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SYNERGY EFFECTS OF NATURAL FUNGAL INHIBITORS CALCULATED BY QUEUING MODEL

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Abstract. *Model is based on the fungal birth and death processes. Model is suited for Petri dish. Growth of fungal colony diameter in Petri dish is described with exponential function. The value of diameter is declared as integer variable. Integer variable with 1 mm increment is a discrete state of the system. Time in the system is continuously. Discrete states, continuous time and exponential growth are basis for the application of queuing systems in the Petri dish. Queuing system clearly separated the intensity of birth and death. Difference between the birth intensity and death intensity is declared as the fungal life cycle. Fungal life cycle variable is extra sensitive to the inhibitors effects. The procedures for parameters calculation are mathematically explained, as well as the significance of the obtained parameters. Application of the model is performed for F. verticilloides in control conditions and at 16% concentration of basil and clove essential oils. Life cycle minimum is the synergetic inhibition maximum. For F. verticilloides, synergetic inhibition maximum is at 42% of basil and 58% of clove in 16% essential oil concentration.*

Key words: *fungi, synergy, inhibition, essential oil, natural extract*

1. Introduction

Exponential probability distribution has exceptional constitutive characteristics such as maximum entropy, constant hazard function and it is memoryless. If the random evolution of a system is exponentially distributed, then this system is memoryless. In memoryless system, future state depends only on its present state, and not on any past states. The exponential distribution is the only distribution that

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has memoryless property. Also, exponential function with the base e = 2.718281, $f(x)=e^x$ has one unique feature. Function $f(x)=e^x$ and her arbitrary derivation are identical, $f(x)' = f(x)'' = \ldots = f(x)^{(n)} = e^x$. This feature is the basis of memoryless.

Mycelium growth (Kang et al., 2003, Vargas-Arispuro et al., 2005; Boyraz and Özcan, 2006, Judith et al., 2008, Villa et al., 2009), hifae growth (Larralde-Corona et al., 1997 ; Kampichler et al., 2004; Diéguez-Uribeondo et al., 2004), spore count (Wagner et al., 2001) and fungal germination under extreme conditions (Onofri et al., 2007) have exponential properties.

Alteration of the fungal colony diameter (Roller and Covill, 1999; Tzortzakis and Economakis, 2007; Taniwaki et al., 2009, Tang et al., 2009), fungal colonies in the presence of bacteria (Brule et al., 2001), the development of fungal biomass (Damar et al., 2006), the influence of various inhibitors (Collopy-Junior et al., 2006), essential nutritienats (Ramirez et al., 2004) and the impact of radiation on the fungal growth (Maity et al., 2008) can be described with exponential distribution.

Indirect evidence about exponential fungal dynamics we can find in the fungal degradation process (Kim et al., 2000; Schober and Trösch, 2000; Mal-Nam et al., 2000; Ruiz-Aguilar et al., 2002; Ishii et al., 2007, 2008; Wakaizumi et al., 2009; Tanaka et al., 2009, Elsherbiny et al., 2017).

Fungal growth occurs in the system of self-replicator species (Scheuring and Szathmáry, 2001; Chertov et al., 2004; Milne, 2008; Boswell, 2008). Self-replicator growth system is the basis of analogy between fungal growth and exponential function. Indirectly, through an exponential distribution, fungal systems are memoryless. Analogously, the fungal growth is the Markovian process.

Management of microbiological systems has significant economic, environmental and health aspects. The microbiological control of foods is particularly significant. In the case of fungi, control of growth by using inhibitors is based on compromise. Inhibition need to meet the requirements of microbiological quality and in the same time, to preserve the nutritional, health and organoleptic properties of food.

The intensity of fungal inhibition is commonly investigated with the agar plate method, based on the measurement of the colony diameter, in the presence of essential oil or herbal extract during the time. (Nielsen and Rios, 2000; Guynot et al., 2003; Suhr and Nielsen, 2003; Benkeblia, 2004; Pereira, et al., 2006; Sheng-Hsien et al., 2007; Lopez-Malo et al., 2007; Fung and Zheng, 2007; Tullio et al., 2007; Soylu et al., 2007; Viuda-Martos et al., 2007, 2008; Tzortzakis, 2009; Reddy et al., 2009; Tatsadjieu et al., 2009, Tanackov S. et al., 2014; Badea et al., 2016; Llana-Ruiz-Cabello et al., 2016 Tancinová et al., 2018, 2019).

Inhibitors can be synthetic and natural. Use of synthetic inhibitors is not always desirable, especially in food. Essential oils and plant extract are the main natural inhibitors. A special analytical challenge is the potential synergic effects in the application of inhibitors. Synergy inhibitors may improve the composition of the combinatorial selection of inhibitors with greater antifungal effect and more acceptable organoleptic characteristics.

The inhibition intensity is determined by the comparative method a posteriori. This method is based on determining the initial birth rate without inhibition. In the presence of inhibitors, a reduced birth rate is obtained. The comparison (difference)

of these two intensities represents the difference in the birth intensity, again. The intensity of dying due to inhibitory effects remains unknown. Individual inhibitor intensities can be estimated by standard procedure, but the synergistic effect of two or more inhibitors is difficult to describe by existing models.

Considering the growth of colonies as a stochastic system opens up the possibility of applying a queuing system (QS). The birth and death intensities in microbiology analysis are analogous to the intensities of clients arrivals and clients servicing from queueing systems. The capacity of QS models has been proven in many fields of research (Fazlollahtabar anf Gholizadeh, 2019a; Fazlollahtabar and Gholizadeh, 2019b; Tanackov I. et al, 2019a, Tanackov I. et al, 2019b)

2. Materials and methods

2.1. Experimental setup

For the antifungal activity testing, commercially available, food grade clove and basil extract was provided from ETOL "Tovarna arom in eteričnih olj" d.d., Celje, Slovenia.

As test microorganisms, the following fungal strain from the genus *Fusarium* was used: *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* Sheld.). The fungal culture were isolated from cakes and maintained on Potato Dextrose Agar (PDA) at 4°C as a part of the collection of the Laboratory for Food Microbiology at the Faculty of Technology, University of Novi Sad, Serbia.

The agar plate method was applied in the testing of the antifungal activity of extracts. The basic medium for the antifungal tests was PDA. The medium was divided into equal volumes (150 ml), poured into Erlenmeyer (250 ml) flasks and autoclaved at 121ºC for 15 min. Concentrations 0 i 0.16% (v/v) were tested self-contained extracts, and basil-clove combinations: 50%:50%; 75%:25% i 25%:75%. The extracts were added to medium after cooling to 45°C. The culture medium was then poured into sterile Petri dishes (ϕ9 cm), 12 ml into each plate.

To prepare the conidial suspension dispute we used the seven-day culture *F. verticillioides* grown on PDA. Suspension of fungi prepared in a medium which contained 0.5% Tween 80 and 0.2% agar dissolved in distilled water and were adjusted to provide initial spore count of 10^6 spores/mL by using a haemocytometer. For each extract dose and fungi species, including the controls were centrally inoculated by spreading 1 µl of spore suspension (10^3 spores/ml) using an inoculation needle. After inoculation, the Petri plates were closed with parafilm. The efficacy of the treatment was evaluated by daily measurement of the diameter of radial colony growth during 14 days of incubation at 25 ±2ºC (table 1.).

2.2. Markovian process in Petri dish

Exponential parameter (Whitt, 2018; Tanackov et al., 2019) of fungal growth ξ is a function of the intensity of birth λ and death μ, $\xi=f(\lambda,\mu)$, provided $\lambda \geq \mu$. In Petri dish queuing system, number of microorganisams determines the state of the system. Description with Markovian birth-death process is based on the exponential intensity of birth λ and death μ. If the initial state of the system is defined with zero

microorganisms, then the initial intensity of death μ is also equal to zero. The system goes into a state one microorganism with intensity λ. Simultaneously with the transition to a state one microorganism, death process with intensity μ is started. In the same time with the transition to a state with one microorganism, process of death with intensity μ start. From the state with one microorganism, system exceeds to the state with two microorganisms by the same intensity of the birth, λ. If the system implemented another birth with intensity λ, and the first micro-organism has not finished the process of dying, the system exceeds in to the state two microorganisms. The dying process of the first microorganism is not complited and the second microorganism begins the process of dying. Therefore, the intensity of death in a state two microorganisms is equal 2μ. System with the same birth intensity exceeds in the next state, and with multiplicity intensity of death exceeds to a previous state. If the number of microorganisms is equal to k in the system, then all k microorganisms are in the process of dying. Therefore, the intensity of dying in system with k microorganisms is equal to $k\mu$. System with k microorganisms cannot realize $(k+1)\mu$ intensity of the death. This relationship between the intensity of birth λ and intensity of death μ, can be described with exponential development of fungal colonies in Petri dish (Fig. 1).

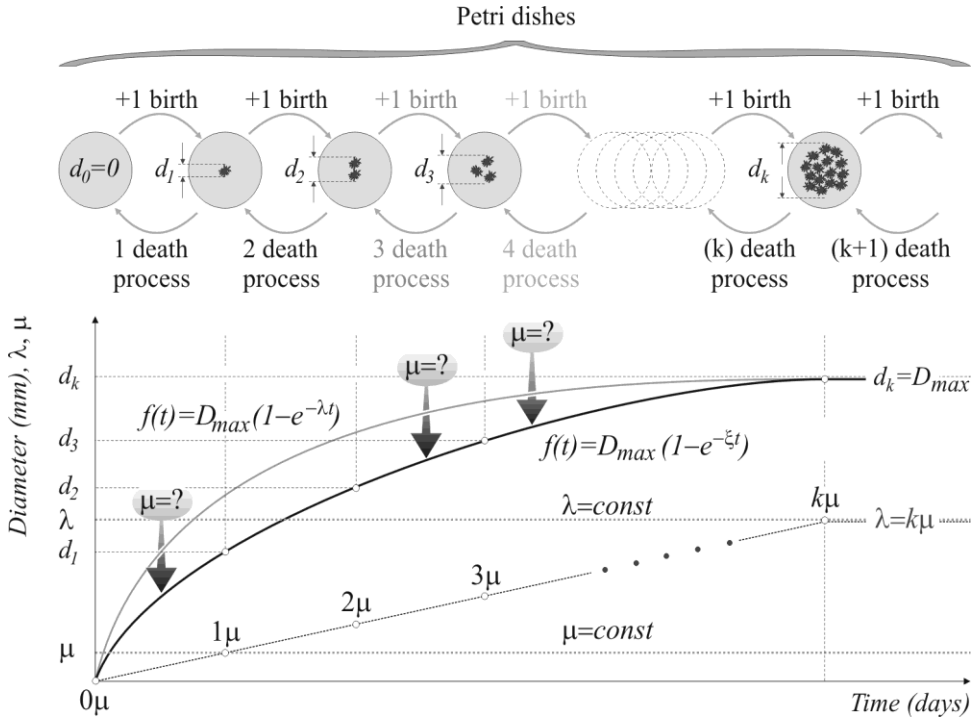

Figure 1. Fugal colony, exponential development

At asymptote diameter, the intensity of birth and k multiplicated death are identical, $\lambda=k\mu$. The value of the colony diameter is equal to asymptote value which is maximal colony diameter D_{max}. This point have a crucial importance for solving the explicit form of the function $\xi=f(\lambda,\mu)$. The solution to the intensity of dying is now

available, initially in steady-state mode. If necessary, the intensity of dying can be considered as non-stationary in time, and additional possibilities are consideration of non-stationary intensity of dying in conditions of different temperatures, humidity, initial inoculation or other important microbiological parameters.

2.3. Petri dish Queuing system

Marcovian processes in the system are determined with the time and state of the system. Time in the system can be discretely and continuously. State of the system can be discrete and continuous, also.

Consecutive time intervals for fungal colony diameter measurements were determined by SI unit, time.

These intervals are discrete, usually 1 day. Measurement of fungal colony diameters in Petri dish, are recorded in the SI unit of length, in millimetres or centimetres. Development of fungal colonies declared this dimension as a variable. Diameters values are represent in the time series.

The time is independent variable, and the diameter is dependent variable. Due to the nature of the fungal colony development, the total number of fungi cannot be precisely determined. All elements of the fungal colony development are synthesized in the diameter.

Diameter is a generalized variable of the system. The state of the fungal colony is continuous variable.

Fungal colony diameter (d_0, d_1, d_2, d_3, …, d_k), $f(t_i)=d_i$ time series in Petri dish, for discrete time intervals Δt, (t_0, t_1, t_2, t_3, …, t_k), $t_{(i+1)} = t_i+\Delta t$, $k\in[0, 1, 2, \dots, n]$ have a form of exponential function:

$$f(t_k) = d_k = D_{max}(1-e^{-\xi t_k}) \quad k\in[0, 1, 2, \dots, n] \tag{1}$$

A high approval of empirical and theoretical data is necessary condition for regular description of the fungal colony diameter with the exponential function. This agreement can be expressed with the correlation coefficient. The linear regression of empirical and theoretical data must be described with fulfil values of parameters $a\approx1$ and $b\approx0$, in addition to the high value of correlation coefficient $r\approx1$.

A fulfilment of these conditions gives a representative description of the empirical time series with exponential theoretical function. Discrete values of colony diameter can be obtained with integer function values from representative function.

$$s(t) = [f(t)] = INT\left[D_{max}(1-e^{-\xi t})\right] \tag{2}$$

Approximation of $f(t)$ with the function $s(t)$ depends from the increment size. Smaller increment has a better approximation. For fungal colony diameter measuring in millimetres, integer increment 1 mm gives a satisfactory approximation. With discrete values of the colony diameter, are fulfilment conditions for the application of Markovian process with continuous time. Continuous time with discrete states of the system allows the Petri dish to formation Markovian queuing system (Fig. 2).

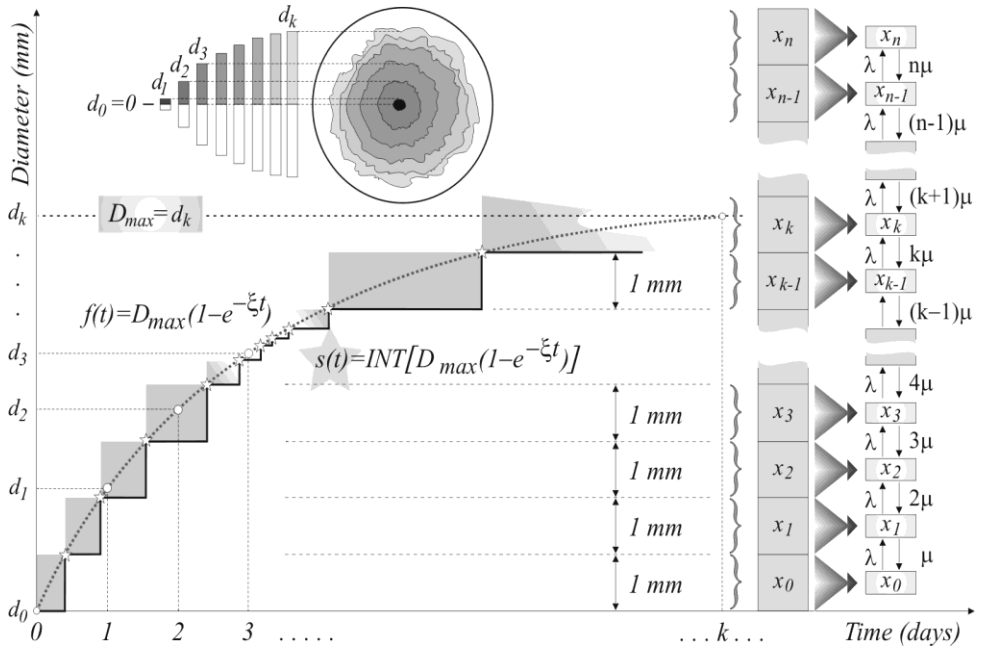

Figure 2. Petri dish Markovian queuing system

Explicit form of the function $\xi=f(\lambda,\mu)$ have two unknown variables, λ and μ. For the calculation of their values, it is necessary to define a system of two equations. The first equation is obtained from initial conditions. At the beginning of growth, at t=0, the intensity of death is equal to zero, μ=0. Intensity of birth λ is equal to:

$$D_{max}(1-e^{-\xi t})'_{t=0} = (D_{max}\xi e^{-\xi t})_{t=0} = \lambda \Leftrightarrow \lambda = D_{max}\xi \tag{3}$$

The second equation can be obtained from the development of state and calculation of the average number of clients in the Markovian system (Fig. 3).

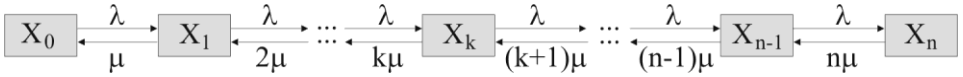

Figure 3. Development of Markovian's system mold colonies in Petri dishes

Differential equations of queuing states in the stationary mode, with constant values of birth and death intensity $\lambda(t)=\lambda=const$ and $\mu(t)=\mu=const$, are:

$$p_0(t)' = 0 = -\lambda p_0 + \mu p_1 \Leftrightarrow p_1 = \frac{\lambda}{\mu} p_0$$

$$p_1(t)' = 0 = \lambda p_0 - \mu p_1 - \lambda p_1 + 2\mu p_2 \Leftrightarrow 0 = -\frac{\lambda^2}{\mu} p_0 + \mu p_2 \Leftrightarrow p_2 = \frac{1}{1\cdot 2}\left(\frac{\lambda}{\mu}\right)^2 p_0$$

$$p_2(t)' = 0 = \lambda p_1 - 2\mu p_1 - \lambda p_1 + 3\mu p_2 \Leftrightarrow 0 = -\frac{\lambda^3}{2\mu^2} p_0 + 3\mu p_2 \Leftrightarrow p_3 = \frac{1}{1\cdot 2\cdot 3}\left(\frac{\lambda}{\mu}\right)^3 p_0$$

. . . . .

$$p_k(t)' = 0 = \lambda p_{k-1} - \mu p_k - \lambda p_k - \mu p_{k+1} \Leftrightarrow p_k = \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k p_0$$

. . . . .

$$p_n(t)' = 0 = +\lambda p_{n-1} - n\mu p_n \Leftrightarrow p_n = \frac{1}{n!}\left(\frac{\lambda}{\mu}\right)^n p_0 \tag{4}$$

In the n previous equations we have $(n+1)$ unknown variables, $k \in [0, 1, 2, \ldots, n]$. Equation needed to solve this system of equations, we can obtaine from the basic requirements of probability states:

$$p_0 + p_1 + p_2 + \ldots + p_{n-1} + p_n = 1 \Leftrightarrow p_0 + \frac{\lambda}{\mu} p_0 + \ldots + \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k p_0 + \ldots + \frac{1}{n!}\left(\frac{\lambda}{\mu}\right)^n p_0 = 1$$

From these $(n+1)$ equations, probability of state p_0 is:

$$p_0\left(1 + \frac{\lambda}{\mu_0} + \ldots + \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k + \ldots + \frac{1}{n!}\left(\frac{\lambda}{\mu}\right)^n = p_0 \sum_{k=0}^{n} \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k = 1 \Leftrightarrow p_0 = \frac{1}{\sum_{k=0}^{n} \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k}\right. \tag{5}$$

Recurrent equation for calculating the probabilities of the queuing system states is obtained from (4) and (5):

$$p_k = \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k p_0 \Leftrightarrow p_k = \frac{\frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k}{\sum_{k=0}^{n} \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k} \tag{6}$$

The average number of clients in the system is obtained from the (6):

$$\sum_{s=0}^{n} s \cdot p_s = \sum_{s=0}^{n} s \cdot \frac{\frac{1}{s!}\left(\frac{\lambda}{\mu}\right)^s}{\sum_{k=0}^{n} \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k} = \sum_{s=0}^{n} \frac{\frac{s}{s!}\left(\frac{\lambda}{\mu}\right)^s}{\sum_{k=0}^{n} \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k} = \left(\frac{\lambda}{\mu}\right) \frac{\sum_{s=1}^{n} \frac{1}{(s-1)!}\left(\frac{\lambda}{\mu}\right)^{s-1}}{\sum_{k=0}^{n} \frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k} \tag{7}$$

The exponential function $e^{\frac{\lambda}{\mu}}$ may be written as a Taylor series:

$$\sum_{n=0}^{\infty}\frac{\left(\frac{\lambda}{\mu}\right)^n}{n!}=\frac{\left(\frac{\lambda}{\mu}\right)^0}{0!}+\frac{\left(\frac{\lambda}{\mu}\right)^1}{1!}+\frac{\left(\frac{\lambda}{\mu}\right)^2}{2!}+\frac{\left(\frac{\lambda}{\mu}\right)^3}{3!}+\cdots=e^{\frac{\lambda}{\mu}} \Rightarrow$$

$$\lim_{n\to\infty}\frac{\sum_{s=1}^{\infty}\frac{1}{(s-1)!}\left(\frac{\lambda}{\mu}\right)^{s-1}}{\sum_{k=0}^{\infty}\frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k}=\frac{e^{\frac{\lambda}{\mu}}}{e^{\frac{\lambda}{\mu}}}=1$$

$$\frac{\sum_{s=1}^{n}\frac{1}{(s-1)!}\left(\frac{\lambda}{\mu}\right)^{s-1}}{\sum_{k=0}^{n}\frac{1}{k!}\left(\frac{\lambda}{\mu}\right)^k}\approx 1$$

For a large enough n, we can adopt

Average number of clients in the system is equal:

$$\sum_{s=0}^{n}s\cdot p_s=\frac{\lambda}{\mu} \tag{8}$$

In stationary mode of ergodic Marcovian queuing system, this value (8) is equal to the average diameter of the fungal colony d_{ave}. From birth intensity initial conditions and from average number of clients, the value of death intensity μ is:

$$d_{ave}=\frac{\lambda}{\mu}\Leftrightarrow d_{ave}=\frac{D_{\max}\xi}{\mu}\Leftrightarrow\mu=\frac{D_{\max}\xi}{d_{ave}} \tag{9}$$

Values D_{max}, ξ and d_{ave} we can calculated from experimental measurements of Petri dish. D_{max} is the parameter of the fungal colony asymptote. ξ is the parameter of the exponential function. ξ is calculated by the heuristic search of the colony diameter time series $d_0, d_1, d_2, d_3, \ldots, d_k$,

Parameter d_{ave} is equal to:

$$d_{ave}=\frac{1}{k}\sum_{i=0}^{k}d_i=\frac{d_0+d_1+d_2+\ldots+d_k}{k} \tag{10}$$

In a long time of measurement, average diameter d_{ave} converge to asymptote of fungal colony D_{max}.

$$\lim_{k\to\infty}d_{ave}=\lim_{k\to\infty}\frac{d_0+d_1+d_2+\ldots+d_k}{k}=D_{\max} \tag{11}$$

Also, the intensity of death converges to the growth rate.

$$\lim_{k\to\infty}\mu=\lim_{k\to\infty}\frac{D_{\max}\xi}{\frac{d_0+d_1+d_2+\ldots+d_k}{k}}=\lim_{k\to\infty}\frac{D_{\max}\xi}{D_{\max}}=\xi \tag{12}$$

Therefore, entry in to the deep asymptotic region should be limited. The introduction of another diameter d_{k+1} from time series in to the d_{ave} calculation (11), should be stopped because of small differences between successive diameter, $d_k \approx d_{k+1}$. Significance of this difference, *p= (1–q)* can be directly set and calculated from the integral equation (13):

$$\int_{t_k}^{t_{k+1}} D_{\max}(1-e^{-\xi t})dt = D_{\max}\xi e^{-\xi t}\Big|_{t_k}^{t_{k+1}} = D_{\max}\xi(e^{-\xi t_{k+1}} - e^{-\xi t_{k+1}}) \le q \tag{13}$$

Adding and subtracting the value of 1, we relate the diameter and significance (14):

$$-D_{\max}\xi(1-1+e^{-\xi t_{k+1}} - e^{-\xi t_k}) = -D_{\max}\xi(1-e^{-\xi t_k} - (1-e^{-\xi t_{k+1}})) \le q \tag{14}$$

Successive diameters are $d_k = D_{max}(1-e^{\xi t_k})$ i $d_{k+1} = D_{max}(1-e^{\xi t_{k+1}})$, and limit of the diameter difference d_{end} is equal to (15) :

$$-D_{\max}\xi(d_k - d_{k+1}) \le q \Leftrightarrow (d_{k+1} - d_k) \le \frac{q}{D_{\max}\xi} = d_{end} \tag{15}$$

Equation (14) provides reliable intensity of birth λ and death μ, with the required significance *p*.

2.4. Results

The calculation of all relevant parameters is given in table 2. Calculated limit difference d_{end} for significance p=0.95 satisfies all the requirements of measuring up to 14 days. Empirical results of measuring diameter and theoretical exponential functions linear regression parameters a and b were $a \approx 1$ and $b \approx 0$, in control conditions and 16% concentration for all compositions of basil and clove essential oils. The correlation coefficient is high $r^2 \ge 0.99$, for all conditions, also. Calculation of the parameters ξ i D_{max} is valid. Parameter d_{ave} is obtained from (11) as the average diameter of fungal colonies. Birth intensity λ is obtained form (3), and death intensity μ is obtained from (9)

3. Discusion

In this example, approximated diameter converges to value 120.154 mm for 50% basil and 50% clove in 16% essential oil concentration. This value is larger than the approximated diameter for control conditions, 117.176 mm. From diameter comparation, synergetic stimulation off fungal growth is deduced by classic approach. However, the diameter does not express explicit morphological changes. Morphological changes are contained in the parameter of the life cycle.

The life cycle of fungi represent the subtraction between birth intensity and death intensity. Subtraction between birth intensity in control conditions $\lambda_{control}$=9,608 mm/day and the death intensity in control conditions $\mu_{control}$=2,058 mm/day is the life cycle of fungi *F. verticilloides* in control conditions $\Delta_{control}$=7,594 mm/day. Life cycle control value is constant for all range of concentration and arbitrary inhibitors

relation. Intensity of birth and death values, λ_{clove}^{basil} i μ_{clove}^{basil} respectively, at 16% concentration for different relations basil and clove, do not have a constant value. These values are functions of relations between basil and clove. The calculations of the *F. verticilloides* life cycles in control condition and basil-clove synergetic conditions are given in table 3.

Graphic presentation of the results in table 3 is given in Fig. 4.

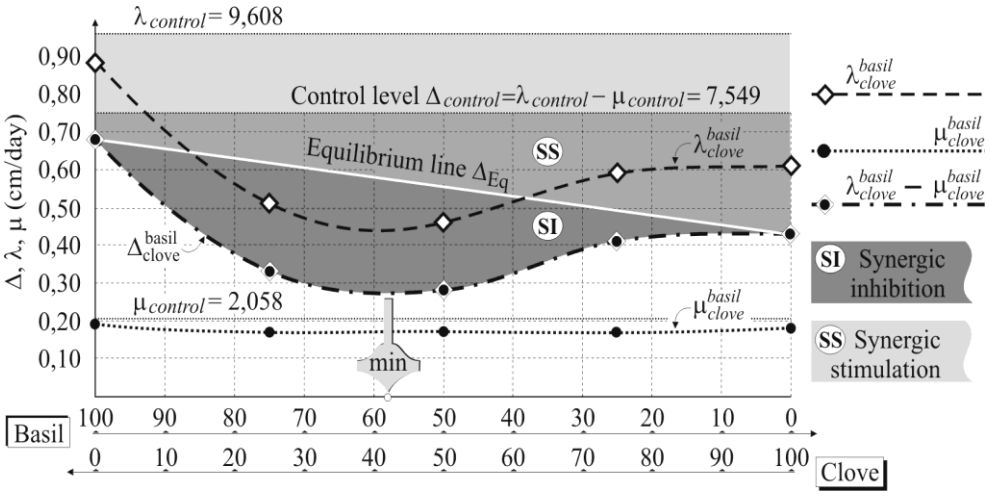

Figure 4. *F. verticilloides* life cycle dynamic

From Fig. 4 is the apparent dynamics of birth and death process for the *F. verticilloides*. Birth process intensity λ_{clove}^{basil} on the 16% concentration has pronounced variations of value. Birth intensity at 100% basil in 16% essential oil concentration (8.850 mm/day) have higher birth intensity from 100% clove in 16% essential oil concentration (6,197 mm/day). With the increasing participation of clove in 16% essential oil concentration, come to a sudden fall of the birth intensity. Minimum intensity birth is about 60% basil and 40% clove participation. With further increase participation of clove, comes an increase in the intensity of birth. Stabilization of about 75% of clove participation, remains constant until the end of the domain.

The intensity of the death μ_{clove}^{basil} at 16% concentration has not pronounced variations. Death intensity have μ_{clove}^{basil} are less than the death intensity in control conditions. Lacking the expected death intensity increase. However, reducing the intensity of growth directly reduces the quantity of the system, and thus the intensity of dying.

Birth intensity minimum is at 50% basil and 50% clove. Death intensity minimum is at 25% basil and 70% clove. Approximate function of the life cycle $\Delta_{clove}^{basil} = \lambda_{clove}^{basil} - \mu_{clove}^{basil}$, has a minimum at 42% basil and 58% clove. *F. verticilloides* life cycle minimum is the maximum basil-clove synergetic inhibition (Fig. 4.).

Equilibrium line Δ_{Eq} gets points through 100% of the selected concentrations of two inhibitors. Equilibrium line is a set of values that is linearly proportional to the inhibitor participation in a 16% essential oil concentration. The synergetic stimulation zone (SS) is above from Equilibrium line

The synergetic inhibitona zone (SI) is below from Equilibrium line. Area from Equilibrium line to the life cycle control level line is the synergic stimulation area. The values of the life cycle can vary about Equilibrium line. In such variations, values above the Equilibrium line are synergistic stimulation, even though they are less from the control level value. Synergetic inhibition values are below the Equilibrium line. In the shown case, for 16% concentration of essential oil relationships Basil and Clove, all values of the life cycle are under Equilibrium line. Selection of essential oils have a distinct inhibiting effect of synergy in the whole area, with a pronounced minimum of 42% basil and 58% clove.

Standard models based on the difference in growth intensity between non-inhibited and inhibited sample, cannot directly express the maximum synergistic effect of the two inhibitors. The formation of the two-dimensional function of the action of two inhibitors using standard models requires an incomparably larger number of experiments with different concentrations, and one post-process application of some heuristic model. The results show that the QS model is more accurate, reliable and less expensive for research.

4. Conclisuions

Queuing model has a high sensitivity. The basis of sensitivity is in intensity differentiation. These intensities are obtained from growth rate. At the same time, it is necessary to percieve a clear distinction between growth rate and life cycle. Growth rate is a feature of the of birth and death process. The life cycle is a feature of the birth and death intensitys.

The queuing model has limitations. In the case of higher concetrations, application of inhibitors may delay the start of fungal growth. During the asymptotic inhibition, birth intensity is equal to zero.

Changes in diameter does not correspond to exponential function. This period lasts until the beginning of delayed growth. Start of growth changes the value of the birth intensity. Existence of two values for the same intensity is a feature of nonstationary queing system. Solving the nonstationary queuing system can not be done by the proposed mathematical procedure. Therefore, the model gives solutions only in the case of small concentrations of inhibitors. These concentrations must be less then MIC (minimal inhibitory concentracion). The model can be applied to the analysis of bacterial inhibition, as well as the simultaneous application of three or more inhibitors.

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