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STATISTICAL ANALYSIS ON THE POTENTIAL ANTIFUNGAL ACTIVITY OF SOME ESSENTIAL OILS OBTAINED FROM MEDICINAL PLANTS

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Abstract

The paper presents descriptive statistics of the experimental results regarding the potential antifungal activity of four essential oils obtained from new varieties of medicinal plants created in Romania (Yellow basil, Red basil, Marigold and Hyssop). Efficacy tests were performed on the phytopathogenic fungus Fusarium oxysporum ZUM 2407, using four concentrations, each with four repeats. It was observed the direct dependence of the efficacy of the antifungal action of the essential oils depending on the concentration used, but also its decrease in time, due to volatilization and consumption in the control process. It was established that the action of the studied essential oils is rather fungistatic for all the analyzed variants. Based on the efficacy of the antifungal action of those four essential oils, the analyzed variants could be ranked as attack intensity and a measure of economic efficiency was developed in order to further apply these solutions in agricultural practice.

***Key words**: medicinal plants, new varieties, essential oils, antifungal action, Fusarium oxysporum.*

INTRODUCTION

Research on medicinal plants can be approached from many different but equally valuable points of view, including traditional and new uses: botanical and phytochemical characterization, metabolic model physiology, crop, disease and pest management, economic and industrial implications. Phytosanitary protection in organic farming is one of the most delicate issues, given that the technical means available are relatively few and do not guarantee the same efficacy as synthetic chemicals, normally used in conventional agriculture.

The approached field is not new, studies on antifungal activity using essential oils obtained from different plants, being made in various conditions by other authors (Yulia, 2005; Lee et al., 2007; Chuah et al., 2010; Jakowienko et al., 2011; Nosrati et al., 2011; Aminifard et al., 2012; Behdani et al., 2012; Sobhani et al., 2014; Dakole et al., 2016; Dianez et al., 2018; Moutassem et al., 2019; Olea et al., 2019; Mutlu-Ingok et al., 2020; Khaledi et al., 2021). Also, the antifungal action of some plants has been studied in various other forms: powder, different types of extracts (Kocic-Tanackov et al., 2011; Kowalska et al., 2020). The action of some essential oils extracted from various plants has also been studied as a possible substitute for fungicides, pesticides (Koul et al., 2008; Moghtader et al., 2011; Santra et al., 2020).

The paper presents the results of the descriptive statistical analysis regarding the antifungal activity of four essential oils (EOs) obtained from medicinal plants (Yellow basil, Red basil, Marigold and Hyssop) comparatively, for the phytopathogenic fungus *Fusarium oxysporum* or blackening and rot of the root and package of plants. Other symptoms include partial yellowing of the basal leaves on one half of the leaf blade; the twisting of the rosette leaves, especially due to the asymmetrical development of the leaf tongue, or the uncharacteristic embossing of the leaves (Gilardi et al., 2016; Velarde et al., 2018).

F. oxysporum develops a white, reddish or purplish mycelium, depending on the composition of the nutrient substrate. The pathogen produces in abundance microconidia, oval or elliptical, attached to the microfialids. It also forms abundant chlamydospores after 2-4 weeks of cultivation. They are often grouped in pairs or in rows (Summerell et al., 2010).

MATERIALS AND METHODS

The experimental method

In order to study the inhibition of the growth of the pathogen *Fusarium oxysporum* ZUM 2407, four new varieties of medicinal plants were tested (Yellow basil, *'Aromat de Buzau'* variety, Red basil, *'Seraphim'* variety, Marigold, *'Nanuk'* variety and Hyssop, *'Catalina'* variety), obtained by the method of hydrodistillation using french equipment. They were used undiluted (100%) or diluted in proportions of 3/4 (75%), 1/2 (50%) and 1/4 (25%).

The test was performed *in vitro* (Figure 1), in sterile, unventilated Petri dishes with a diameter of 9 cm. The vessels were loaded with 20 ml of CGA nutrient medium. The plates were seeded centrally with the fungal inoculums, from a 14-day culture, represented by mycelia rounds calibrated to 6 mm in diameter. At a distance of 2 cm from the fungal inoculums, 4 sterile paper rounds were placed, equidistant on each plate, loaded with a 10 µl sample of essential oil. At the same time, control plates were prepared according to the same protocol, in which it was tested the solvent used to dilute essential oils to analyze the possible influence on mycelia growth. Control cultures with *F. oxysporum* were also prepared by seeding the pathogen according to the same protocol. It should be noted that during the course of the experiments, no additional doses of essential oils were added. Samples were incubated at 27°C and analyzed for the first 10 days after inoculation.

Antifungal activity (Figure 2) was assessed biometrically by measuring mycelia growths in the test plates, compared to growths in the control plates, according to the formula proposed by Lahlali et al., 2010:

$$E = \frac{100 \cdot (R_C - R_I)}{R_C}, \% \qquad (1)$$

where: R_C is the radius of the mycelia colony in the control plate, R_I is the radius of the mycelia colony in the interaction zone.

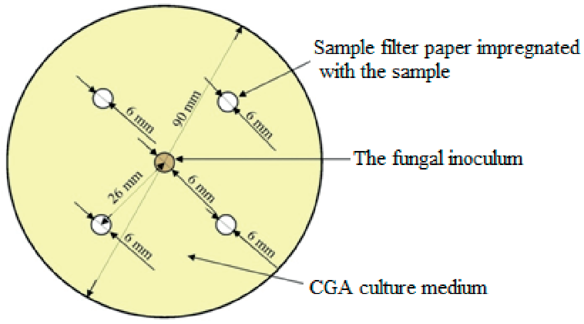

Figure 1. Essential oils inoculation model for testing antifungal activity

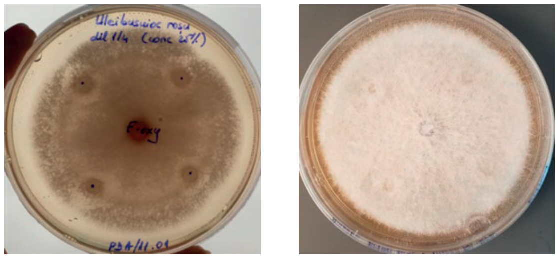

Figure 2. Reduction in the density of *Fusarium oxysporum* mycelium in the presence of Red basil essential oil (left) compared to untreated control (right)

The statistical analysis performed for the interpolation of the experimental data on the antimicrobial activity of essential oils (EOs) were performed with the MathCAD 2000 programs (Reference manual, Math Soft, 2000) and Excel from the MS Office 2007 package.

RESULTS AND DISCUSSIONS

This chapter presents the experimental data in the form provided by descriptive statistics: mean values, variation of mean values as a function of time and concentration, linear regression analysis. According to (Butanescu, 2018), descriptive statistics include methods that allow the collection of data collections in a simple and explicit form. Descriptive statistics methods can be divided into numerical and graphical methods. Numerical summaries are obtained by numerical methods such as mean, standard mean deviation, etc. Graphical methods provide data visualizations, useful for identifying data structure. The statistics correspond to the populations formed by the values of the growth radius of the mushroom

colony, values measured during the experiment. The series of four repetitions performed for each reading over time, for each of the 4 essential oils (with 4 concentrations) tested and the control variant, were studied by elementary statistical estimators: arithmetic means, median, quartiles. Box plot representations were made. This preliminary stage shows us that no aberrant values were recorded and it was not necessary to delete the data. Therefore, in the following calculation, the average values of the series consisting of the 4 essential oils (with 4 concentrations, each analyzed in 4 repetitions) and the control variant were used.
The average values obtained together with the efficacy formula (1) lead to estimates of the variation of efficiency according to time and concentration and to the ranking of the efficiency of the action of the four essential oils analyzed.

Variation in the growth of Fusarium oxysporum and the efficacy of the treatment with Yellow basil, 'Aromat de Buzau' variety *essential oil*

In Figure 3 are shown the average increases of the radius of the fungi colony as a function of time, for the 4 concentrations tested and for the control. Each point marked on the graph in Figure 3 corresponds to an average value for the four repetitions of the experiment. Experimental points are marked with symbols of different shapes and colours, according to legend. The regression trend lines (Butanescu, 2018), are shown for each oil concentration in the corresponding colour (see the legend). Figure 4 shows the variation of efficacy (calculated with formula 1), for each experimental point depending on the time (period 3-10 days). The experimental points and regression lines have the same colour code as in Figure 3, according to the legend of Figure 3.
Figure 5 shows the variation of the average temporal efficacy, i.e. the way in which the average efficacy varies depending on the time and the concentration of Yellow basil essential oil used.
From the graphic representations in figure 3 it results that, in time, the rays of the fungi colony increase, in the interval of 3-10 days. The highest growth is the fungi colony corresponding to the untreated control. Fungi colonies that are given Yellow basil essential oil increase in proportion to the inverse of the concentration of essential oil used. This means that the higher the concentration of Yellow basil essential oil, the slower the growth of the fungus (3-10 days) - the fungistatic action of Yellow basil essential oil. Figure 5 shows that the effectiveness of any of the 4 concentrations used of Yellow basil essential oil decreases over time. The probable causes of this phenomenon are volatilization and consumption of essential oil in antifungal action. Figure 4 also shows that the antifungal efficacy of Yellow basil essential oil increases in proportion to its concentration.

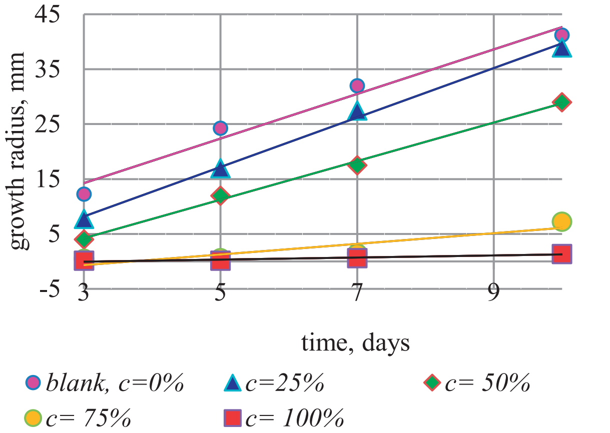

Figure 3. Variation of the average values of the growth radius of the fungi colony and their trend lines depending on the time, for the control and the 4 tested concentrations of the Yellow basil essential oil

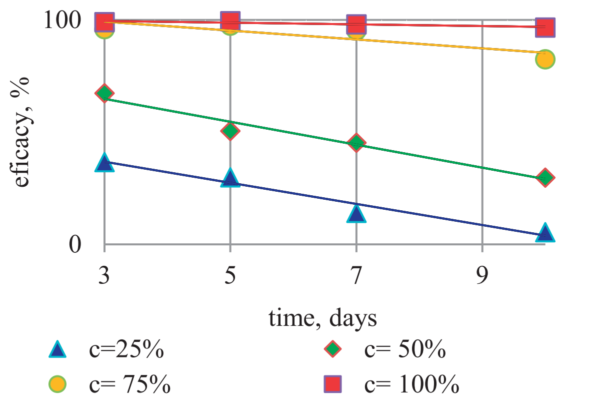

Figure 4. Variation over time in the efficacy of Yellow basil essential oil (4 different concentrations)

Figure 5 shows that the weighted average efficiency over time increases with the concentration of Yellow basil essential oil used to control the phytopathogenic fungus *F. oxysporum*. The shape of the curve in Figure 5 suggests that at a concentration of 80%, the increases are small, negligible, compared to the increases corresponding to concentrations between 25% and 80%.

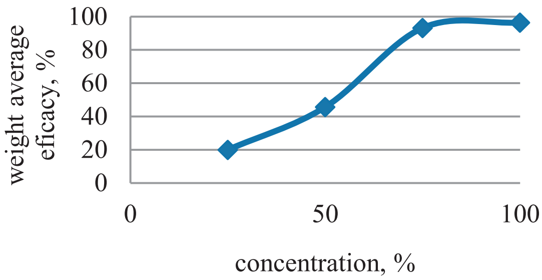

Figure 5. Dependence on the average temporal concentration of the concentration, for the essential oil of Yellow Basil

Variation in the growth of Fusarium oxysporum and the efficacy of antifungal treatment with Red basil, 'Seraphim' variety essential oil

The rules for graphical representation of data and regression lines are the same as for Yellow basil, the results being of the same type and data in the same order, both for Red basil and for Marigold and Hyssop.

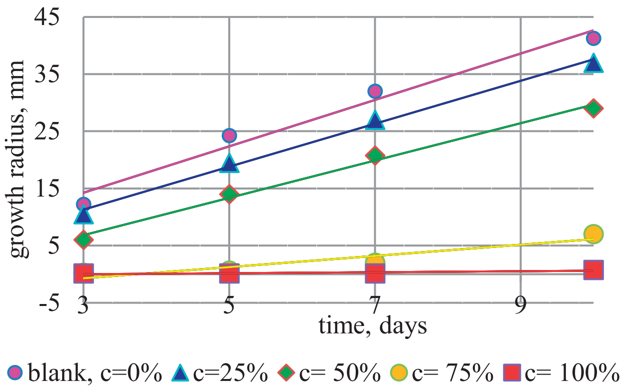

Figure 6. The time values for the control and the 4 tested concentrations of Red basil essential oil were dependent on the average values of the growth rate of the fungi colony and their trend lines

As with Yellow basil, Figure 6 shows that for Red basil oil, the radius of the colony increases with time (3-10 days). Here, too, the largest growth is the fungi colony corresponding to the untreated control. Fungi colonies treated with Red basil essential oil increase in proportion to the inverse of the concentration of the solution used for testing. This means that the higher the concentration of Red basil essential oil, the slower the growth of the phytopathogenic fungus (3-10 days).

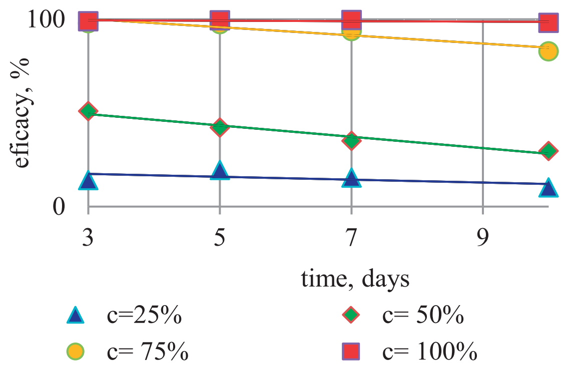

Figure 7. Time dependence of Red basil oil efficacy (4 different concentrations)

Figure 7 shows that the efficiency of any of the solutions with different concentrations of Red basil essential oil decreases over time (the probable causes being volatilization and consumption of essential oil) - therefore decreasing the intensity of the fungistatic action of Red basil essential oil. Figure 7 also shows that the efficacy of antifungal solutions based on Red basil essential oil increases in proportion to its concentration.

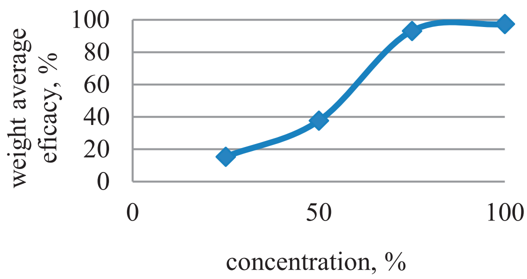

Figure 8. The dependence of the average temporal efficacy, on the concentration, for Red basil essential oil

Variation in the growth of Fusarium oxysporum and the efficacy of the treatment performed with the essential oil of Marigold, 'Nanuk' variety essential oil

The rules for graphical representation of data and regression lines are the same as for Basil (Yellow and Red), the figures being in correspondence with those for Yellow basil.

As in the case of Basil (Yellow and Red), Figure 9 shows, for the essential oil of Marigold, that the growth rays of the *F. oxysporum* fungi colony increase in time, in the interval of 3-10 days, and the greatest growth is the control (untreated sample). *Fusarium* colonies treated with essential oil increase in proportion to the inverse of the concentration of the Marigold essential oil solution. This means that the higher the concentration of Marigold essential oil used, the slower the growth of the fungus during the time the observations were made (3-10 days).

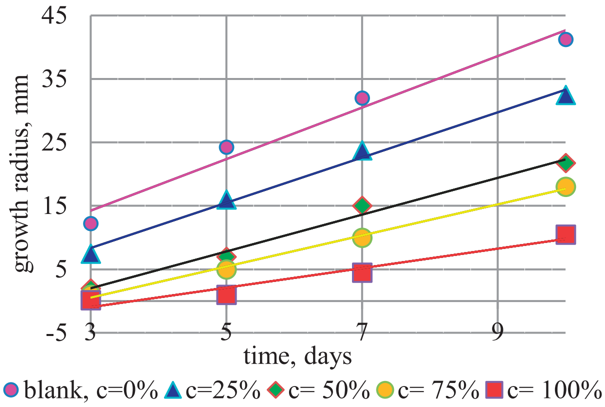

Figure 9. Dependence of the average values of the growth radius of the fungi colony and their trend lines, depending on the time, for the control and the 4 tested concentrations of Marigold essential oil

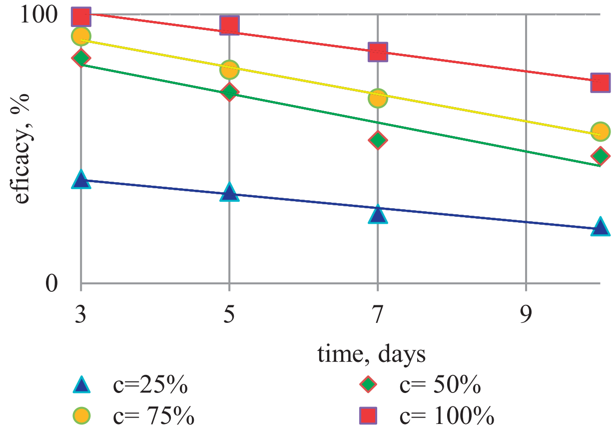

Figure 10. Time dependence of the efficacy of Marigold essential oil (4 different concentrations)

Figure 10 shows that the efficiency of any of the solutions containing different concentrations of Marigold essential oil decreases over time (the probable cause being the consumption of the essential oil in the antifungal action). Also, Figure 10 results that the efficacy of the antifungal solutions based on Marigold essential oil increases proportionally with the oil concentration.

Figure 11 shows that the time-weighted average efficiency increases with the concentration of Marigold essential oil used. The shape of the curve in Figure 11 suggests that for concentrations greater than 50%, the growths are slower compared to the growths found for oils with concentrations between 25% and 50%.

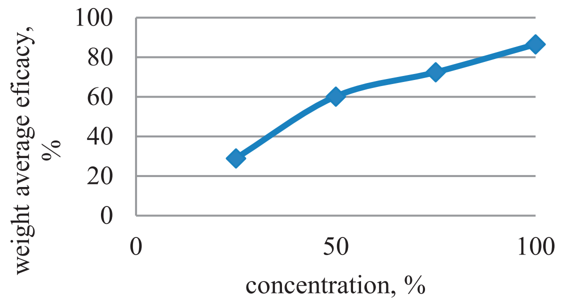

Figure 11. Concentration dependence of the temporal efficacy of Marigold essential oils

Variation in the growth of Fusarium oxysporum fungi and of the efficacy of Hyssop, 'Catalina' variety essential oil treatment

The rules for graphical representation of data and regression lines are similar to those previously performed for the 3 essential oils. The figures also correspond to the others.

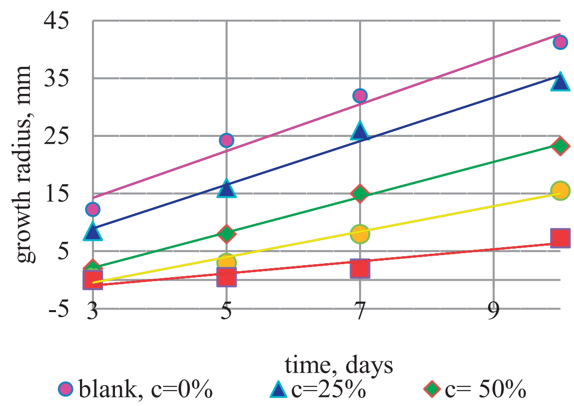

Figure 12. Dependence of the average values of the growth radius of the fungi colony and their trend lines, of time, for the control and the four concentrations of Hyssop essential oil

As in the previous cases presented, Figure 12 shows, for the Hyssop essential oil, that the radius of the fungi colony increases over time (interval 3-10 days). The highest increase is recorded for the control sample, and the other colonies of *F. oxysporum*, treated with Hyssop essential oil, increase in proportion to the inverse of the concentration of the oil used solution. This means that the higher the concentration of Hyssop essential oil, the slower the growth of the fungus (3-10 days).

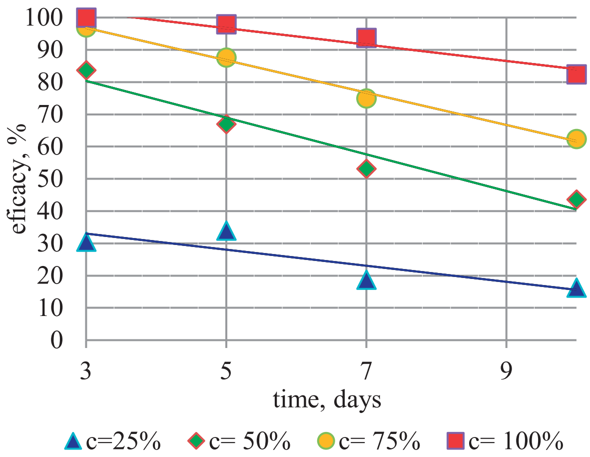

Figure 13. Time dependence of the efficacy of Hyssop essential oil (in 4 different concentrations)

Figure 13 shows that the efficacy of any of the concentrations used of Hyssop essential oil decreases over time, the probable causes being the volatilization and consumption of essential oil in the antifungal action) Also, Figure 13, results that the efficacy of the antifungal solutions based on Hyssop essential oil increases proportionally with the increase of the oil concentration in the used solution.

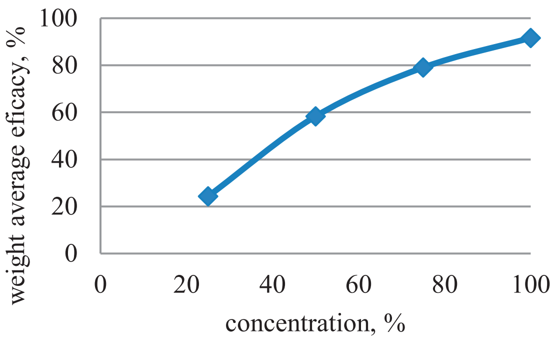

Figure 14. Dependence of the average temporal efficacy on the concentration, for the essential oil of Hyssop

Figure 14 shows that the time-weighted average efficiency increases with the increasing concentration of Hyssop essential oil to use for controlling the phytopathogenic fungus. The shape of the curve in Figure 14 suggests that as the concentration increases, the rate of increase in the efficacy of the treatment with Hyssop essential oil decreases slightly.

Following the paper, a study is made on the comparative estimation of the performances of the 4 essential oils (Yellow basil, Red basil, Marigold, Hyssop), each with 4 concentrations (25%, 50%, 75%, 100%).

Figure 15 shows the temporal dependencies of the efficacies of the 16 variants of essential oil solutions (4 essential oils with 4 concentrations each). The variations represented in figure 15 allow some conclusions, but also some questions regarding some directions for further research.

It is observed that for all the studied essential oils, obtained from the 4 medicinal plants, the efficacy of the treatment is proportional to the concentration of essential oil used. The best efficacy of the treatment is obtained at the value of 100% (pure oil), and the lowest is achieved at the value of the concentration of 25%.

This is a somewhat expected conclusion and validates the experimental results and the statistical calculation. The conclusion is confirmed by the graphical representations in Figures 4, 7, 10, 13.

At the level of the graphical representations of the descriptive statistics, Figure 15 results that, for most of the observation interval (3-10 days), all the essential oils analyzed with 100% concentration have the maximum efficacy, in the following order: Red basil, Basil yellow, Hyssop and Marigold. In the interval (of variable length between these curves), the oils with a concentration value of 75% extracted from Yellow and Red basil are interspersed (with compatible efficacy) in order, followed by Hyssop and Marigold. The oil solutions with a concentration value of 50% form a relatively compact beam in relation to the other curves, in order: Marigold essential oil, then Hyssop, and finally Yellow and Red basil. In the end, the lowest efficiency (where the bundle of curves of the solutions of the four oils falls), have the solutions with a concentration value of 25%, in order: Hyssop, Marigold, Yellow and Red basil.

The meaning of the order of the curves in Figure 15 is mathematically vague, because the order of the second curves may vary in the range of observation. The meaning of the notion of order in this paragraph is based on the observation of the time interval, in which a certain curve is "above" another. The precise mathematical term can be introduced in the case of knowing the optimal interpolation expressions and can be expressed in the case of the statistical study of nonlinear regression or surfaces of influence. However, such a study is not appropriate now, as very accurate results make sense only after a series of repeated experiments, to validate and increase the resolution over time and in the range of solution concentrations.

Also related to the graphical representation in figure 15, it is specified that the variation curves of the concentrations used are cubic spline curves, i.e. they have polynomial expressions of the third degree between every two experimental points where the neighbouring curves are connected, having first continuous derivative. These curves are automatically calculated by the program used for graphics, only for the purpose of making a nice-looking graphic. For this reason, there is no discussion of intersection points or extremes that appear on the graph.

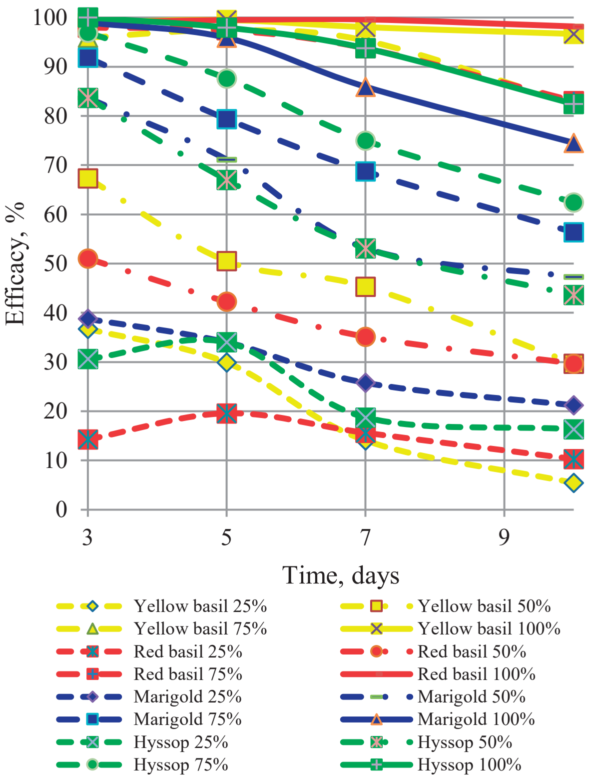

Figure 15. Time dependence of the efficacy of the 4 types of essential oils, each of them with 4 concentrations

Figure 16 shows the variations of the temporal mean values of the efficacy of the 4 essential oils studied and which allow the following conclusions:

The efficacy of the 4 essential oils (Yellow basil, Red basil, Marigold and Hyssop) increases with concentration, the growth being monotonous. This behaviour is confirmed by the graphical representations in Figures 5, 8, 11 and 14.

A less reliable observation (many other experiments with modifications to the above must be performed for consolidation) is that, up to a concentration value of 60-65%, control solutions based on Marigold and Hyssop essential oil, have a higher efficacy than Yellow basil and Red basil. At concentrations above 65%, the order changes and becomes similar to that shown in Figure 15.

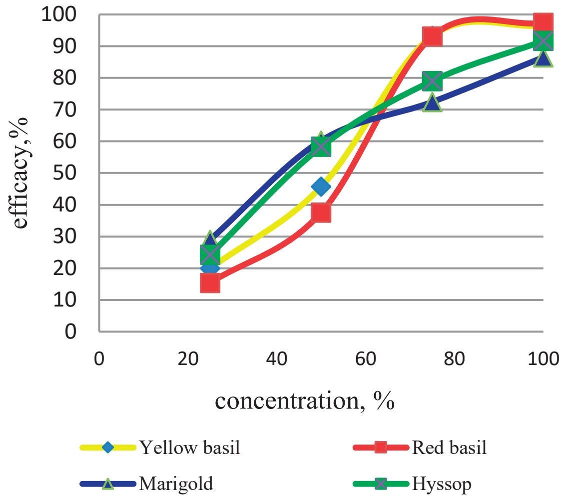

Figure 16. Time dependence of the average temporal value of efficacy, comparatively, for the 4 types of essential oils

The mean temporal efficacy across the concentration range of the solutions used in the experiments is given comparatively in the graphical representation in Figure 16. The calculation of this temporal mean over the entire observation interval is made according to the formula of the time average value of a variable, in the discrete formulation:

$$e_T = \frac{\sum_{i=1}^{N-1} \frac{(e_{i+1}+e_i)(t_{i+1}-t_i)}{2}}{T} \qquad (2)$$

where, in our case, e_T is the mean temporal efficacy over the observation interval, T=7 days, $\{e_i\}_{i=1,\dots,N}$, $\{t_i\}_{i=1,\dots,N}$, are the strings of the experimental results, in order of efficacy and reading times of the growth rays, and N is the number of concentrations (N = 4). It should be noted that e_T depends on the concentration used, which is why these average values allow representations as concentration functions (Figures 4, 7, 10, 13, 15). Considering then the series of time averages $\{e_{Tk}\}_{k=1,\ldots,N}$ and of the concentrations $\{c_k\}_{k=1,\ldots,N}$ (N having also the value four, corresponding to the concentrations: 25, 50, 75 and 100%), a similar calculation leads to the average value of efficacy over time and concentrations:

$$e_{TC} = \frac{\sum_{k=1}^{N-1} \frac{(e_{Tk+1} + e_{Tk})(c_{k+1} - c_k)}{2}}{C} \quad (3)$$

The range of concentrations that were experimentally worked on and calculated after (3) was $C \in \{25\%, 50\%, 75\%, 100\%\}$. The average value, depending on the time and concentrations, is a parameter of the global performance of the solutions based on essential oils obtained from the four medicinal plants, a parameter on the basis of which a hierarchy of the intensity of their control activity can be made. The graphical representation of the e_{TC} parameter for the four types of essential oils obtained from medicinal plants is given in Figure 17.

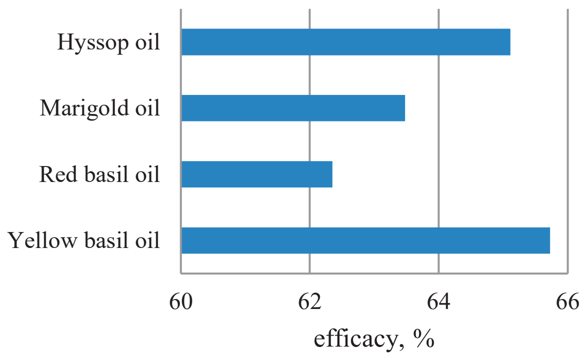

Figure 17. Average temporal efficacies of the 4 essential oils (comparative representation)

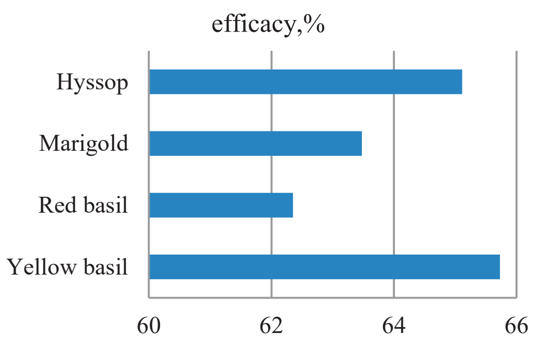

Figure 18. The dependences of efficacy values (%) on time and concentration

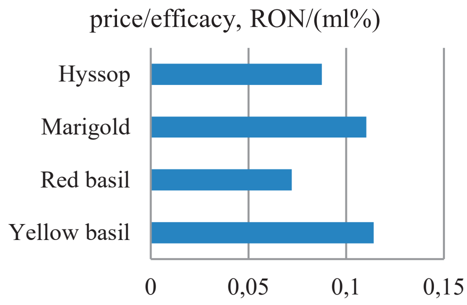

Figure 19. Price/efficacy ratios for the four types of essential oils

$$ef = \frac{e_{TC}}{p} \quad (4)$$

where ef is a measure of economic efficiency, and p is the price, both for each type of essential oil analyzed.

The ef parameter can be considered an approximate measure of efficiency. The graphical representation of the global efficiency was shown in Figure 18. Using these calculations, two more interesting conclusions can be drawn:

The total efficacy indicates the following order of action to control the fungus *F. oxysporum* (Figure 18): Yellow basil, Hyssop, Marigold, Red basil.

A measure of economic efficiency (*ef*), shows the following descending order: Yellow basil, Marigold, Hyssop and Red basil (Figure 19).

Similar research conducted in recent decades has led to similar or convergent conclusions to those stated above. For example, (Dakole et al., 2016) shows that the germination rate of *F. oxysporum* and *P. infestans* conidia decreases as the concentration of essential oils obtained from *C. citrinus*, *C. citratus*, *E. tereticornis* and *O. gratissimum* increases. Also in (Dakole et al., 2016), it is shown that the percentage of inhibition of mycelia growth in *F. oxysporum* and *P. infestans* obtained after treatments performed with ethanolic extracts and essential oils obtained from thirteen plants, increases with the concentration used. The increase in the inhibitory effect of some oils extracted from basil (*Ocimum basilicum* L.) in controlling the fungus *F. oxysporum* is clear from the numerical data presented in (Kocic-Tanackov et al., 2011).

Similar conclusions were obtained in (Nosrati et al., 2011) for essential oils from mint, in the control of the same. fungi, *F. oxysporum*. In

(Şesan et al., 2017) assessments are made similar to those resulting from this study, on the effects of oils extracted from various plants, including Hyssop. The doctoral thesis (Svecova, 2010) is dedicated to the phytopathogenic control of some phytopathogenic fungi existing in horticultural crops using natural plant extracts, among the results being given inhibition curves similar to those presented in this paper, but with an observation time interval that includes the moment initially of the experiments.

In the study conducted by (Pawar et al., 2007), 75 different essential oils (including Basil) were evaluated against two phytopathogenic fungi (*F. oxysporum* sp. *cicer* and *Alternaria porri*). The results showed that among the most active was the essential oil of Basil, at which the maximum inhibition rate was 17.25 mm.

CONCLUSIONS

The results obtained using the experimental results and the tools of descriptive statistics, allow obtaining conclusions regarding the process of controlling the fungus *Fusarium oxysporum*, using four essential oils obtained from new varieties of medicinal plants (Yellow basil, Red basil, Marigold and Hyssop):

- The intensity of the process of controlling the phytopathogenic fungus *Fusarium oxysporum* using four essential oils obtained from medicinal plants, depends on two essential variables: the time and the concentration used in the control process;
- It should be noted that the essential oils tested do not have fungicidal activity, but only fungistatic, being able to delay mycelia growth, the degree of inhibition depending on the concentration of essential oil and its composition;
- The calculated parameters and the graphical representations allowed the hierarchy of the action of the essential oils in different concentrations, but also global hierarchies over the considered time interval and over the time and concentration interval;
- A global parameter was also developed as an approximate measure of economic efficiency. For example, Yellow basil essential oil best meets the requirements for inhibiting fungal growth, but has the lowest economic efficiency of all studied.

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