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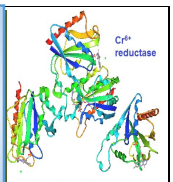

Modelling the Effect of Picloram on the Growth Kinetics of Cell Suspension Cultures of *Ficus deltoidea* L.

Ling, A.P.K.[1], Halmi, M.I.E.[2], Hussein, S.[3*] and Ong S.L.[4]

[1]Department of Biomedical Sciences and Biotechnology, School of Health Sciences, International Medical University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
[2]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3]Agrotechnology and Bioscience Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia.
[4]School of Biosciences, Taylor's University, 47500 Subang Jaya, Selangor, Malaysia.

*Corresponding author:
Dr. Sobri Hussein,
Agrotechnology and Bioscience Division,
Malaysian Nuclear Agency,
Bangi, 43000 Kajang,
Selangor, Malaysia.
Email: sobri@nuclearmalaysia.gov.my

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ABSTRACT

Ficus deltoidea (or commonly known as mistletoe fig) in various parts of the world mainly serves as an ornamental shrub or houseplant and found native mainly in Asia tropical region, for example, Malaysia, Indonesia, Philippines and Thailand. Studies on the effect of plant growth regulators on cells production from this plant is important as optimization of cells production can lead to efficient production of secondary products characterization and production. An important aspect of the sigmoidal cells growth curve is the growth parameters. In this work, we model the effect of picloram (4-amino-3,5,6-trichloropicolinic acid) on the growth kinetics of the cell suspension cultures of *Ficus deltoidea* according to the modified Gompertz model. The adjusted coefficient of determination showed good agreement between experimental and predicted data with values ranging from 0.97-0.99. Parameters obtained from the fitting exercise were maximum cells growth rate (μ_m), lag time (λ) and maximal cells production (Y_{max}). The results showed that picloram at concentrations of 3 mg/L and above were optimal for giving the highest cells growth rate measured as PCV (packed cell volume). The parameter growth rate obtained from the modelling exercise will be helpful for additional secondary modelling implicating the consequence of media conditions as well as other factors on the effect of picloram on the growth rate of cell suspension from this plant.

INTRODUCTION

Ficus deltoidea (or commonly known as mistletoe fig) in various parts of the world mainly serves as an ornamental shrub or houseplant and found native mainly in Asia tropical region, for example, Malaysia, Indonesia, Philippines and Thailand [1]. However, in Malaysia, *Ficus deltoidea* (commonly known as 'Emas Cotek', 'sempit-sempit' by the native society) is a medicinal plant which believe to be beneficial for the women reproductive system (BERNAMA, 2006). Besides, the traditional medicine practitioners in Malaysia believe that this herb is good for improving blood circulation and for rejuvenation and it can be processed into herbal tea [2]. The juice of this plant is a remedy for several ailments like gout, hypertension and diabetes apart from improving blood circulation as well as reducing cholesterol and toxins in the body [3].

Under particular circumstances, cells can go through a process in which embryos are produced by adult somatic cells or a process more commonly known as somatic embryogenesis [4]. An immediate uses of cell suspension is micropropagation where stock plant materials are multiplied to produce progeny plants in large numbers using modern methods of plant tissue culture. Micropropagation is used to multiply plants that have been bred through conventional plant breeding methods or genetically modified, plants from a seedless stock plant and plants that are not easily vegetatively produced. Other uses of cell suspension include production of medicinally important

compounds, bioactive plant metabolites or vaccines and useful proteins in biadiognostics or other applications [5–10].

Growth of cell suspension, like bacterial growth, is a linked process that displays unique phases where the specific growth rate, which initially has a value of zero producing a lag time (λ) then accelerates in a certain time period to a maximal value. The growth curves also include a final phase where an asymptote (*A*) is achieved where the rate gets to zero. Eventually, cells growth reaches a stage where the cells started to die and entering the death phase. The overall profile of the growth rate appears sigmoidal curve [11].

One of the most important parameters of the growth curve is μ_{max} (or μ_m). In biological systems, this value is used to develop secondary models such as the effects of product, pH, temperature, substrate on growth rate of the organism. The μ_{max} or μ_m is usually given by the slope of the line at the exponential phase [12]. The most popular method in estimating this value is through conversion of the exponential phase to a linearized form usually via transforming the y values into logarithm or natural logarithm and then determining the slope of this curve using linear regression. A better method, but often neglected, is to model all of the set of data with nonlinear regression growth model and then getting the values of μ_{max}, λ, and A from the model [13].

The modified Gompertz model is one of the classical growth models that include model such as the Verhulst [11,14]. The Gompertz function, named in 1844-1845 by Pierre François Verhulstis, is based on an exponential relationship between specific growth rate and population density. The initial stage of growth is approximately exponential; then, as saturation begins, the growth slows, and at maturity, growth stops. Gibson et al. [15] were the first to use the Gompertz equation to fit microbial growth curves and the equation was successfully used to describe the exponential and stationary phases of the microbial growth curves that is sigmoidal. However, the model was not adequate to describe the lag phase. The model was modified by Gibson et al. [15] to incorporate the lag phase, and have been successfully used in modelling many microbial growth curves to the point where its dominance in mathematically modelling bacterial growth and product formation curves have been acknowledged [11,13,16].

Modelling of the growth curves can yield important parameters that can be used for further optimisation works for cell suspension such as determination of specific growth rate, lag period and maximum cells formation. In this study, the cell suspension cultures from *Ficus deltoidea* was modelled according to the modified Gompertz model.

MATERIALS AND METHODS

Data acquisition

Data was acquired from Figure 1 from our previously published work where cell suspension was initiated from the female leaf explants [3] and then replotted (**Fig**. 1).

Fitting of the data

To find out regardless of whether there is a statistically substantial distinction between models with many amount of parameters, according to the quality of fit, data was statistically examined by the coefficient of determination (R^2).

The modified Gompertz model (**Eqn.** 1) is expressed as follows:

$$y=A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda-t)+1\right]\right\} \quad (1)$$

A= Cells growth lower asymptote;
μ_m= maximum specific callus growth rate;
λ=lag time
y_{max}= Cells upper asymptote;
e = exponent (2.718281828)
t = sampling time

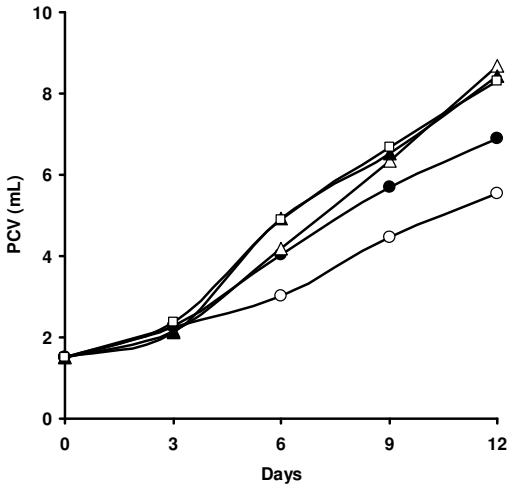

Fig. 1. The effect of picloram concentrations at 1 (○), 2 (●), 3 (△), 4 (▲) and 5 (□) mg/L, on the production of cell suspension cultures of *Ficus deltoidea.*

RESULTS AND DISCUSSION

Plants as a result of stresses, produce disorganized cell masses, for example, tumors or callus upon pathogen infections or injury. The phrase "callus" emanates from the Latin word callum, meaning hard, and in medicine, it means dermal tissue thickening [17,18]. These days, unorganized cell masses are jointly known as callus, and the same word is utilized more generally. Callus can be made from just one differentiated cell, and many callus are totipotent, which means they are able to bring about whole plant regeneration [4,19]. Most importantly, fribale callus has always been an excellent source of cell suspension culture.

The cells production from this plant was weakly sigmoidal in shape with a noticeably absence lag phase (**Fig.** 2). The cells production over time profile was fitted to the modified Gompertz model. The resultant fitting shows visually acceptable fitting with adjusted coefficient of determination (R^2) of between 0.97 to 0.99 indicating good fitting (Figure 2). Parameters obtained from the fitting exercise were maximum cell growth rate (μ_m), lag time (λ) and maximal cell production (Y_{max}) (**Table** 1). The results showed that picloram at concentrations of 3 mg/L and above were optimal for giving the highest cells growth rate, which was similar to the conclusion reached in the original study based on visual observation [2].

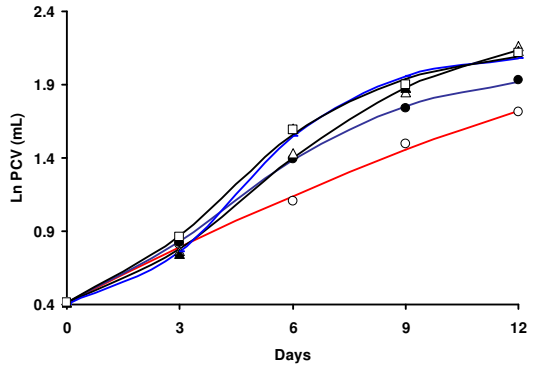

Fig. 2. The effect of picloram concentrations at 1 (○), 2 (●), 3 (△), 4 (▲) and 5 (□) mg/L, on the production of cell suspension culture of *Ficus deltoidea* fitted to the modified Gompertz model.

Table 1. Cell suspension production coefficients from the effect of the plant growth regulator, picloram on cells growth from *Ficus deltoidea* fitted to the modified Gompertz model. Values include 95% confidence interval.

Constants	Picloram (mg/L)				
	1.0	2.0	3.0	4.0	5.0
Asymptote (PCV (mL))	12.76 (6.85-18.66)	7.73 (5.13-11.63)	10.56 (1.61-68.99)	8.41 (1.63-43.42)	8.71 (2.58-29.43)
μ_m (d^{-1})	0.124 (0.113-0.362)	0.198 (0.11-0.287	0.215 (0.078-0.508)	0.286 (0.473-1.045)	0.25 (0.172 -0.672
lag (days)	-6.98 (wide)	0.57 (-2.87-4.02)	1.09 (-8.66-10.85)	1.76 (-7.79-11.32)	1.02 (-7.71-9.75)

The study carried out here attempt to optimize cell suspension production using mathematical model as cells growth has not been modelled properly using any primary growth models. Other growth models that are available including Baranyi-Roberts [20,21] and Logistic, modified Gompertz [22–24,24,25,25–27], Richards, Schnute [11,28], Von Bertalanffy [29,30], Buchanan three-phase [31–37] and more recently the Huang model [38].

The use of other growth models need to be statistically weighed in against the modified Gompertz model in the future [24,39], and this is currently being carried out. Despite this, the modified Gompertz model is the most popular model as it is the simplest (having three parameters) and allows comparison with published results to be carried out. It is anticipated that many more works on plant secondary products utilizing plant's callus and tissue culture [4,6,7,17,18,40–45] can benefit from this work.

The asymmetrical sigmoidal shape of the modified Gompertz represents and may offer greater flexibility than the logistic. Sigmoidal models such as the logistic and Gompertz differ chiefly at the point of inflection between the lower and the upper asymptotes with the logistics and Gompertz models having the distance of 1/2 and 1/e between the lower and the upper asymptotes, respectively [16].

In an essence, other growth models provide flexible slope function and variable point of inflection between the lower and upper asymptotes. These functions are either special or simpler cases of a parent growth model. For instance, the Richard model incorporates the logistics, Gompertz or von Bertalanffy growth models [11,15,16]. The model has its drawbacks and is not perfect with several main issues. Firstly, in the static version, $y_{(t=0)}$ is not equal to y_o. Secondly, an inflection point is the intrinsic property of the sigmoidal curve causing the model to have a systematic problem in describing the exponential phase (Baranyi et al., 1993). Finally, the model tend to over-estimates its parameter values [46–48]. Despite this, the modified Gompertz model has been extensively used to model the growth of bacteria and bacterial secondary products production such as biohydrogen, methane, lactic acid, biofuel and bacterioricin to name a few [49–53] including callus growth [24,54,55].

Parameters obtained from the fitting exercise would be later used for further secondary modelling. These mechanistic models are aimed to reach a better understanding of the chemical, physical, and biological processes. Compared to empirical model, mechanistic models including the modified Gompertz are more powerful since they tell you about the underlying mechanism or processes that drives the change in growth rates observed [56].

CONCLUSION

In this study, the effect picloram on cells growth profile has been successfully modelled using the modified Gompertz model. Parameters obtained from the fitting exercise were maximum cells growth rate (μ_m), lag time (λ) and maximal cells production (Y_{max}) of 0.193 d^{-1}, 2.91 days and 0.38 g cells/25 mL culture, respectively. The use of the modified Gompertz growth model to obtain useful growth constants is important for future cells growth and secondary product optimization from this plant.

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