MATHEMATICAL MODEL FOR COMPARISON OF THE INFLUENCE OF ESSENTIAL OILS AND HERBAL EXTRACTS ON THE MOULDS GROWTH

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ABSTRACT

The model for numerical comparison of the influence of essential oils and herbal extracts on the moulds growth, as exponential function with three parameters (S, λ, A), was proposed in this work. The model's basis is the functional relationship of the colony diameter dependent of the time and concentration of essential oils or herbal extracts. Three basical types of inhibition have been defined: inhibition to the Abscissa (IAb), inhibition of the Intensity (IIn), and inhibition to the Asymptote (IAs). The practical aplication of proposed model was done according to the results obtained after examination of the influence of different concentrations of *Allium ampeloprasum* (elephant garlic) essential oil on the growth of food spoilage causing moulds *Aspergillus tamarii*, *Penicillium griseofulvum* and *Eurotium amstelodami*. Function parameters of antifungal activity were calculated in view of 16-days time series of colony diameters, with 0, 1, 4, 7, 10 and 15% v/v essential oil of *A. ampeloprasum*. All obtained functions have high coefficient of correlation.
By comparison of parameters of obtained exponential functions, the activity of *A. ampeloprasum* essential oil to the *Aspergillus tamarii*, *Penicillium griseofulvum* and *Eurotium amstelodami* is numerically comparable. The defined functions could be used in matrix of inhibition and optimization of activity time and minimum inhibitory concentracione (MIC time) of antifungal agens.

1. INTRODUCTION

Moulds have been the most often contaminants and cause of food spoilage. They influence the health, nutritive and sensory quality of food, and cause the economical losses. Besides, some species from genus *Aspergillus, Penicillium, Fusarium, Alternaria, Eurotium* have the capability of synthesizing the toxic and cancerogenic metabolits (aflatoxins, ochratoxins, zearalenone, deoxinivaleol, fumonizine, sterigmatocystine, aleternariol, pianozonic acid, etc.), potentially harmful to human and animal health (IACR,1999; van Egmond, 2005).
The pogress of moulds have being tried to control by the addition of synthetic preservatives. However, due to their possible harmful effects, there have been rising the increasing interes in natural antimicrobial agents, extracted from spices and herbs, as alternative preservatives for lengthening the shelf life of food. This is especially the case in short shelf life products, as well as those packed in modified atmosphere. A number of works report about the influence of essential oils or extracts and their constituents to the moulds growth and micotoxin biosynthesis. There are few methods, describing the

examination of their influence on the moulds growth: agar plate method, disc-diffusion method (Rasooli and Abyaneh, 2004; Rasooli *et al.*, 2006), agar-diffusion, macro-, and microdilution method (Lopez-Malo *et al.*, 2007; Omidbeygi *et al.*, 2007), poisoned food (PF) technique (Chuita *et al.*, 2009). The most often applied is the agar plate method, based on the measurement of the colony diameter, in the presence of essential oil or herbal extract, during the time [Lopez-Malo *et al.*, 2007; Benkeblia, 2004; Vagi *et al.*, 2005; Fung and Zheng, 2007; Dimic *et al.*, 2007; Viuda-Martos *et al.*, 2008).

In this literature rewiev, a number of variables exists (different mould species, different types and concentration of essential oils and extracts, different activity time). Besides inhibition, stimulation of the moulds growth was stated in some cases. Sinergy and individual effects of essential oils and extracts to the moulds growth can be significantly different. Also, there is no standardized analytical method to compare the inhibitory activity of the most important variables, activity time and concentration of essential oils and extracts to different types of moulds.

The aim of this work was to determine the mathematical model which could enable the numerical comparison of influence of the activity time and concentration of essential oils and herbal extracts to the growth of the moulds, with the possibility of determination of MIC and MFC.

2. EXPERIMENTAL

Allium ampeloprasum *essential oil was obtained by water vapour distillation of the green plant (grown at the Institute of Field and Vegetable Crops, Novi Sad, Serbia). The plant was chopped in small pieces and mashed with domestic blender (Braun Minipimer MR 400). Sample was transfered into a 2L flask and mixed with distilled water (1:1 ratio), and the Clevenger apparatus was installed. The system was heated during 3h and the essential oil was collected in the petroleum ether layer, in the oil separator tube. When the extraction time run out, petroleum ether layer was collected in a centrifuge tube and left at room temperature to evaporate the solvent. The tube, containing essential oil, was sealed with rubber stopper and stored in refrigerator.*

Three moulds: *Aspergillus tamarii*, *Penicillium griseofulvum* and *Eurotium amstelodami* (isolated from spices) were used for antifungal investigations.

Microorganisms were stored in Potato dextrose agar (PDA), at temperature of 4°C. For this experiment, 7 days old cultures of moulds were used.

For each isolate, a conidial spore suspension (10^6 spores/ml) was prepared in medium which contained 0.5% v/v Tween 80 and 0.5% agar w/v in distillated water (Nielsen and Rios, 2000). Inoculation was performed with 1µl of spore suspension (10^3 spores/ml) in centred PDA medium in standard Petri dishes. Steril disc (5 mm) was placed in the centre of every dish cover, and 10µl of essential oil in concentracions of 0, 1, 4, 7, 10 and 15% v/v were added. Then, the plates were closed with parafilm and left to incubate at 25 °C for up to 16 days. Every day colony diameters were measured. All tests were performed in duplicate. Colony diameter was measured using orthogonal scales, and the mean value was calculated.

3. MODEL SELECTION FOR THE DESCRIPTION OF MOULDS GROWTH IN THE PRESENCE OF ESSENTIAL OILS AND HERBAL EXTRACTS

The moulds growth can be described using the exponential (Scheuring and Szathmáry, 2001) and Gompertz-type function (Gutiérrez-Jáimez et al., 2007). The exponential nature

of moulds development was described by models with constant and variable coefficients (Boswell, 2008). Description of the moulds growth, by everyday measurement of colony diameter, without inhibitors, can be expressed by exponential function $f(t) = 1 - e^{-\lambda(t)}$ where is: $\lambda(t)$ - growht intensity in the function of time. When the growth intensity is constant, i.e. λ=*const*, functional expression becomes
The maximal colony diameter is described by multiplying the expression (1) by the maximal value of diameter, A. The expression which considers maximal, asymptotical value of colony diameter, when λ=*const* and parameter A, is:

$$f(t) = A \cdot (1 - e^{-\lambda t}) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

Under hypothesis that moulds growth in the presence of inhibitors can be described by mentioned exponential distribution, three basic types of inhibition had been noticed. Those are: inhibition of moulds growth to the Abscissa – **IAb** (Figure 1a). inhibition of moulds growth intensity – **IIn** (Figure 1b), and inhibition of moulds growth to the Asymptote - **IAs** (Fig. 1c). The first type, inhibition of moulds growth to the Abscissa – **IAb** (Fig. 1a), occurs in the early phase moulds growth. Depending on the inhibition intensity, the beginning of growth is being shifted along Abscissa, at which the independent variable - time, is presented.
The second type of inhibition is presented by the change in intensity of moulds growth, which is noticeable the most in the mid phase mould growth **Iin** (Fig. 1b).
The third type of inhibition is noticeable the best in the late phase mould growth. In the case of inhibition of mould growth to the Asymptote - **IAs**, colony diameter converge to the value lower than the value in control conditions (Fig. 1c).

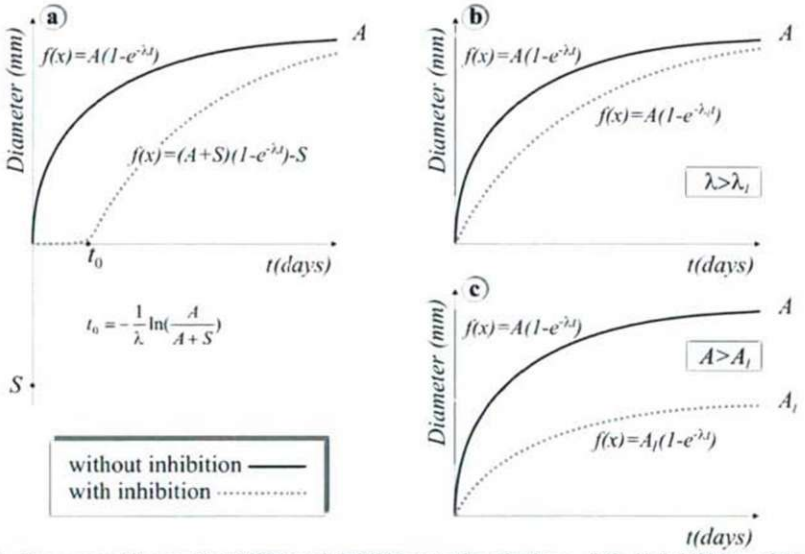

Figure 1. Types moulds growth inhibition a) Inhibition to the Abscissa – IAb, b) Inhibition of the moulds growth Intensity – Iin, c) Inhibition of the moulds growth to the Asymptota – Ias

The proposed exponential function (2) has two parameters, which can be used to describe the moulds growth inhibition Asymptote (parameter A) and inhibition of the moulds growth intensity (parameter λ). To describe the Inhibition moulds growth to the Abscissa -

IAb, it is necessary to introduce the third parameter into the exponential function (1). Theoretically, the beginning of the growth can be shifted to negative part of the ordinate (diameter axis). This is achievable by adding the S value to the value of Asymptote parameter, A. When the S value is subtracted from the function (1), the final function with three parameters, which can be used to describe the **IAb**, is derived is:

$$f(t)=(A+S)\cdot(1-e^{-\lambda t})-S=A-e^{-\lambda t}(A+S) \tag{2}$$

Now, it is necessary to calculate the period of **IAb**, i.e. to calculate the t_0 value, in which the function $f(t_0)=0$. The t_0 value presents the period of the **IAb**:

$$A-e^{-\lambda t_0}(A+S)=0 \Leftrightarrow e^{-\lambda t_0}(A+S)=A; \qquad t_0=-\frac{1}{\lambda}ln(\frac{A}{A+S}) \tag{3}$$

The exponential function with three parameters and time as independent variable, which describes all three types of inhibition, is equal to:

$$f(t,c)=\begin{Bmatrix} 0, & t\le t_0 \\ A-e^{-\lambda t}(A+S), & t>t_0 \end{Bmatrix}=\begin{Bmatrix} 0, & t\le t_0, c\ge 0 \\ A(c)-e^{-\lambda(c)t}(A(c)+S(c)), & t>t_0, c\ge 0 \end{Bmatrix} \tag{4}$$

Intensities of inhibition, in dependence on the inhibitors concentration, can be followed by the change of parameters S, λ and A. Introduction of the second independent variable into the function (4) is ensured by forming the functional connection of parameters S, λ and A, and inhibitors concentration c, $S(c)$, $\lambda(c)$ and $A(c)$.

4. APPLICATION OF THE PROPOSED MODEL TO RESULTS OF THE EXPERIMENT

Time series of empirical values of the moulds colony diameters in the presence of different concentrations of *A. ampeloprasum* essential oils for *A. tamarii*, *P. griseofulvum* and *E. amstelodami* are presented in Figures 2-4. Parameters S, λ and A are obtained by heuristic searching in EXCEL. Inhibition time t_0 is calculated using the formula (4). The ratio of the diameter values of time series of control (0% v/v of *A. ampeloprasum* essential oil) and other concentrations (1, 4, 7 and 10% v/v of *A. ampeloprasum* essential oil) with theoretical values of the function and calculated parameters S, λ and A, is described by the coefficient of correlation R^2 and parameters of linear regression line (a, b).

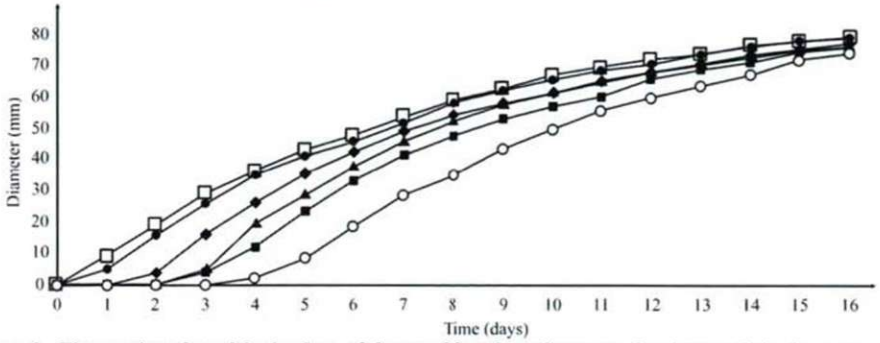

Figure 2. Time series of empirical values of the moulds colony diameters for A. tamarii in the presence of different concentrations of A. ampeloprasum essential oil.
Fat contents: (□) 0%, (●) 1%, (◆) 4%, (▲) 7%, (■) 10% and (○) 15%

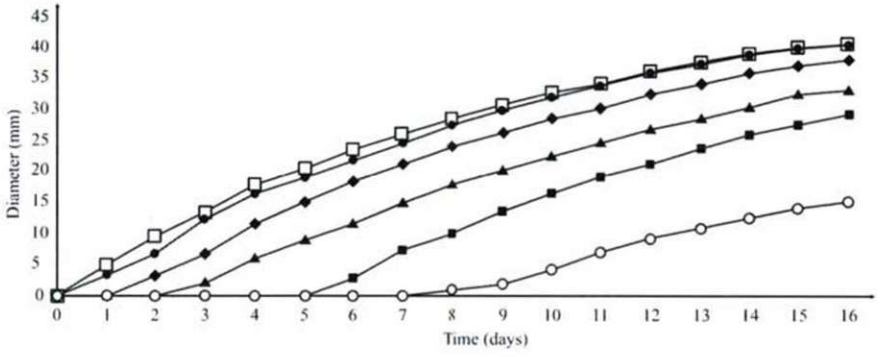

Figure 3. Time series of empirical values of the moulds colony diameters for P. griseofulvum in the presence of different concentrations of A. ampeloprasum essential oil.
Fat contents: (□) 0%, (●) 1%, (◆) 4%, (▲) 7%, (■) 10% and (○) 15%

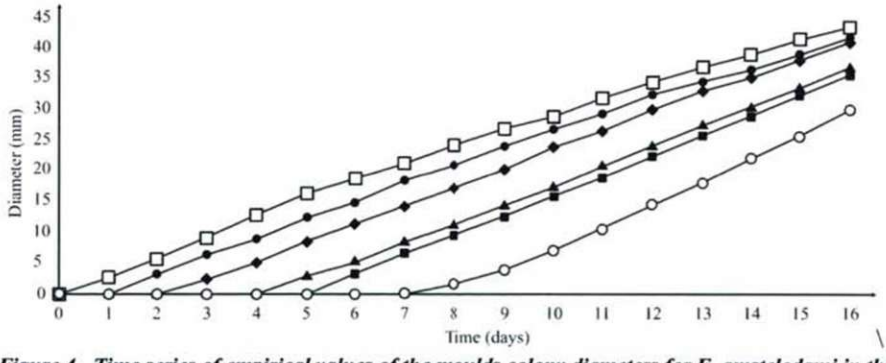

Figure 4. Time series of empirical values of the moulds colony diameters for E. amstelodami in the presence of different concentrations of A. ampeloprasum essential oil.
Fat contents: (□) 0%, (●) 1%, (◆) 4%, (▲) 7%, (■) 10% and (○) 15%

5. MOULDS COLONY DIAMETERS IN FUNCTION OF TIME AND CONCENTRATION OF *A. AMPELOPRASUM* ESSENTIAL OIL

IAb with *A. ampeloprasum* essential oil is found for all three examined moulds. Changes of parameter S in function of concentrations of 0, 1, 4, 7 and 10% v/v *A. ampeloprasum* essential oils (Table 1.) are presented by regression lines with calculated coefficient of correlation. If c is the concentration of *A. ampeloprasum* essential oil concentration, then the functional connections of parameter S (dependent variable) for examined moulds and concentrations c (independent variable) are:

Table 1. Parameters and coefficient values for A. tamarii, P. griseofulvum and E. amstelodami in conditions of inhibition with different concentrations of A. ampeloprasum essential oils

	Concentration (%)	S	λ	A	t_0	R^2	a	b
A. tamarii	0	0.00	0.1526	86.74	0.000	0.9975	0.973	3.310
	1	12.30	0.1526	86.74	0.869	0.9982	1.019	-0.712
	4	26.07	0.1526	86.74	1.721	0.9992	1.010	-0.790
	7	39.32	0.1526	86.74	2.449	0.9991	0.964	0.069
	10	50.86	0.1526	86.74	3.023	0.9988	0.994	-0.822
	15				for validation			
P. griseofulvum	0	0.00	0.1109	43.98	0.000	0.9997	1.014	-0.181
	1	2.66	0.1075	43.98	0.546	0.9991	0.974	0.272
	4	4.79	0.0972	43.98	1.064	0.9988	0.955	1.127
	7	10.88	0.0850	43.98	2.586	0.9981	0.980	0.901
	10	21.10	0.0777	43.98	5.044	0.9716	0.886	2.543
	15				for validation			
E. amstelodami	0	0.00	0.0403	91.86	0.000	0.9992	0.998	0.757
	1	4.15	0.0403	91.86	1.094	0.9989	1.004	0.323
	4	9.59	0.0403	91.86	2.460	0.9989	0.982	0.225
	7	17.96	0.0403	91.86	4.424	0.9991	0.995	0.023
	10	19.94	0.0403	91.86	4.868	0.9985	0.969	0.422
	15				for validation			

$$S_{A.\ tamarii} = 4.8382\ c + 4.4421,\ R^2 = 0.9765 \quad (5)$$

$$S_{P.griseofulvum} = 1.9578\ c - 0.7288,\ R^2 = 0.9385 \quad (6)$$

$$S_{E.astelodami} = 2.0291\ c + 1.3999,\ R^2 = 0.9625 \quad (7)$$

The only change of growth intensity was observed for *P. griseofulvum*. The intensity of growth of *P. griseofulvum* is indirect proportional to applied concentration of *A. ampeloprasum* essential oil. The intensity of growth did not change in dependence of applied essential oil concentration for *A. tamarii* ($\lambda = 0.1526 = const$) and *E. amstelodami* ($\lambda = 0.0403 = const$). It means that essential oil inhibited *P. griseofulvum* also by inhibition of the growth intensity. That is the reason to find out the functional connection of parameter λ and the *A. ampeloprasum* essential oil concentration. Functional dependence of parameter λ and concentration c now becomes: $\lambda_{P.griseofulvum} = -0.0034\ c + 0.1107$.

For none of examined moulds the **IAs** with essential oil was not detected. Two-dimensional function for description of the *A. tamari* colony diameter growth in conditions of inhibition with different concentrations of *A. ampeloprasum* essential oil (independent variable c) and time (independent variable t) is:

$$f(t,c)_{A.tamarii} = 84.76 - e^{0,1526t}(84.76 + (4.8382\,c + 4.4421)), \quad t > t_0, c \geq 0$$

$$\text{where: } t_0^{A.tamarii} = -\frac{1}{0.526} ln(\frac{84.76}{84.76 + (4.8382\,c + 4.4421)}) \tag{8}$$

Two-dimensional function for description of *P. griseofulvum* colony diameter growth in conditions of inhibition with different concentrations of *A. ampeloprasum* essential oil (independent variable *c*) and time (independent variable *t*) is:

$$f(t,c)_{P.griseofulvum} = 43.98 - e^{(0,1107-0,0034c)t}(43.98 + (1.9578\,c - 0.7288)), t > t_0, c \geq 0$$

$$\text{where: } t_0^{P.griseofulvum} = -\frac{1}{0.1107 - 0.0034c} ln(\frac{43.98}{43.98 + (1.9578\,c - 0.7288)}) \tag{9}$$

Two-dimensional function for description of *E. amstelodami* colony diameter growth in conditions of inhibition with different concentrations of *A. ampeloprasum* essential oil (independent variable *c*) and time (independent variable *t*) is:

$$f(t,c)_{E.amstelodami} = 91.86 - e^{0,0403t}(91.86 + (2.0291\,c + 1.3999)), t > t_0, c \geq 0$$

$$\text{where: } t_0^{E.amstelodami} = -\frac{1}{0.0403} ln(\frac{91.86}{91.86 + (2.0291\,c + 1.3999)}) \tag{10}$$

6. APPLICATION OF MODEL AND MATRIX OF INHIBITION

From the formula (5), the parameter *S*, which is in functional connection with *A. ampeloprasum* essential oil concentration, can be expressed. The obtained formula gives the possibility of calculation of essential oil concentration necessary for requested period of **IAb**. In the following calculations, the period of **IAb** of t_0=10 days is adapted. Function of time and *A. ampeloprasum* essential oil concentration for *A. tamarii* is (8):

$$4.8382c + 4.4421 = 86.74\frac{1 - e^{-0,1526t_0}}{e^{-0,1526t_0}} \Leftrightarrow c = \frac{86.74(1 - e^{-0,1526 \cdot 10})}{4.8382e^{-0,1526 \cdot 10}} - \frac{4.4421}{4.8382} = 63.62$$

IAb of the growth of *A. tamarii* for t_0=10 days is being achieved with *c*=63.62% v/v of essential oil, which is the MIC. For calculation of **IAb** of *P. griseofulvum* in period of t_0=10 days, it is necessary to introduce the function of parameter λ in ascertained form: $\lambda_{P.griseofulvum}$ = 1.9578·*c*–0.7288=0. It is obvious from equation that total inhibition of *P. griseofulvum* growth begins at concentration of *c*=32.55% v/v of essential oil. The concentration of essential oil of 32.55% v/v is the MFC for *P. griseofulvum*. **IAb** of the growth of *P. griseofulvum* for t_0=10 (3, 9) days is being obtained by numerical interpolation for the essential oil concentration value of *c*=16.59% v/v . **IAb** of the growth of *E. amstelodami* of t_0=10 (10) days is being achieved with *c*=21.77% v/v of essential oil (MIC). On the basis of derived analyses matrix of inhibition is formed (Table 2):

Table 2. Matrix of inhibition of A. ampeloprasum essential oil for A. tamarii, P. griseofulvum and E. Amstelodami, t_0=10 days

Moulds	Inhibition parameters				
	S (**IAb**)	λ (**IIn**)	A (**IAs**)	MIC(%)	MFC(%)
A.tamarii	*4.8382c+4.4421*	–	–	63.62	–
P.griseofulvum	*1.9578c–0.7288*	*0.1107–0.0034c*	–	16.59	32.55
E.amstelodami	*2.0291c+1.3999*	–	–	21.77	–

A. ampeloprasum essential oil has the singular system of inhibition for *A. tamarii* i *E. amstelodami*, and double system of inhibition for *P. griseofulvum*. For 10 days growth inhibition the highest concentration is necessary for *A. tamarii* (63.62% v/v), and the lowest for *P. griseofulvum* (16.59% v/v). *A. ampeloprasum* essential oil is capable to completely inhibit onlu *P. griseofulvum*, with MFC=32.55% v/v.

7. MODEL VALIDATION

Theoretical functions for c=15% v/v of *A. ampeloprasum* essential oil were carried out for validation on the basis of obtained functional dependence of essenatial oil concentration and parameter S, from formulae (5, 6, 7) and λ for *P. griseofulvum*.. The results of calculation of parameters, coefficients of correlation of empirical and theoretical moulds colony diameter values and coefficients of linear regression line are given in Table 3.

Table 3. Values of parameters and coefficients for examined moulds in conditions inhibition with 15% v/v of A. ampeloprasum essential oil

Moulds	S	λ	A	R^2	a	b
A. tamarii	76.9951	0.1526	86.74	0.9971	1.0033	0.7241
P. griseofulvum	28.6382	0.0597	43.98	0.9975	0.8680	0.1140
E. amstelodami	34.5364	0.0403	91.86	0.9946	1.1129	0.1346

The results of empirical values of time series of *A. tamarii* colony diameters, *P. griseofulvum* and *E. amstelodami* with 15% v/v of *A. ampeloprasum* essential oil and theoretical values of time series of colony diameters calculated for c=15% v/v on the basis of function (8) for *A. tamarii*, function (9) for *P. griseofulvum*, and function (10) for *E. amstelodami*, are given in Figures 2, 3 and 4, respectively.
Inhibition of *A. tamarii* with *A. ampeloprasum* essential oil has been excellently described by the proposed model. For *P. griseofulvum*, the time of **IAb** of empirical and theoretical values altered solution is needed. Due to high coefficient of correlation value, the alteration is being performed by multiplying the theoretical function with coefficient of linear regression line a=0.8680. For *E. amstelodami*, application of altered solution is needed. High coefficient of correlation enables the simple alteration by multiplying the total theoretical function by coefficient a=1.1129.

8. CONCLUSION

The proposed model has been synchronized with system of defined types of inhibition, by three parameters, S, λ and A: **IAb** , **IIn** and **IAs**, respectively. In spite of this, in case of stimulative activity of essential oil or herbal extracts to moulds growth, the model acts by positive logics.

The practical application of model for description of the growth of examined moulds in the presence of *A. ampeloprasum* essential oil showed successful interpolation and partially successful, but easy adoptable, extrapolation with experimental results. The applied model transparently compares the influence of essential oil to examined moulds.
The most possible intervals of MIC and MFC could be predicted by extrapolation of model values. The ratios of activity time and essential oil or extract concentrations to moulds growth can be optimized by interpolation of model values.
For application of proposed model, it is needed to examine the concentrations of essential oil from 0% to MIC or MFC, according to type of inhibition. With this, the calculations of essential oil inhibitory activity are being adjusted to interpolation. The defined functions could be used in matrix of inhibition and optimization of activity time and concentration of antifungal agens.

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