

INFLUENCE OF THE APPLICATION OF SELECTED ESSENTIAL OILS ON THE GERMINATION OF SOYBEAN (*GLYCINE MAX*) SEEDS

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Abstract. The present scientific paper presents the results of the effects of treatments with essential oils of crimson beebalm (*Monarda didyma*) and wild thyme (*Thymus pulegioides*), at different concentrations (25 µl/ml and 50 µl/ml), on the germination of soybean (*Glycine max*) seeds, considering the antifungal and antibacterial properties of the essential oils mentioned above. Soybean seeds represent one of the most important sources of vegetable protein, widely cultivated throughout the world. To evaluate the phytotoxicity of essential oils on the seed germination process, two experiments were realized: one *in vitro* and one *in vivo*. It was observed that the application of *Monarda didyma* and *Thymus pulegioides* essential oil treatments, at different concentrations, did not negatively influence the germination percentage of soybean seeds, but caused a reduction/inhibition of root size development. The *in vivo* experiment showed an inhibitory effect of the tested oils on the development of vegetative organs of soybean plants compared to plants in the control group not treated with essential oils. Based on the antifungal and antibacterial properties of the oils analysed, as well as the results obtained in the *in vitro* experiment following the application of essential oil treatments on soybean seeds, the use of the oils studied, can be taken into account as possible ecofriendly fungicides in grain storage.

Keywords: *Monarda didyma*, *Thymus pulegioides*, phytotoxicity

INTRODUCTION

Today, there is a growing interest in ecofriendly products such as essential oils. These are able to fight pathogens and control the growth of plants that are harmful to agricultural crops. Thus the use of essential oils is arousing great interest for their bactericidal, virucidal, fungicidal, antiparasitic, insecticidal and herbicidal applications (RICCI *et al.*, 2017; NIKOLOVA *et al.*, 2021; PUSKAROVA *et al.*, 2017). Essential oils are complex oily volatile liquids characterised by a strong odour. They are synthesised by all aromatic plant organs as secondary metabolites and stored in secretory cells, cavities, ducts, epidermal cells or glandular trichomes. They may contain 20 to 60 active compounds responsible for their biological activities (RASSEM *et al.*, 2016; GWIAZDOWSKA *et al.*, 2022; RATHORE & KUMAR, 2022).

Both in the field and during post-harvest storage, cereals, fruits and vegetables are highly susceptible to fungal spoilage. The use of volatile oils, as biopesticides, in agriculture, especially in post-harvest practices, can be a sustainable solution to protect agricultural crops, the environment and not least to protect the health of the final consumers (DE MARTINO *et al.*, 2009; DHIFI *et al.*, 2016; ELSHAFIE & CAMELE, 2017; AGRIPOULOU *et al.*, 2020; BOUKHATEM *et al.*, 2020; EL-ALAM *et al.*, 2020; EL-ALAM *et al.*, 2020; ACHIMÓN *et al.*, 2021).

The control of fungal diseases as well as crop pests (weeds) is currently mainly achieved by the application of synthetic pesticides (AKTAR *et al.* 2009; AIMAD *et al.* 2022). The widespread use of synthetic herbicides/products has led to an increase in weed resistance to herbicides, and thus to a directly proportional increase in risks to human and animal health, since intensive/excessive use of synthetic herbicides in high concentrations also increases the risk of toxic residues both in agricultural products and in the environment (AKTAR *et al.*, 2009; HUA *et al.*, 2014; AIMAD *et al.*, 2022; FRATERNALE *et al.*, 2022; FINETTI *et al.*, 2022). Natural plant derived compounds have a short half-life because they are readily biodegradable and thus

do not harm the environment (pollution) or consumers of agricultural products treated with natural compounds (RICCI *et al.*, 2017; ZHOU *et al.*, 2021; AELENEI *et al.*, 2016).

Considering the urgent need to identify new ecofriendly products with applicability in sustainable agriculture, such as essential oils, we propose in this paper to evaluate/test the *in vitro* phytotoxicity of two essential oils, which following the study of literature have shown inhibitory effects on fungal and bacterial phytopathogens, at different concentrations, extracted by hydrodistillation of aerial parts, from different plants belonging to the botanical family *Lamiaceae*, on cereal seed. Thus, following the evaluation of the phytotoxicity degree of the oils studied on cereal seeds, we can identify ecofriendly substances that could be applied in sustainable/ecological agriculture (ADEBAYO *et al.*, 2013; ESPER *et al.*, 2014; HAN *et al.*, 2022).

The aim of the present work is to evaluate the effects of essential oils of *Monarda didyma* and *Thymus pulegioides*, at different concentrations, on the germination of soybean (*Glycine max*) seeds, taking into account the antifungal and antibacterial properties of the essential oils mentioned above.

MATERIAL AND METHODS

The study of the effects of two essential oils, of different concentrations 25µl/ml and 50µl/ml, respectively, on the seed germination process was carried out on soybean seeds. The tested oils were obtained by hydrodistillation of the aerial parts of *Monarda didyma* (cultivated in Timișoara) and *Thymus pulegioides* (plants harvested from Carașova).

To evaluate the phytotoxicity of essential oils on the seed germination process, two experiments were carried out: one *in vitro* and one *in vivo*.

The first experiment, *in vitro*, was carried out in sterile Petri dishes of size 90x15mm. A double layer of filter paper was added inside the boxes. Ten soybean seeds were placed on top of the filter paper in each Petri boxes, selected beforehand.

The experimental material was placed in Petri boxes, which were labelled as follows:

Control (Petri boxes with 10 soybeans that were not subjected to the vapour of the essential oils tested),

M1 (Petri boxes with 10 soybean seeds which were subjected to *Monarda* essential oil vapour at a concentration of 25µl/ml for eight days),

M2 (Petri boxes with 10 soybean seeds which were subjected to *Monarda* essential oil vapour at a concentration of 50µl/ml for eight days),

C1 (Petri boxes with 10 soybeans which were subjected to vapour of spontaneous thyme essential oil at a concentration of 25µl/ml for eight days) respectively,

C2 (Petri boxes with 10 soybean seeds which were subjected to the vapour of spontaneous thyme essential oil at a concentration of 50µl/ml for eight days).

All Petri boxes were isolated/sealed with clamshell tape to prevent evaporation of essential oil and maintained in a laboratory at 25±2°C.

After eight days of exposure of soybeans to the vapours of different concentrations of essential oils of *Monarda* and *Thymus*, the Petri boxes were unsealed and the samples were placed in other sterile Petri boxes with two layers of filter paper added. Each Petri box was pipetted with 10 ml of distilled water. Petri boxes were left to germinate in the same laboratory where they were fumigated for 8 days at the same temperature 25±2°C until seed germination. Each experimental batch was evaluated after germination. The data obtained were statistically analysed.

The second experiment, *in vivo*, involved the evaluation of the effects of the two essential oils, previously studied, at concentrations of 25 and 50 µl/ml respectively, on the germination of soybean seeds (belonging to the same variety, previously used in the first experiment) in soil. The

test was carried out in pots filled 2/3 with universal potting soil and 1/3 with a mixture of universal potting soil and Terawet in a ratio of 1:1.

The mixture of soil and Terawet was placed in pots. In each pot ten soybean seeds were added. Each vessel has been labelled as follows:

Control (vessel that was subsequently wetted with distilled water only throughout the experiment),

M1 (vessel into which monarda essential oil of 25µl/ml concentration was pipetted using a micropipette),

M2 (vessel into which monarda essential oil of 50µl/ml concentration was pipetted),

C1 (vessel into which spontaneous thyme essential oil of 25µl/ml concentration was pipetted) respectively,

C2 (vessel into which spontaneous thyme essential oil of 50µl/ml concentration was pipetted).

The vessels were then periodically moistened with distilled water. Eleven days after the start of the experiment, the plants were removed from the plastic containers, cleaned, evaluated/measured and compared with the control batch, and the data were tabulated and statistically analysed.

RESULTS AND DISCUSSIONS

Chemical composition of essential oils

The essential oils were extracted from the dried aerial plant parts (herba) of *Monarda didyma* and the dried aerial plant parts (herba) of *Thymus pulegioides*. Extraction of volatile compounds was done using a steam hydrodistillation plant located in the laboratory of the Crop Department of Agricultural Faculty of USVT.

The chemical composition analysis of *Monarda didyma* essential oil, which was determined by chromatograph mass spectrometer (GC/MS), identified a total of 28 chemical compounds, of which the following compounds stood out quantitatively: linalool 48.27%, thymol 18.71%, o-cymol 13.36% and carvacrol 2.01% (BOJINESCU, 2022).

The essential oil extracted from the dried aerial parts of the *Thymus pulegioides* plant showed a characteristic, yellowish odour. The yield of essential oil obtained was 0.44±0.021%. Chemical composition analysis (GC/MS) of the essential oil identified a total of 36 compounds, of which 6 were found in higher percentage: carvacrol (25.43%), thymol (13.93%), p-cymene (0.43%), gamma-terpinene (7.47%), cis-geraniol (13.12%) and linalool (0.47%). Due to the high percentage of carvacrol contained, the essential oil obtained by hydrodistillation of the dried aerial parts of the *Thymus pulegioides* ssp. *pulegioides* plant can be classified as an essential oil belonging to the carvacrol chemotype (BEICU *et al.* 2021; ANGELINI *et al.* 2003).

Effect of essential oil vapours on soybean germination - *in vitro* experiment

In this experiment, the effects produced by the vapours of essential oils of *Monarda didyma* and *Thymus pulegioides* at concentrations of 25µl/ml and 50µl/ml respectively were investigated. Soybean samples were subjected to the vapour treatments for eight days. At the end of the eight days of fumigation, soybean samples were germinated in other sterile Petri dishes at 25±2°C. In each sample container 10 ml of distilled water was pipetted.

After for days of germination, the samples were evaluated/measured and compared with the control samples. To evaluate the effects of treatments with different concentrations of essential oils, the germination percentage of soybean seeds from the control and each experimental batch was evaluated. As germination index we referred to the appearance of the root (radicle). Subsequently, the size (cm) of each rootlet was also assessed. The data obtained are presented in Table 1.

Table 1

Evaluation of the radicle dimension (cm), treated with *Monarda* and *Thymus* oil of different concentrations, compared to the control group

No.	Control	<i>Monarda</i> oil 25 μ L/ml	<i>Monarda</i> oil 50 μ L/ml	<i>Thymus</i> oil 25 μ L/ml	<i>Thymus</i> oil 50 μ L/ml
1.	4.2	3.5	3.4	4.5	4.1
2.	4.0	2.4	4.0	3.2	4.0
3.	3.8	2.5	3.2	3.4	2.2
4.	3.5	0.9	2.6	2.9	2.3
5.	3.7	0.8	2.6	2.0	2.7
6.	3.5	0.9	3.0	2.0	3.0
7.	3.5	0.6	1.7	0.7	2.0
8.	3.7	0.5	2.1	1.0	1.9
9.	3.8	0.8	0.3	0.8	1.6
10.	0	0	1.1	0.6	0.6
Average	3.37	1.29	2.4	2.11	2.44

In the control lot the germination percentage of soybean seeds was 90% as shown in Table 1. Compared to the control group, in the experimental groups treated with *Thymus* essential oil (25 μ L/ml respectively 50 μ L/ml) and *Monarda* essential oil 50 μ L/ml, no changes in germination percentage were observed. On the other hand, the experimental lot subjected for eight days to *Monarda* essential oil vapour at a concentration of 25 μ L/ml showed a germination percentage of 90%, compared to the control lot where a percentage of 100% was obtained.

If we refer to the average root size, according to the data obtained, treatments with different concentrations of essential oils produced a slight inhibition of root growth, compared to the control group. On the other hand, wild thyme oil applied at a concentration of 50 μ L/ml, resulted in a stimulation of root (radicle) growth, compared to the mean root growth of the control group.

Effect of essential oil vapours on soybean germination - *in vivo* experiment

Several determinations/measurements were made for the control and for each experimental group, which aims to highlight the effects of treatments with different concentrations of the essential oils taken in the study, on the germination of soybean seeds, but also on the subsequent development of the plants (Table 2, Table 3).

The soybean seed samples from the control group showed a germination percentage of 90%, an average plant length of 18.35 cm, an average plant root length of 5.83 cm and an average soybean plant stem length of 10.92 cm.

In comparison, the lots exposed to the essential oils, although showing a similar percentage germination as the control samples, when comparing the size of the vegetative organs, a reduction/inhibition of the growth/development of the vegetative organs of the soybean plants was observed for all oils tested.

If we refer strictly to the germination percentage, we can state that the tested oils, at concentrations of 25 and 50 μ L/ml respectively, do not influence the germination rate of soybean seeds.

After evaluation of soybean plants from the lot treated with essential oil of spontaneous thyme at a concentration of 25 μ L/ml, compared to the control lot (which was not treated with essential oil) we observe a significant reduction in the size of germinated plants, but also of each vegetative organ. Percentage of 50% of the plants, although they germinated (the appearance of the radicle could be observed), could not continue their development. An inhibition of growth/development of soybean plants after germination was also observed in soybean samples treated with *monarda* essential oil at a concentration of 50 μ L/ml.

Table 2

Evaluation of the parameters of soybean seedlings, treated with *Monarda* oil of different concentrations, compared to the control group

	Control			<i>Monarda</i> oil 25 µL/ml			<i>Monarda</i> oil 50 µL/ml		
	Plant length	Root length	Stem length	Plant length	Root length	Stem length	Plant length	Root length	Stem length
1.	19.9	7.0	12.0	28	9.2	17.5	16.8	2.6	12.9
2.	20.8	6.5	12.0	25.6	2.4	16.5	12.7	2.6	9.5
3.	21.0	6.9	14.0	17.6	3.0	17.5	14.7	2.6	10.1
4.	19.3	6.1	11.9	17.6	1.8	10.3	3.4	1.5	1.5
5.	19.8	6.3	11.7	8.8	2.5	6.5	2.8	0.7	2.1
6.	20.5	6.3	12.0	5.0	1.3	2.7	1.7	1.1	0.5
7.	20.7	6.4	12.1	3.2	1.5	1.8	0	0.5	0
8.	20.9	6.6	11.8	4.9	1.5	3.0	0	0.5	0
9.	20.6	6.2	11.7	0	0.5	0	0	0.5	0
10.	0	0	0	0	0.6	0	0	0	0
Average	18.35	5.83	10.90	11.07	2.43	7.58	5.21	1.26	3.66

Table 3

Evaluation of the parameters of soybean seedlings, treated with *Thymus* oil of different concentrations, compared to the control group

	Control			<i>Thymus</i> oil 25 µL/ml			<i>Thymus</i> oil 50 µL/ml		
	Plant length	Root length	Stem length	Plant length	Root length	Stem length	Plant length	Root length	Stem length
1.	19.9	7.0	12.0	11.6	1.5	7.0	30.3	17.7	14.5
2.	20.8	6.5	12.0	9.2	3.1	2.0	25.0	2.7	10.0
3.	21.0	6.9	14.0	5.5	1.5	4.0	29.2	15.0	14.0
4.	19.3	6.1	11.9	3.2	1.5	1.7	13.8	2.5	11.0
5.	19.8	6.3	11.7	0	1.9	0	13.5	2.0	8.8
6.	20.5	6.3	12.0	0	1.3	0	9.2	3.3	6.0
7.	20.7	6.4	12.1	0	1.1	0	4.5	2.3	2.5
8.	20.9	6.6	11.8	1.6	1	0.6	3.2	2.0	1.6
9.	20.6	6.2	11.7	0	0.4	0	0	1.5	0
10.	0	0	0	0	0	0	2.3	0.8	1.3
Average	18.35	5.83	10.90	3.11	1.33	1.53	13.1	4.98	6.97

This inhibition of plant development produced by synergism (CÔTÉ *et al.* 2021) between the compounds identified in the chemical composition of the essential oils tested by us, is in agreement with the results obtained by other researchers (RICCI *et al.* 2017).

Compared to the control group, the experimental group treated with wild thyme essential oil, at a higher concentration, 50µl/ml, showed an increase in the growth of soybean plants after germination (Figure 1).

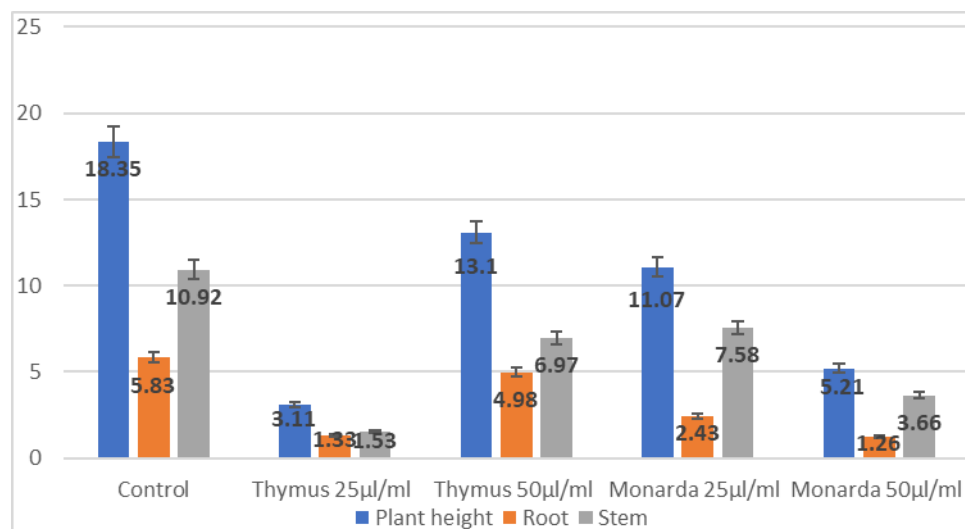


Figure 1. Graphical representation of the average size of the vegetative organs, compared to the control group

The analysis of the seed samples from the control lot showed a germination rate of 90%, the average plant size was 18.35 cm, the average root size was 5.83 cm and the average stem size was 10.92 cm.

The *in vivo* experiment showed an inhibitory effect of the tested oils on the development of the vegetative organs of soybean plants, compared to the plants of the control group not treated with essential oils.

CONCLUSIONS

Analysis of soybean samples that were subjected to treatment with essential oil of spontaneous thyme, *Thymus pulegioides*, concentration of 25µl/ml, showed a germination percentage of 90% and resulted in inhibition of vegetative organ development, compared to the control lot samples. The germination percentage of the samples from the batch treated with *Thymus pulegioides* essential oil of 50µl/ml concentration was also 90%, instead inhibition of soybean plant development was observed.

The germination percentage of samples from the batch treated with *Monarda didyma* essential oil of 25µl/ml concentration was 90%, inhibition of soybean plant development was observed, compared to the soybean plant size of the control batch.

The germination percentage of the samples from the batch treated with *Monarda didyma* essential oil of 50µl/ml concentration was 90% and a decrease in the average size of vegetative organs was observed.

Considering the antifungal and antibacterial properties of the oils analysed, as reported in the literature, as well as the results obtained in the first *in vitro* experiment following the application of essential oil treatments on soybean seeds, the use of the oils studied, can be taken into account as possible ecofriendly fungicides in grain storage.

It is necessary to continue the *in vitro* and *in vivo* tests regarding the essential oils influence on seed germination, for other crops and also on some weed species, due to the inhibitory effect observed.

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