalool (24-35%) contained in lavandin oil (RATHORE & KUMAR, 2022), the oil tested by us, contains a percentage of 35.86% linalool, which corresponds to the standards stated above.

The chemical composition, obtained by GC-MS analysis, of lavandin essential oil grown in open field at a farm in Egypt, resulted in the identification of a higher number of chemical compounds (29 in number), compared to the number of chemical compounds identified in the oil tested by us. The principal constituent was eucalyptol, 51.08%, followed by camphor (24.60%), α-pinene (5.58%), β-pinene 4.00% and linalool 0.62% (ELDEGHEDY *et al.,* 2022).

In another study by Varona et al. (2013), 16 chemical compounds were identified in the chemical composition of lavandin essential oil, also obtained by hydrodistillation. The principal chemical compounds identified by GC-MS analysis were linalool in 33.2%, linalyl acetate 29.7%, followed by camphor in 7.1% (VARONA *et al.,* 2013).

The difference in both quality and quantity of the compounds identified in the chemical composition of essential oils is influenced by several factors such as: pedo-climatic conditions in the growing areas which vary from year to year, oil extraction technique, vegetative organs of the plants from which the volatile oil is extracted, the degree of development of the plant from which the volatile compounds are obtained, etc (APROTOSOAIE *et al.,* 2017; VALKOVÁ *et al.,* 2021; RATHORE & KUMAR, 2022).

The principal chemical compounds of marjoram (*Origanum majorana)* essential oil identified by GC-MS analysis are: carvacrol 57.35%, gamma-terpinene 14.46%, thymol 5.32%. Percentage wise, they represent 77.13% of the total 25 chemical compounds identified (GURIȚĂ, 2019). Marjoram essential oil, analyzed by Hassanin and co-workers (2017), showed a lower percentage of γ-terpinene (13.32%), compared to the oil taken in our study (HASSANIN *et al.*, 2017).

Germination of maize seed - *in vitro* experiment

Most essential oils, due to their chemical compounds and the synergistic mode of action between compounds (ESPER *et al.,* 2014), have a high degree of phytotoxicity. With this *in vitro* experiment, performed in Petri boxes, we evaluated the phytotoxicity degree of the essential oils of lavandin and marjoram, at two different concentrations, 25 µl/ml respectively 50 µl/ml, on maize seeds. Maize samples were fumigated for 8 days. After 8 days of fumigation, maize probes were returned to other sterile Petri boxes for germination at 25±2°C. In the control lot and experimental lots, 10 ml distilled water was added. Evaluation of the pores was done after root emergence (considered as germination index).

The germination percentage of maize samples from the control batch, which were not subjected to the vapours of different concentrations of essential oils, was 90%, compared to the experimental batches, where the germination percentage was lower following 8-day treatments under the influence of *Lavandula x intermedia* and *Origanum majorana* essential oil vapours.

In addition to the percentage evaluation of seed germination, the size (reported in cm as a unit of measure) of the coleoptile, the size of the embryonic root and the average adventitious root were analysed for each sample (Tables 1, 2, 3, 4 and 5).

Table 1

Germinated seeds of maize from the control lot

Seed	1	2	3	4	5	6	7	8	9	10
Coleoptile	1.3	1	1	0.9	0.8	0	0.9	0.8	1.1	1.2
Embryonic root	2.8	2.5	2.5	2.1	2.8	0	2.0	2.4	2.0	2.2
Adventitious roots	1.5	0.6	0.9	0.5	0.8	0	0.1	0.8	0.8	1.0

Table 2

Germinated maize seeds, previously fumigated with essential oil of lavandin 25 µl/ml concentration

Seed	1	2	3	4	5	6	7	8	9	10
Coleoptile	1.2	1.2	1.5	1.3	1.6	0.5	0.5	1.3	1.5	1.0
Embryonic root	3.0	3.1	3.5	5.0	3.3	2.0	1.2	3.4	3.4	2.0
Adventitious roots	0.6	1.0	1.0	2.0	1.4	1.0	0	1.3	1.5	0.4

Table 3

Germinated maize seeds, previously fumigated with essential oil of lavandin 50 µl/ml concentration

Seed	1	2	3	4	5	6	7	8	9	10
Coleoptile	0	0	0	0.8	0.5	0.7	1.2	1.0	1.6	1.0
Embryonic root	0	0	0	1.9	0.3	2.0	1.7	0	3.0	2.6
Adventitious roots	0	0	0	0.5	0	0.6	0	0	1.5	1.7

Table 4

Germinated maize seeds, previously fumigated with essential oil of marjoram 25 µl/ml concentration

Seed	1	2	3	4	5	6	7	8	9	10
Coleoptile	0	0	0	1.3	0.3	0.5	0.4	1.0	0.7	0.6
Embryonic root	0	0	0	3.0	1.0	2,5	1.2	2.0	1.5	1.7
Adventitious roots	0	0	0	2.0	0	0,7	0	0.8	0	0

Table 5

Germinated maize seeds, previously fumigated with essential oil of marjoram 50 µl/ml concentration

Seed	1	2	3	4	5	6	7	8	9	10
Coleoptile	0	0	1.0	0.4	0.4	0.5	0.4	0.9	0	0.6
Embryonic root	0	0	1.8	1.6	2.5	1.2	1.0	1.3	0	0.7
Adventitious roots	0	0	0.8	0	0	0	0	0	0	0

The evaluation of the seeds from the experimental group in comparison with the samples of the groups that were treated with *Lavandula x intermedia* essential oil, at a concentration of 25µl/ml (Table 1 and 2), showed a stimulation of germination, but also a stimulation of the average increase in the size of the coleoptile, the embryonic root and the adventitious roots, compared with the average results of the samples from the control group. On the other hand, increasing the concentration from 25 to 50 µl/ml (doubling it) resulted in an inhibition of germination of maize root balls, thus obtaining a germination percentage of only 70%, compared to the control group where a percentage of 90% was obtained. Also, analysis of the mean sizes of adventitious roots, embryonic roots and coleoptile of the germinating samples of the experimental batch compared to the control batch samples also showed a significant inhibition/reduction in the development of the target parameters/vegetative organs (Figure 1, 2 and 3 and Table 3).

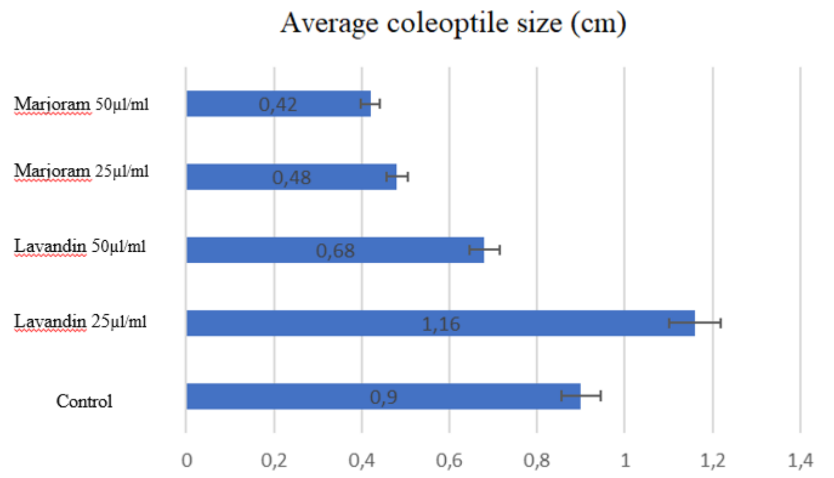

Figure 1. Effects of essential oil treatments on coleoptile development of maize seeds

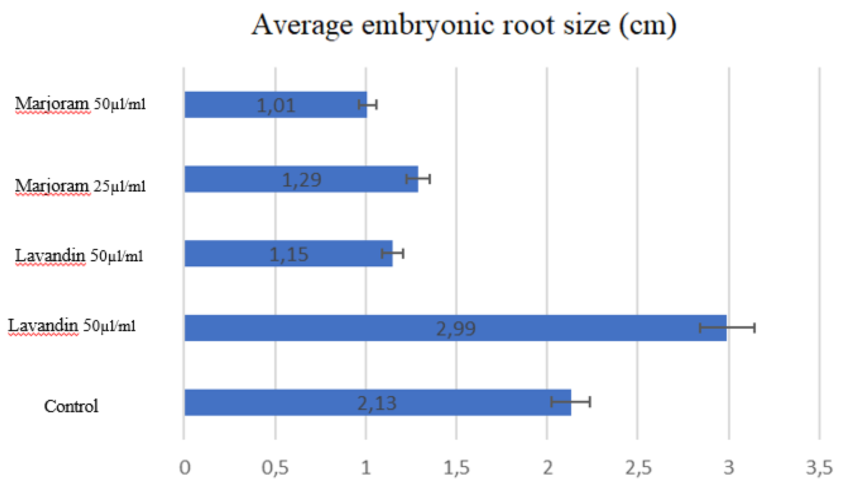

Figure 2. Effects of essential oil treatments on maize seed embryonic root development

Seeds from the experimental lots subjected to treatments with *Origanum majorana* essential oil vapour (at both 25 µl/ml and 50 µl/ml concentrations, respectively), produced germination inhibition of maize samples compared to untreated maize samples from the control lot. In percentage terms, only 70% of the treated samples germinated. A significant reduction in embryonic root development, adventitious root development and coleoptile development was also observed in germinated maize compared to untreated samples, as can be seen from the graphical representations, Figure 1, 2 and 3 (Table 4 and 5).

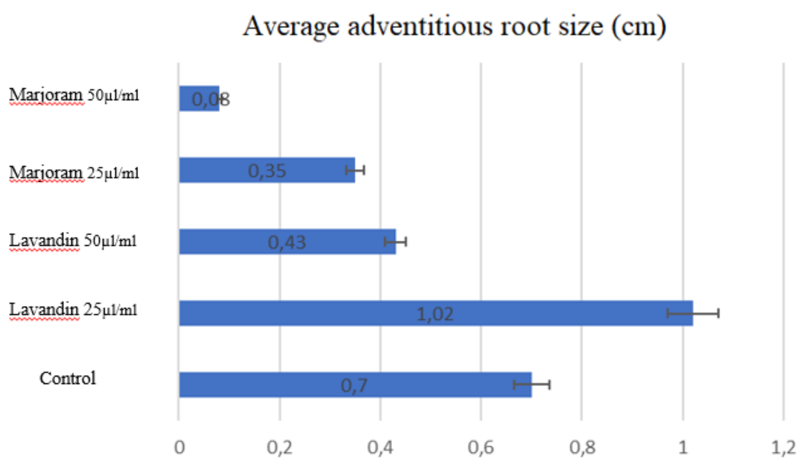

Figure 3. Effects of essential oil treatments on adventitious root development of maize seeds

CONCLUSIONS

The analysis of the seed samples from the control lot, showed a germination percentage of 90%, the average coleoptile size was 0.9 cm, the average embryonic root size was 2.13 cm and the average adventitious root size was 0.7 cm.

Analysis of maize samples that were treated with *Lavandula x intermedia* essential oil of 25µl/ml concentration, showed 100% germination percentage and stimulation of growth/development of vegetative organs, compared to the control batch samples.

The germination percentage of the samples from the lot treated with *Lavandula x intermedia* essential oil of 50µl/ml concentration is reduced (70%), compared to the germination percentage of the control lot pots, also a significant reduction in the size of the vegetative organs of the germinated pots was observed.

The germination percentage of the samples from the group treated with *Origanum majorana* essential oil at a concentration of 25µl/ml was 70%, reduced by 20% compared to the germination percentage of the control group, and a significant reduction in the size of the vegetative organs of the germinated sows was also observed.

The germination percentage of the samples from the group treated with *Origanum majorana* essential oil at a concentration of 50µl/ml is 70%, reduced by 20% compared to the germination percentage of the control group, and a significant reduction in the size of the vegetative organs of the germinated sows was also observed.

Considering the results obtained from the *in vitro* experiment, we noticed that *Lavandula x intermedia* essential oil, at a concentration of 25µl/ml, has a beneficial effect on the germination process, stimulating the development of vegetative organs and can be considered as an ecofiendly fungicide in grain warehouses.

Following these results, it is necessary to continue both *in vitro* and *in vivo* testing, regarding the stimulating effect of *Lavandula x intermedia* essential oil on the germination on several crop seeds and also the inhibitory effect of *Origanum majorana* essential oil on the germination of weed seeds, with applicability in organic farming.

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