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Research Article

Analysis of Germination Curves of *Cinchona officinalis* L. (Rubiaceae) Using Sigmoidal Mathematical Models

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Seed germination is the fundamental phenomenon that determines the successful growth and development of each plant species, even more so in *Cinchona officinalis*, which is a forest species that stands out for its medicinal importance. The objective of this work was to determine the best sigmoidal mathematical model describing the germination of C. *officinalis*. For the germination test, a completely randomized design was used with six treatments and three replicates per treatment; 100°*C. officinalis* seeds were used per replicate, and 1800 seeds were needed in the trial. Gompertz sigmoidal, logistic, and von Bertalanffy models were used to analyse the germination curves of *C. officinalis*. The results of these adjustments were analysed based on the graphic representation and statistical criteria (Akaike's value (AIC), R^2, and R^2_{ai}). The results suggest that the Gompertz and logistic models have a better graphic representation, showing values close to those observed, while the von Bertalanffy model shows negative germination values. According to the statistical criteria, the lowest AIC and the highest were obtained. R^2 and R^2_{ai} with the Gompertz model, followed by the logistic model and von Bertalanffy. It is concluded that the Gompertz model can represent the shape of the germination curves of *C. officinalis* for the six treatments of the test.

1. Introduction

Cinchona officinalis is a forest species that stands out for its medicinal importance, as its bark contains alkaloids such as quinine, which was used as the only treatment to combat malaria for more than 300 years [1]. This species was unsustainably harvested for centuries; its bark was exported to different countries; the most conservative data report that between the 17th and 18th centuries, around 500 tons were exported annually to Europe [2].

Seed germination is the fundamental phenomenon that determines the successful growth and development of each plant species [3]. It is regulated by several environmental factors, such as temperature, soil moisture, soil pH, light intensity, and photoperiod [4]. Despite the importance of plant germination, detailed research to predict seedling emergence is still lacking [5]. The germination process forms an S-like curve, which is characterized by a recess phase in which seedling emergence does not occur, followed by an exponential period of emergence and ends with a plateau which is when emergence is complete [6].

Research has shown that seed germination can be estimated through modeling techniques [6]. Sigmoidal nonlinear regression models (Weibull, Gompertz, logistic, etc.) are the most widely used empirical approach to predict seed germination [7–9]. For example, thermal weather models

Table 1: Types of substrates used in the germination of *C. officinalis*.

Treatments	Descriptions
T0	Witness (forest soil)
T1	50% of forest soil + 35% sand + 15% humus
T2	50% forest soil + 50% sand
T3	50% humus + 50% sand
T4	50% of forest soil + 50% of humus
T5	100% sand

have been developed to predict seedling emergence [10]; chickpea germination patterns have been determined based on a mathematical model [11]; monomolecular, logistic, and Gompertz mathematical models have been compared to analyse cumulative germination curves over time, and as a result, it was obtained that the logistic model allows describing the shape of germination curves of aged fennel seeds [12]; three-parameter sigmoidal models fitted to the germination data of two glyphosate-susceptible and two glyphosate-resistant populations of *Chloris virgata* in response to different day/light temperature regimes and osmotic potential levels were determined [13].

In this regard, it is important to generate and validate models that can explain biological phenomena mechanistically and allow important new questions to be asked about the elusive nature of plant biology [14]. Since there are no models described in the literature consulted that allow predicting the germination of *C. officinalis,* and taking into account that the propagation of this species is mainly by seeds [15], it was proposed as an objective to determine the best sigmoidal mathematical model that describes the germination of *C. officinalis.*

2. Materials and Methods

2.1. Location of the Study. The experimental part of the research began on November 20, 2021, and concluded on January 16, 2022, in the community of La Cascarilla (UTM coordinates 732697.45 W, 9372588.42 S), province of Jaen, Peru, at 1810 m. Annual precipitation is 1730 mm, with a minimum temperature of 13.0°C and a maximum of 20.5°C [16, 17].

2.2. Germination Test. Seeds of *C. officinalis* collected in October 2021 from a single population existing in the community of San Luis (UTM coordinates 714805.6 W, 9295674.9 S) at 2489 m altitude were used. We collected 0.5 kg of mature capsules, which were packed in cloth bags and transported to the community of La Cascarilla. The capsules were placed in the shade for 20 days, and then seeds in perfect phytosanitary condition were selected for use in the germination test.

2.2.1. Test Preparation. A subirrigation chamber was used, as described in [17], which was divided into 18 experimental units. In each experimental unit, the substrate was prepared to take into account the percentages of forest soil, sand, and humus established in Table 1 for each treatment. Subsequently, the substrate was moistened to field capacity, and 100 seeds were sown for each treatment. Finally, daily irrigation was applied (0.10 L m^{-2}) so that the humidity inside the chamber remained constant during the trial. Inside the subirrigation chamber, the temperature was controlled at 24°C and the humidity at 95%.

Table 2: Sigmoidal mathematical models used to describe the germination of *C. officinalis*.

Sigmoidal models	Equations
Gompertz	$y = ae^{-be^{-cx}}$
Logistic	$y = (a/1 + be^{-cx})$
von Bertalanffy	$y = a(1 - be^{-cx})^3$

2.2.2. Experimental Design. A completely randomized design with six treatments and three replicates per treatment was used. One hundred seeds of *C. officinalis* were required for each replicate, and 1800 seeds were required for the entire trial.

2.2.3. Evaluation and Data Recording. The experiment was monitored on a daily basis for 57 days in order to count the number of germinated seeds on a daily basis. The presence of the root apex was taken into account as an indicator of germination. The germination percentage was determined according to the following equation proposed in [18]:

$$germination\ percentage = \frac{number\ of\ germinated\ seeds}{number\ of\ seeds\ sown} \text{x}100. \tag{1}$$

2.3. Model Selection. Regression analysis of the experimental data was performed using sigmoidal mathematical models described in the easynls library of RStudio Software (see Table 2) [19]. A comparison was made between the results of the Gompertz, logistic, and von Bertalanffy models. The evaluation of the behavior of the three models was performed using experimental data from the germination trial.

2.4. Evaluation of Model Performance. Statistical criteria have been used to compare the time series, one produced by the model for each treatment, and the second one coming from the measurements. These statistics provide unbiased indicators of model performance [20], which have been the Akaike information criterion (AIC), the coefficient of determination (R^2), and the adjusted coefficient of determination (R^2_{aj}).

3. Results

Figure 1 shows the germination of *C. officinalis* in all treatments, which started 19 days after sowing the seeds and ended on day 43 for treatments T0 (Figure 1(a)) and T1 (Figure 1(b)) and on day 47 for treatments T2, T3, T4, and T5 (Figures 1(c)–1(f)). The cumulative germination curves

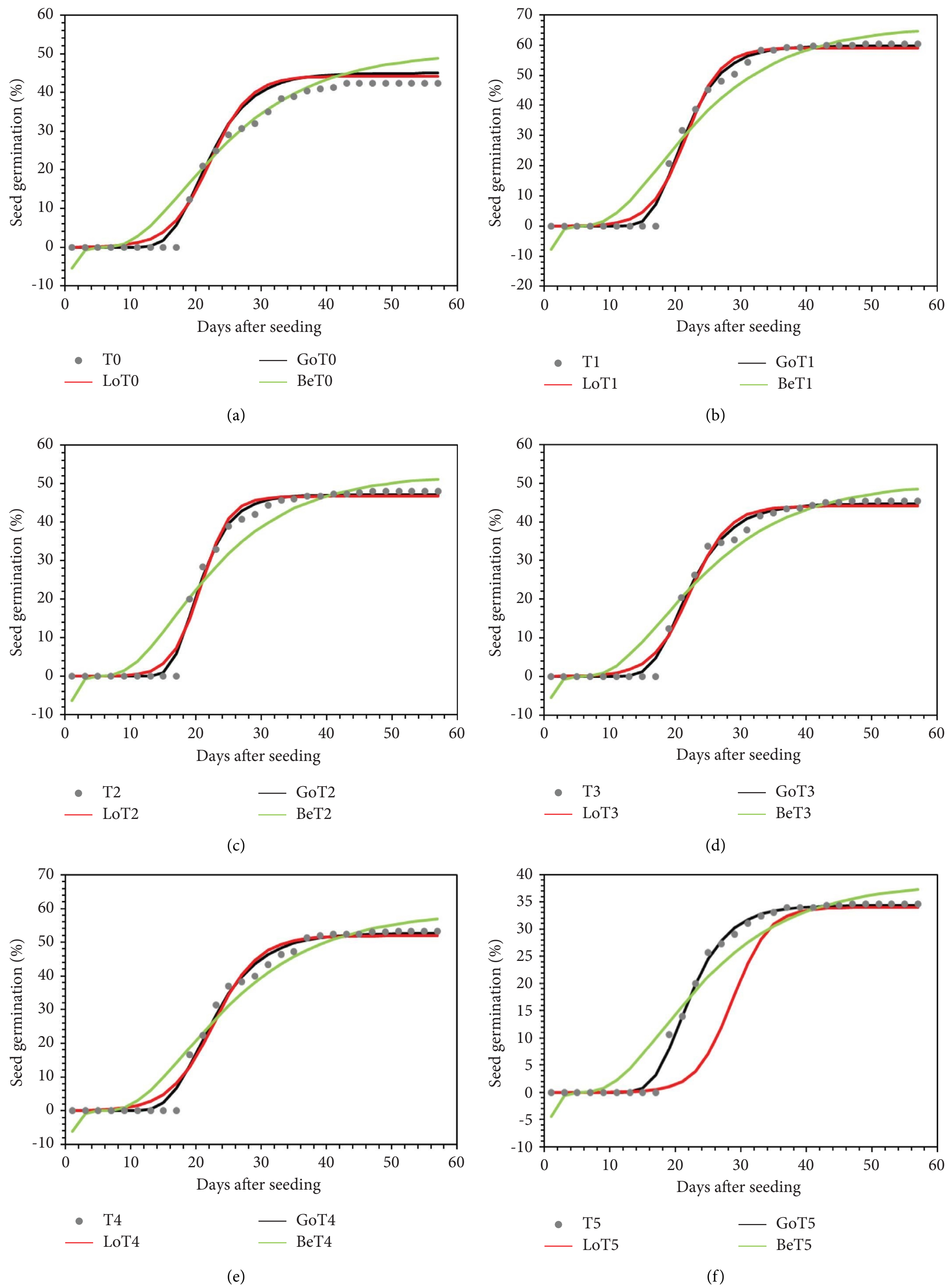

Figure 1: Gompertz model, the logistic model, and the von Bertalanffy model fitted the cumulative germination curves of *C. officinalis* seeds for the six treatments studied. (a) T0, (b) T1, (c) T2, (d) T3, (e) T4, and (f) T5. The solid line indicates the values predicted by each model, and the solid symbol indicates the data observed during the germination test. The abbreviations Go, Lo, and Be allude to the Gompertz, logistic, and von Bertalanffy models, respectively.

Table 3: Parameter estimation for Gompertz, logistic, and von Bertalanffy sigmoidal models.

Treatments	Gompertz			Logistic			von Bertalanffy		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
T0	44.997	92.977	0.224	44.424	1385.243	0.327	50.498	1.612	0.087
T1	59.683	170.787	0.258	59.108	3319.215	0.376	66.519	1.631	0.091
T2	47.028	401.896	0.310	46.645	11966.88	0.454	52.293	1.643	0.095
T3	44.676	110.027	0.229	44.087	2047.754	0.341	50.273	1.613	0.087
T4	52.630	60.262	0.199	51.844	778.999	0.293	59.071	1.600	0.085
T5	34.378	147.579	0.243	33.998	3233.069	0.362	38.535	1.623	0.088

show a certain degree of similarity between the 6 treatments. No notable differences are observed in the shape of the germination curves predicted by the Gompertz and logistic models; however, between these two models and the von Bertalanffy model, differences in the shape of the germination curves are observed. In addition, it is evident that the germination curves predicted by the von Bertalanffy model underestimate the germination of *C. officinalis* by showing negative values.

Table 3 shows the results of the estimation of parameters *a*, *b*, and *c* for the Gompertz, logistic, and von Bertalanffy models. There were no statistical differences in the values of *a* (asymptote of the curve) of the three models analysed; however, for *c* (inflection point of the curve) there were significant differences between the von Bertalanffy model and the Gompertz and logistic models. In addition, there were large differences between the three models for parameter *b* (rate of increase).

Table 4 shows a statistical comparison between the curves fitted by the three models. The goodness-of-fit for the three models was statistically compared through the AIC, R^2, and R^2_{aj}. Low AIC and R^2 values close to unity mean that the model represents the data better. Under these assumptions, it is observed that the lowest AIC values and the highest R^2 for each treatment are in the Gompertz model, followed by the logistic and von Bertalanffy models, so the Gompertz model better describes the cumulative germination of *C. officinalis*.

4. Discussion

Seed emergence of *C. officinalis* began on day 19 and ended on day 47 after sowing. These results differ slightly from those obtained in [17], whose substrate was collected from cinchona forest relicts, in which germination started at 12 days and ended at 42 days after sowing, while the study [21] reported that *C. officinalis* begins to germinate five days after sowing; these differences are related, for example, to the days of seed storage, which in this study was four months; in addition, germination is contingent on the morphological and genetic characteristics of the seeds [22]. However, the results obtained in this study were similar to those reported in [23], who found that *C. officinalis* seeds remained stable with their germination curve 50 days after sowing.

The highest germination (60.31%) was achieved by T1. This value turns out to be higher than that reported in [24], who reported germination percentages ranging between 30 and 50% for a clayey and loamy substrate, respectively. The high germination percentage reported for T4 is presumably due to the physical and chemical characteristics of the soil since the substrate that forms T4 is the one with the highest amount of humus, which provides an adequate amount of nutrients and facilitates the circulation and retention of water, characteristics necessary during germination [25]. In addition, the properties of the substrate used as a germination medium influence seed imbibition [26], a process that promotes the activation of substances in the embryonic system and thus accelerates and increases the germination percentage [27].

Mathematical models are used to estimate the germination percentage of plant species [5]. S-shaped curves have been proposed to describe germination in a variety of plants, bryophytes, fungi, etc.; these curves are known as sigmoidal and are fitted by nonlinear regression models [28]. The results showed graphically a similar fit for the Gompertz and logistic models in the six germination treatments; however, the same was not evident for the von Bertalanffy model, whose graphical representation differs from the previous models. This is attributed to the fact that the mathematical models Gompertz and logistic have similar properties that allow the empirical representation of growth phenomena since each equation has three arbitrary constants (Table 2), which essentially correspond to the upper asymptote, the time origin, and the time unit, or "rate constant" [29]. The two models represent sigmoidal curves that are initially concave, and after passing the inflection point, they become convex. The main nuance that differentiates them is the location of the inflection point, located between 35% and 40% of the growth in Gompertz models and at 50% in the logistic one [30].

In the literature, there are various parameterizations and re-parameterizations of the Gompertz model, which are divided into two groups: In type I, a single parameter controls the time at which a specific point of the curve occurs; this point represents a fixed proportion (or percentage) of the upper asymptote, and the time at which this point occurs is not affected by the other parameters in some models. This point falls at the inflection, which in the Gompertz model occurs at 36.8% of the upper asymptote. In type II models, a single parameter controls the initial value of the curve; in these reparametrizations, the other parameters do not affect the starting point [31].

Table 4: Statistics of sigmoidal models used to predict cumulative germination of *C. officinalis*.

Treatments	Gompertz			Logistic			von Bertalanffy		
	AIC	R_{aj}^2	R^2	AIC	R_{aj}^2	R^2	AIC	R_{aj}^2	R^2
T0	125.626	0.991	0.991	143.529	0.983	0.984	171.207	0.955	0.958
T1	129.796	0.994	0.994	149.882	0.988	0.989	192.589	0.947	0.951
T2	121.902	0.993	0.993	138.718	0.987	0.988	183.641	0.938	0.943
T3	112.653	0.994	0.994	133.722	0.987	0.988	170.852	0.955	0.958
T4	131.301	0.991	0.992	150.046	0.984	0.985	174.856	0.961	0.964
T5	83.296	0.996	0.997	106.002	0.992	0.993	158.580	0.950	0.954

The statistical parameters that allowed evaluating the behavior between each of the three sigmoidal models in the six treatments showed that the Gompertz model better represents the germination process of *C. officinalis* because it has a lower AIC value and the coefficients of determination are close to one. On the contrary, the von Bertalanffy model presents the highest AIC values and lower determination coefficients. This result has been reported in different studies, in which the Gompertz model has satisfactorily described the germination of different plant species, for example, the cumulative germination of spores of ferns *Blechnum serrulatum, Blechnum yungense, Cheilanthes pilosa, Niphidium macbridei*, and *Polypodium feuillei* [32]; the germination of leaf lettuce grown in a greenhouse environment [33]; and the prediction of *Lolium rigidum* emergence, which can be used as a predictive tool to improve the control of this weed in rainfed cereal fields of Mediterranean climate zones [34].

5. Conclusions

The sigmoidal mathematical models proved to be valuable tools for analyzing the germination curves of *C. officinalis*. Of the three models evaluated, the Gompertz model best represents germination for the six treatments proposed in the experimental design since it shows a lower AIC value and higher coefficients of determination (R^2 and R_{aj}^2).

The Gompertz model represents better the germination for the six treatments proposed in the experimental design, since it shows a lower value for *AIC* and higher coefficients of determination (R^2 and R_{aj}^2). The information obtained through the Gompertz method is a tool to understand and predict the germination patterns of *C. officinalis* under certain conditions in the substrate.

On the other hand, the curve represented by the von Bertalanffy model projects negative germination data, which is why it is not useful for describing the germination of *C. officinalis.*

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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