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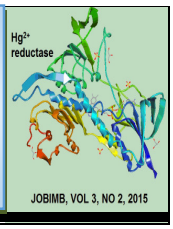

Modelling the Effect of 2-4, D on the Growth Kinetics of Cell Suspension Cultures of *Ficus deltoidea* L.

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ABSTRACT

The mistletoe fig (*Ficus deltoidea*) is frequently found in several areas of the world, and primarily functions as houseplant or an ornamental shrub. The plant is discovered indigenous generally in Asia tropical region for example Indonesia, Philippines, Malaysia, and Thailand. Scientific studies on the effect of plant growth regulators on cells production from this plant are vital as optimization of cells production may result in effective production of secondary products characterization and output. The growth of cell suspension cultures from this plant shows sigmoidal property. In this work, we model the effect of the plant growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D) on the growth kinetics of the cells from this plant according to the modified Gompertz model. The coefficient of determination showed good agreement between experimental and predicted data with values ranging from 0.97-0.98. The results showed that 2,4-D at 2 mg/L was optimal for achieving the highest cells growth rate. It is anticipated that the growth parameter constants extracted from the modelling exercise will be helpful in the future for additional secondary modelling on the effect of media conditions as well as other factors on cells growth.

INTRODUCTION

Ficus deltoidea (or commonly known as mistletoe fig) is an important component of medicinal herb repertoire in Asia [1]. The herbs can be processed into herbal tea. In Malaysia, it is locally known as 'Emas Cotek' or 'sempit-sempit', and based on anecdotal evidences are beneficial for the women reproductive system, improving blood circulation and for rejuvenation [2]. Its juice on the other hand has been touted as a remedy for gout, hypertension and diabetes as well as reducing cholesterol and toxins in the body [3]. Several studies have been carried out to characterize important secondary metabolites using callus and cell suspension from this plant [4–9].

Generally speaking, cell suspension growth, is a connected process that demonstrates unique stages where the specific growth rate, which in the beginning has a value of zero producing a lag time (λ) then accelerates in a certain time period to a maximal value. The final phase of the growth curve includes a final phase where the rate gets to zero, and an asymptote (*A*) is reached. 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 [10]. 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 [11]. 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 [12]. The modified Gompertz model is one of the classical growth models that include model such as the Verhulst [10,13]. The Gompertz function, was named in 1844 by Pierre François Verhulstis, is founded on an exponential connection between specific growth rate and population density. The initial stage of growth is roughly exponential; then, as saturation commences, the growth slows down, and at maturity, growth ceases.

Gibson et al. [14] were the first person to use the Gompertz equation to suit microbial growth curves, and the equation was used successfully to explain the exponential and stationary phases of the microbial growth curves which is sigmoidal. Nevertheless, the model was not satisfactory to explain the lag phase. The model was altered to feature the lag phase, and have been proven to work in modelling many microbial growth curves so much that its popularity in mathematically modelling bacterial growth and product formation curves have been recognized [10,12,15].

It is anticipated that modelling of the growth curves will yield important growth parameters that can be used for further optimisation works for cells such as determination of specific growth rate, lag period and maximum cells production. In this study, the effect of the plant growth regulator, 2,4-D on the growth of the cell suspension cultures from the leaf explants of *Ficus deltoidea* was successfully modelled according to the modified Gompertz model.

MATERIALS AND METHODS

Data acquisition

Data were obtained from our previously published work where cell suspension culture was initiated from the female leaf explants [3] from Figure 4 and then replotted (**Fig.** 1).

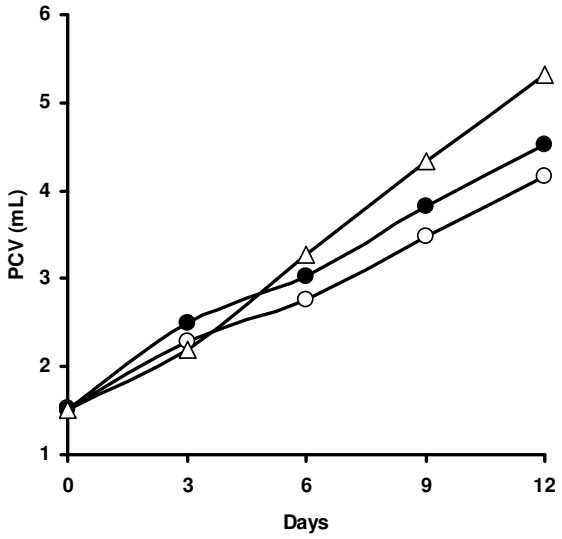

Fig. 1. Effect 1 (○), 2 (●) and 3 (△) mg/L of 2,4-D on growth of cell suspension of *Ficus deltoidea.*

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:

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

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

RESULTS AND DISCUSSION

Plants because of stresses, generate unorganized cell masses, for instance tumors or callus upon pathogen infections or injury. The word "callus" hails from the Latin word callum, which means hard, and in medicine it means dermal tissue thickening [16,17]. 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 cells really are totipotent, which means they are able to bring about whole plant regeneration [18,19]. Callus, particularly friable callus is an important initial source in establishing a fine cell suspension culture.

The cells production from this plant was weakly sigmoidal in shape with a near absence of a lag phase (**Fig.** 1). The cells production over time profile was fitted to the modified Gompertz model. The coefficient of determination showed good agreement between experimental and predicted data with values ranging from 0.97-0.98 (**Fig.** 2).

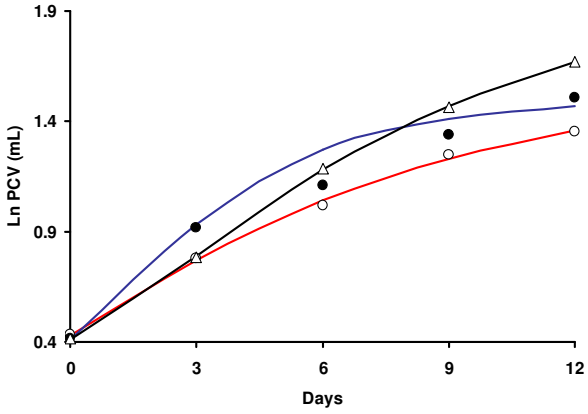

Fig. 2. The effect of 1 (○), 2 (●) and 3 (△) mg/L of 2,4-D on the production of cell suspension culture of *Ficus deltoidea* fitted to the modified Gompertz model.

Parameters obtained from the fitting exercise were maximum cell growth rate (μ_m), lag time (λ) and maximal cell production (Y_{max}). The results showed that 2,4-D at 2 mg/L was

optimal for giving the highest cell growth rate (**Table** 1); a similar conclusion reached in the original study based on visual observation [2]. Another observation was the absence of lag period.

Table 1. Callus production coefficients from the effect of plant growth regulator, 2,4-D on growth of cell suspension of *Ficus deltoidea* fitted to the modified Gompertz model. Values include 95% confidence interval.

Constants	2,4-D (mg/L)		
	1.0	2.0	3.0
Asymptote (PCV (mL))	4.99 (0.10-241.77)	4.48 (0.64-31.50)	6.82 (2.66-17.50)
μ_m (d^{-1})	0.13 (-1.07-1.31)	0.18 (-0.47-0.83)	0.14 (0.08-0.20)
lag (days)	-7.99 (wide)	-1.65 (wide)	-1.57 (wide)

Literature search showed that cell growth has not been modelled properly using any primary growth models. The study carried out here attempts to optimize cell suspension culture using mathematical model. Other growth models that are available including Baranyi-Roberts [20,21] and Logistic, modified Gompertz [22–24,24,25,25–27], Richards, Schnute [10,28], 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 [5,6,16–18,40–45] can benefit from this work.

Compared to the logistic model, which have been used to model plant or plant's callus growth [46–48], the asymmetrical sigmoidal shape of the modified Gompertz offers 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 [15]. 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 [10,14,15].

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 [49–51]. 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 [52–56] including callus growth [24,57,58].

Parameters extracted from the fitting exercise will be later employed for further secondary modelling. These mechanistic models are targeted to achieve a better knowledge of the chemical, physical, and biological processes governing callus and cells growth. In comparison to empirical model, mechanistic models for example the modified Gompertz tend to be more powerful since they tell you about the actual mechanism or processes that drives the alteration in growth rates observed [59].

CONCLUSION

In conclusion, the effect of the plant growth regulator, 2,4-D on the cell suspension growth profile has been successfully modelled using the modified Gompertz model. Parameters obtained from the fitting exercise were maximum callus growth rate (μ_m), lag time (λ) and maximal callus 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 novel for this plant.

REFERENCES

1. Kiong ALP, Then CN, Hussein S. Callus induction from leaf explants of *Ficus deltoidea* Jack. Int J Agric Res. 2007;2(5):468–75.
2. Ong SL, Kiong ALP, Poospooragi R, Hussein S. Production of Flavonoid compounds in cell cultures of *Ficus deltoidea* as influenced by medium composition. Int J Med Aromat Plants. 2011;1(2):62–74.
3. Ling OS, Kiong ALP, Hussein S. Establishment and optimisation of growth parameters for cell suspension cultures of *Ficus deltoidea*. Am-Eurasian J Sustain Agric. 2008;2(1):38–49.
4. Hussein S, Ibrahim R, Kiong ALP, Fadzillah NM, Daud SK. Multiple shoots formation of an important tropical medicinal plant, *Eurycoma longifolia* Jack. Plant Biotechnol. 2005;22(4):349–51.
5. Ibrahim R, Hussein S, Noordin N, Azlan E, Manan MA, Adrian H, et al. Advanced cell culture technology for essential oil production and microarray studies leading to discovery of genes for fragrance compounds in *Michelia alba* (Cempaka putih). Acta Hortic. 2008;765:95–100.
6. Kiong ALP, Yeo ST, Gansau JA, Hussein S. Induction and multiplication of callus from endosperm of *Cycas revoluta*. Afr J Biotechnol. 2008;7(23):4279–84.
7. Ling APK, Ong CPL, Tee CS, Hussein S. Establishment of protoplast isolation protocols of *Orthosiphon staminues*. Am-Eurasian J Sustain Agric. 2009;3(3):587–96.
8. Hussein S, Ling APK, Ng TH, Ibrahim R, Paek KY. Adventitious roots induction of recalcitrant tropical woody plant, *Eurycoma longifolia*. Romanian Biotechnol Lett. 2012;17(1):7026–35.
9. Laere E, Ling APK, Wong YP, Koh RY, Mohd L, Hussein S. Plant-based vaccines: Production and challenges. J Bot. 2016;2016.
10. Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K. Modeling of the bacterial growth curve. Appl Environ Microbiol. 1990;56(6):1875–81.
11. Fujikawa H. Development of a new logistic model for microbial growth in foods. Biocontrol Sci. 2010;15(3):75–80.
12. Johnsen AR, Binning PJ, Aamand J, Badawi N, Rosenbom AE. The Gompertz function can coherently describe microbial mineralization of growth-sustaining pesticides. Environ Sci Technol. 2013;47(15):8508–14.
13. Gompertz B. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philos Trans R Soc London. 1825;115:513–85.
14. Gibson AM, Bratchell N, Roberts TA. The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry. J Appl Bacteriol. 1987;62(6):479–90.
15. López S, Prieto M, Dijkstra J, Dhanoa MS, France J. Statistical evaluation of mathematical models for microbial growth. Int J Food Microbiol. 2004;96(3):289–300.

16. Kiong ALP, Huan HH, Hussein S. Callus induction from leaf explants of *Melaleuca alternifolia*. Int J Agric Res. 2007;2(3):227–37.
17. Ling APK, Phua GAT, Tee CS, Hussein S. Optimization of protoplast isolation protocols from callus of *Eurycoma longifolia*. J Med Plants Res. 2010;4(17):1778–85.
18. Hussein S, Ibrahim R, Kiong ALP. Potential biochemical markers for somatic embryos of *Eurycoma longifolia* Jack. J Plant Biol. 2006;49(1):97–101.
19. Kiong ALP, Leong SW, Hussein S, Ibrahim R. Induction of somatic embryos from different explants of *Citrus sinensis*. J Plant Sci. 2008;3(1):18–32.
20. Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol. 1994;23(3–4):277–94.
21. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Evaluation of several mathematical models for fitting the growth of the algae *Dunaliella tertiolecta*. Asian J Plant Biol. 2014;2(1):1–6.
22. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Modeling the growth curves of *Acinetobacter* sp. strain DRY12 grown on diesel. J Environ Bioremediation Toxicol. 2014;2(1):33–7.
23. Halmi MIE, Shukor MS, Masdor NA, Shamaan NA, Shukor MY. Test for the presence of autocorrelation in the modified gompertz model used in the fitting the growth of sludge microbes on PEG 600. J Environ Microbiol Toxicol. 2015;3(1):6–8.
24. Shukor MS, Masdor NA, Halmi MIE, Ahmad SA, Shukor MY. Test of randomness of residuals for modified Gompertz model used for modelling the growth of callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Asian J Plant Biol. 2015;3(1):11–3.
25. Shukor MS, Shukor MY. Statistical diagnostic tests of residuals from the Gompertz model used in the fitting of the growth of *E. coli* measured using a real-time impedimetric biosensor. Nanobio Bionano. 2015;2(2):58–62.
26. Halmi MIE, Shukor MS, Masdor NA, Shamaan NA, Shukor MY. Evaluation of several mathematical models for fitting the growth of sludge microbes on PEG 600. J Environ Microbiol Toxicol. 2015;3(1):1–5.
27. Shukor MS, Shukor MY. Modelling the growth kinetics of *E. coli* measured using real-time impedimetric biosensor. Nanobio Bionano. 2014;2(2):52–7.
28. Gibson AM, Bratchell N, Roberts TA. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int J Food Microbiol. 1988;6(2):155–78.
29. Cloern JE, Nichols FH. A von Bertalanffy growth model with a seasonally varying coefficient. J Fish Res Board Can. 1978;35(11):1479–82.
30. Darmani Kuhi H, Kebreab E, Lopez S, France J. A derivation and evaluation of the von Bertalanffy equation for describing growth in broilers over time. J Anim Feed Sci. 2002;11(1):109–25.
31. Buchanan RL, Whiting RC, Damert WC. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol. 1997;14(4):313–26.
32. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Modeling the growth kinetics of *Chlorella vulgaris* cultivated in microfluidic devices. Asian J Plant Biol. 2014;2(1):7–10.
33. Shukor MS, Shukor MY. Test for the presence of autocorrelation in the Buchanan model used in the fitting of the growth of the catechol-degrading *Candida parapsilopsis*. J Environ Microbiol Toxicol. 2014;2(2):45–6.
34. Ahmad SA, Shukor MS, Masdor NA, Shamaan NA, Roslan MAH, Shukor MY. Test for the presence of autocorrelation in the Buchanan-three-phase model used in the growth of *Paracoccus* sp. SKG on Acetonitrile. J Environ Bioremediation Toxicol. 2015;3(1):6–8.
35. Sabullah MK, Shukor MS, Masdor NA, Shamaan NA, Shukor MY. Test of randomness of residuals for the Buchanan-three-phase model used in the fitting the growth of *Moraxella* sp. B on monobromoacetic acid (MBA). Bull Environ Sci Manag. 2015;3(1):13–15.
36. Shukor M., Shukor MY. The growth of *Paracoccus* sp. SKG on acetonitrile is best modelled using the Buchanan three phase model. J Environ Bioremediation Toxicol. 2015;3(1):1–5.
37. Shukor MS, Masdor NA, Shamaan NA, Ahmad SA, Halmi MIE, Shukor MY. Modelling the growth of *Moraxella* sp. B on monobromoacetic acid (MBA). Bull Environ Sci Manag. 2015;3(1):1–6.
38. Huang L. Growth kinetics of Escherichia coli O157:H7 in mechanically-tenderized beef. Int J Food Microbiol. 2010;140(1):40–8.
39. Shukor MS, Masdor NA, Halmi MIE, Ahmad SA, Shukor MY. Outlier analysis of the modified Gompertz model used for modelling the growth of callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Asian J Plant Biol. 2015;3(1):14–6.
40. Hendra R, Ahmad S, Sukari A, Shukor MY, Oskoueian E. Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. Int J Mol Sci. 2011;12(6):3422–31.
41. Wazir D, Ahmad S, Muse R, Mahmood M, Shukor M. Antioxidant activities of different parts of *Gnetum gnemon* L. J Plant Biochem Biotechnol. 2011;20(2):234–240.
42. Chern LY, Shukor MY, Muse R. Monoterpenes in plants- a mini review. Asian J Plant Biol. 2013;1(1):15–9.
43. Ling APK, Tan KP, Hussein S. Comparative effects of plant growth regulators on leaf and stem explants of *Labisia pumila* var. alata. J Zhejiang Univ Sci B. 2013;14(7):621–31.
44. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. Antiartherosclerotic effects of plant flavonoids. BioMed Res Int. 2014;2014.
45. Hussin N, Muse R, Ahmad S, Ramli J, Mahmood M, Sulaiman M, et al. Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L. (Lecythidaceae). Afr J Biotechnol. 8(12):2835–2842.
46. Garrett SD. Rhizomorph behaviour in Armillaria mellea (vahl) quél: II. Logistics of infection. Ann Bot. 1956;20(2):193–209.
47. Pequeño-Granado IL, Ojeda-Zacarías MC, Olivares-Sáenz E, Zavala-García F, Alvarado-Gomez OG, Iracheta-Donjuan L. In vitro petiole morphogenesis of *Jatropha curcas* L. (Morfogénesis in vitro de peciolos de *Jatropha curcas* L.). Agrociencia. 2015;49(7):775–85.
48. Ji Z-D, Tu Y-S, Chen M, Ye L-N. Conditional optimization and kinetic research on producing huperzine A using *Huperzia serrata* in vitro. Chin Tradit Herb Drugs. 2016;47(3):499–492.
49. McKellar RC, Knight K. A combined discrete-continuous model describing the lag phase of Listeria monocytogenes. Int J Food Microbiol. 2000;54(3):171–80.
50. Membré J-M, Ross T, McMeekin T. Behaviour of Listeria monocytogenes under combined chilling processes. Lett Appl Microbiol. 1999;28(3):216–20.
51. Whiting RC. Modeling bacterial survival in unfavorable environments. J Ind Microbiol. 1993;12(3–5):240–6.
52. Espeche MC, Tomás MSJ, Wiese B, Bru E, Nader-Macías MEF. Physicochemical factors differentially affect the biomass and bacteriocin production by bovine Enterococcus mundtii CRL1656. J Dairy Sci. 2014;97(2):789–97.
53. Kargi F, Eren NS, Ozmihci S. Effect of initial bacteria concentration on hydrogen gas production from cheese whey powder solution by thermophilic dark fermentation. Biotechnol Prog. 2012;28(4):931–6.
54. Karthic P, Joseph S, Arun N, Varghese LA, Santhiagu A. Biohydrogen production using anaerobic mixed bacteria: Process parameters optimization studies. J Renew Sustain Energy. 2013;5(6).
55. Mathias SP, Rosenthal A, Gaspar A, Aragão GMF, Slongo-Marcusi A. Prediction of acid lactic-bacteria growth in Turkey ham processed by high hydrostatic pressure. Braz J Microbiol. 2013;44(1):23–8.
56. Mohammadi M, Mohamed AR, Najafpour GD, Younesi H, Uzir MH. Kinetic studies on fermentative production of biofuel from synthesis gas using clostridium ljungdahlii. Sci World J. 2014;2014.
57. Omar R, Abdullah MA, Hasan MA, Rosfarizan M, Marziah M. Kinetics and modelling of cell growth and substrate uptake in *Centella asiatica* cell culture. Biotechnol Bioprocess Eng. 2006;11(3):223–9.

58. Shukor MS, Masdor N., Halmi MIE, Ahmad SA, Shukor MY. Modelling the growth of callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Asian J Plant Biol. 2015;3(1):20–5.
59. Bolker BM. Ecological Models and Data in R. Princeton, N.J: Princeton University Press; 2008. 408 p.