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Research Paper / Araştırma Makalesi

Mathematical Modelling on Survival of *Cryptococcus albidus* in Fuji Apple Wounds for Biocontrol Applications

Serap Türkoğlu, Adem Zengin, Arzu Çağrı Mehmetoğlu ✉

Sakarya University, Faculty of Engineering Department of Food Engineering, Esentepe Campus, Sakarya, Türkiye

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✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): acagri@sakarya.edu.tr (A. Çağrı Mehmetoğlu)*
+90 264 295 59 20 +90 264 295 59 50

ABSTRACT

In this study, the method of Artificial Neural Networks (ANN) was used to model the survival ability of *Cryptococcus albidus* SAS157 as a biocontrol agent against mold spoilage on Fuji apple fruit. *C. albidus* (6, 9, or 11 log CFU/mL) and *P. expansum* (6 log CFU/mL) were inoculated the punctured holes on the surface of the apples and stored at various temperatures (4, 10, 15, and 25°C) and relative humidity (RH) levels (85% or 95%) for 14 days. *C. albidus* survived the best in apple wounds when the initial inoculum level was 9 log CFU/mL ($p<0.05$). RH did not significantly change the survival ability of *C. albidus* ($p>0.05$). The growth of *C. albidus* was improved when the temperature was 4°C. A high correlation between actual values was calculated (R^2~0.99) for the *C. albidus* survival within the range of the predicted conditions found by the model. These results indicated the potential of using *C. albidus* for reducing apple spoilage, with considerations for food safety practices due to its rare association with human infections.

Keywords: Survival, Apple, *C. albidus,* Mathematical model, Biocontrol

Elma Üzerindeki Yaralanmış Bölgelerde *Cryptococcus albidus*'un Canlı Kalma Oranının Matematiksel Modelleme ile Belirlenmesi

ÖZ

Bu çalışmada, küf gelişimine karşı biyolojik kontrol aracı olan *Cryptococcus albidus* SAS157'nin Fuji elmalarında canlı kalma yeteneğini modellemek için Yapay Sinir Ağları (YSA) yöntemi kullanılmıştır. *C. albidus* (6, 9 ve 11 log CFU/mL) ve *Pennicillum expansum* (6 log CFU/mL) elmaların yüzeyinde açılan deliklere aşılanarak çeşitli sıcaklık (4, 10, 15 ve 25°C) ve bağıl nem (%85 veya %95) koşullarında 14 gün boyunca depolanmıştır. Elde edilen sonuçlar, *C. albidus*'un elma yaralarında başlangıç inokulum seviyesi 9 log CFU/mL olduğunda en iyi canlı kalma performansını gösterdiğini ortaya koymuştur ($p<0.05$). Ayrıca, farklı nem seviyelerinin *C. albidus*'un canlı kalma yeteneği üzerinde önemli bir etkisi olmadığı belirlenmiştir ($p>0.05$). Bununla birlikte, depolama sıcaklığının 4°C olması, *C. albidus*'un çoğalmasını anlamlı şekilde artırmıştı ($p<0.05$). Model tarafından tahmin edilen koşullar aralığında *C. albidus*'un canlı kalmasına ait deneysel sonuçlar arasında yüksek bir korelasyon hesaplanmıştır (R^2~0.99). Bu bulgular, elma çürümelerinin azaltılmasında *C. albidus*'un potansiyelini vurgulamakta, ancak insan enfeksiyonlarıyla nadir ilişkisi nedeniyle gıda güvenliği önlemlerine dikkat edilmesi gerektiğini önermektedir.

Anahtar Kelimeler: Canlı kalma kabiliyeti, Elma, *C. albidus*, Matematiksel model, Biyokontrol

INTRODUCTION

Mold spoilage on apples in post-harvest storage causes significant economic losses. *Penicillium expansum* is an important factor that causes this type of spoilage on apples, known as blue rot, and also produces a mycotoxin called patulin during this spoilage [1]. Patulin is highly resistant to food processing conditions and has a toxic effect when consumed with apples or apple juice [2]. Synthetic fungicides are used to prevent diseases that can occur in apples. The regular use of fungicides not only damages the ecological balance but also poses a risk to human health due to possible residues that may remain on the produce [3]. In recent years, biological applications such as antagonistic yeasts to prevent mold infestation on fruit have attracted much attention [4, 5]. The main reasons for the use of yeast as biocontrol agents in fruit are their high inhibitory effects against molds and not producing allergenic spores [6, 7]. Yeasts show their antagonistic effect against molds by producing lytic enzyme, preventing the formation of mold micelles and competing for nutrients and space by growing faster than molds [8].

The potential of the yeast antagonist to prevent postharvest spoilage of fresh fruit and vegetables has been demonstrated in many studies [5, 9]. The fact that *Cryptococcus* spp., important antagonistic yeast, can also be used against mold spoilage was reported in several studies [10, 11]. For example, *C. albidus*, *C. laurentii* or *C. infirmominiatu* have been shown to significantly reduce *P. expansum*-induced blue rot in Golden Delicious apples [10-12]. Similarly, in our previous study, the relationship between the ability of the yeast *C. albidus* to reduce the growth of *P. expansum* in the wound formed on the apple surface and storage temperature, RH and storage duration was found [13]. In the same study, it was found that with the increase in the number of initially inoculated *C. albidus* cells, the antagonistic properties also increased. When antagonistic yeasts are applied to fruit surfaces, their survivability is also important so that they can have an effect throughout their shelf life. It is assumed that the survival of these organisms depends on some factors such as RH and temperature in the environment [14]. Studying the effect of different storage conditions on the viability of antagonistic yeasts as bio control agents on apple surface will provide important data for the design of uses in this area. Analyzing the effect of storage parameters is time consuming and costly in the classical method and does not reveal interactions between parameters. Mathematical models, on the other hand, allow the system parameters to be tested with many combinations at regular intervals, while also determining the optimum point where the yeast can survive best.

The use of ANNs has gained increasing attention in recent years due to their powerful capabilities in predicting and optimizing the survival, growth, and behavior of microorganisms under diverse and complex environmental conditions. Unlike classical statistical models, which often rely on linear assumptions or require simplified interactions, ANNs are capable of modeling non-linear, multi-dimensional relationships without prior knowledge of the system dynamics. This makes them especially suitable for biological systems, where interactions among variables such as temperature, relative humidity, time, and microbial load are inherently complex and dynamic. In the field of food microbiology, ANN-based models have been widely applied to predict microbial growth kinetics, evaluate the risk of spoilage or contamination, and, notably, to simulate and optimize the performance of biocontrol agents. These models allow researchers to conduct in silico experiments, reducing the need for extensive laboratory trials while still providing reliable predictions. For example, studies have shown that ANNs can successfully model and optimize the effectiveness of antagonistic microorganisms, such as yeasts and bacteria, against pathogens like *P. expansum* or *Botrytis cinerea* by identifying ideal storage parameters, including inoculum level, temperature, and relative humidity [15–17].

While the use of ANN to model the survival of *C. albidus* specifically on apple wounds is limited several studies have highlighted the potential of ANNs in similar contexts [18]. Therefore, the aim of this study was to apply ANN to determine the influence of key environmental factors such as temperature, RH, time, and yeast inoculum number on the survival of *C. albidus* on apple wounds. By incorporating ANN into this study, we aim to provide a more efficient and comprehensive method for predicting the best conditions for biocontrol applications in postharvest fruit preservation.

MATERIALS and METHODS

Preparation of Apples for Analyses

The apples (*Malus domestica* cv. Fuji) used in this study were purchased from a local greengrocery in Sakarya, Türkiye. Morphologically similar Fuji apples in terms of shape and size were selected for the study. After washing with normal tap water, the apples were immersed in water with 2% chlorine added for a minute. Rinsed with sterile distilled water, the apples were dried at room temperature in a safety cabinet for 15 min.

Inoculation of Apples with *C. albidus*

After centrifugation of 48 hours *C. albidus* SAS157 cell culture (obtained from the Department of Food Engineering culture stock (Sakarya University) in triptych soy broth (Merck, Darmstadt, Germany) containing 0.6% yeast extract (Merck) at 10,000×*g* for 15 min at 4°C, the culture was prepared at the level of 10^6, 10^9 or 10^{11} CFU/mL from the pellet in sterile distilled water using the hemocytometer (Sigma Aldrich, St. Louis, MO, USA). These inoculum levels were selected based on findings from previous studies that emphasize the critical role of initial cell concentration in determining the biocontrol efficacy of antagonistic yeasts and their competition with pathogens, such as *P. expansum* [19]. Using the pipette tip, two holes with a diameter of approximately 3 mm and a depth of 3 mm were opened on both sides of the apple samples [11]. The apples ere inoculated with 10 µL of *C. albidus* culture (10^6, 10^9 and

10^{11} CFU/mL) and 10 µL of *P. expansum* (10^6 CFU/mL), and stored in a container at 85 and 95% RH at 4, 10, 15, and 25°C [11]. These storage conditions and durations were selected based on their relevance to typical post-harvest storage conditions and to evaluate the broad adaptability of antagonistic yeasts under varying environmental factors [14]. The storage conditions of 85±3 and 95±3% RH were created by adding NaCl (Sigma Aldrich) and KNO_3 (Sigma Aldrich) saturated solutions to the containers, respectively [20].

Growth of *C. albidus* on Apples

The number of *C. albidus* was determined in the apple wound at 0, 7, and 14 days of storage. For analysis, a 10 g piece of damaged part of the wounded fruit was placed in the stomacher bag containing 90 mL of peptone water solution (0.1% wt/v) and homogenized for 2 min using a stomacher (Interscience, Bagmixer 400, France). Serial dilution was plated on OGEY Agar (Merck) and incubated at 30°C for 2 days. After incubation, cream-colored colonies grown on agar were counted as *C. albidus* [21].

Statistical Analyses

Each analysis was repeated twice, and the experiment was conducted three times. JMP (13.0 version; SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. The analysis of variance (ANOVA) was performed to analyze the data and the Tukey-HSD multiple comparison test revealed the statistical difference at the $p<0.05$ level.

Datasets

The independent variables in this study were the inoculum level of *C. albidus*, RH, time, and temperature. The effect of these parameters on the survival of *C. albidus* was investigated. The studied inoculum levels were 10^6, 10^9, and 10^{11} CFU/mL. The RH levels were 85% and 95%. The time periods in which the number of *C. albidus* was examined were 0, 7 and 14 days. The temperature variable was tested for 4, 10, 15, and 25°C. The full factorial design was used in the experimentation of these variables, which yields 96 datasets in total.

Artificial Neural Networks Model

The relationship between the four independent variables (the inoculum level of *C. albidus*, RH, time, and temperature) and the dependent variable (the growth of *C. albidus*) was modeled using an ANN model. The model was trained and optimized using the Neural Net Fitting application provided by the Deep Learning Toolbox of MATLAB software (MATLAB, 2021. version 9.10.0.1710957 (R2021a) Update 4. Natick, Massachusetts: The MathWorks Inc.). The procedure of the Neural Net Fitting application and its mathematical basis were provided by Beale et al. [22].

Figure 1 shows the network architecture with one hidden layer and an output layer. The ANN model is based on the feed-forward backpropagation algorithm. The normalization of the x and y values in the datasets was implemented to make them in the range [-1,1].

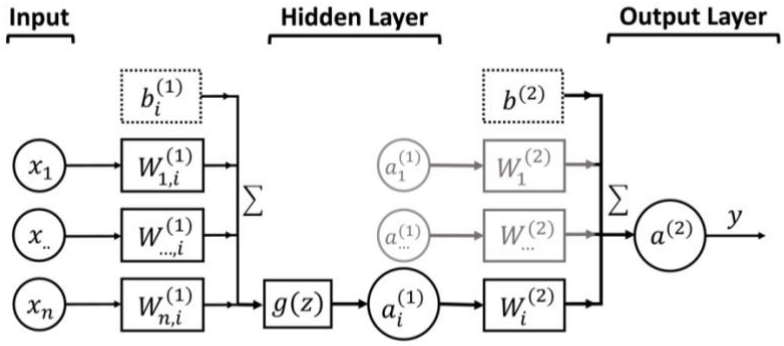

Figure 1. Schematic diagram of an ANN architecture with two layers (x_n denotes the nth parameter, $a_i^{(1)}$ denotes the ith node in the hidden layer, $b_i^{(1)}$ denotes the bias for the $a_i^{(1)}$, $W_{n,i}^{(1)}$ denotes the weight for the relationship between the x_n and the $a_i^{(1)}$. $a^{(2)}$ denotes the output node, $b^{(2)}$ denotes the bias for the $a^{(2)}$, and $W_i^{(2)}$ denotes the weight for the relationship between the $a_i^{(1)}$ and $a^{(2)}$)

The $g(z)$ is the hyperbolic tangent function given in Equation1 that introduced the nonlinearity to the model. Equation 2 takes the sum of the weighted parameters and the biases and makes the sum nonlinear It defines the non-linear relationship between the input parameters and the nodes in the hidden layer. The nodes in the hidden layer can be considered as artificial parameters. Equation 3 is composed of the sum of the weighted hidden nodes and the bias of the output node. Equation 3 defines the linear relationship between the hidden nodes and the output node.

$$g(z) = \frac{1 - e^{-z}}{1 + e^{-z}} \tag{1}$$

$$a_i^{(1)} = g\left(\sum_{n=1}^{N} W_{n,i}^{(1)} x_n + b_i^{(1)}\right) \tag{2}$$

$$a^{(2)} = \sum_{i=1}^{I} W_i^{(2)} a_i^{(1)} + b^{(2)} \tag{3}$$

The development of an ANN model involves two separate processes for the networks with one hidden layer. The first process includes optimizing the weights and biases, the second optimization of the network architecture (i.e., determination the number of nodes in the hidden layer). The experimental datasets were randomly divided into three subsets for these optimizations: Training, Validation, and Test data sets. The training and validation data sets were used to optimize the weights and biases, which included 70% and 15% of the whole data sets, respectively. The test data sets were used to optimize the network architecture and included 15% of the total datasets. The Levenberg-Marquardt algorithm was chosen for the optimization of the weights and biases due to its efficiency with small networks. The Mean Square Error (MSE) and the Coefficient of Determination (R^2) values were used in the network architecture optimization.

In Equations 4 and 5, the S denotes the number of data sets. The y_s notation was used for the predicted value of the dependent variable, which was calculated with the input values in the sth data set. The r_s notation was used for the actual value of the dependent variable in the sth data set. The $\bar{r}$ notation in Equation 5 was used for the mean of the actual values. The e_s in Equation 4 represents the deviation between the predicted and actual values of the dependent variable for the sth data set.

$$MSE = \frac{1}{N}\sum_{s=1}^{S}(e_s)^2 = \frac{1}{N}\sum_{s=1}^{S}(y_s - r_s)^2 \quad (4)$$

$$R^2 = 1 - \left[\frac{\sum_{s=1}^{S}(y_s - r_s)^2}{\sum_{s=1}^{S}(r_s - \bar{r})^2}\right] \quad (5)$$

The procedure in MATLAB was as follows: network architecture with some number of nodes in its hidden layer was chosen by us. The experimental datasets were randomly divided into the three subsets, and the weights and biases were randomly initialized by MATLAB. Then the training started, and the Levenberg-Marquardt algorithm carried out its optimization task. The optimization is an iterative process. More iteration provides more learning and makes the model more fitted to the training data sets. However, highly fitted models lose their generalization. This is known as the over fitting problem. Therefore, when the validation error is at its minimum, the weights and biases are saved and accepted as optimum by MATLAB. At this point, MATLAB uses these weights and biases and calculates the test error and the test accuracy in the forms of MSE and R^2 values, which we used manually to evaluate the performance of the chosen network architecture.

RESULTS and DISCUSSION

The Survival of *C. albidus* on Apple Wound

C. albidus applied to the apples at the different concentrations demonstrated the ability to survive throughout the 14-day storage period under all tested conditions (Figure 2). Notably, under certain environmental parameters, the yeast not only maintained viability but also proliferated. For instance, storage at 4°C and 95% RH significantly promoted growth, with cell counts increasing from 9 to 9.8 log CFU/mL by the end of the storage period ($p<0.05$). This suggests that low temperature and high humidity can create favorable microenvironments within apple wounds, likely due to reduced competition from native microbiota and decreased metabolic stress on the yeast.

In contrast, when apples were stored at 15°C under both 85% and 95% RH, *C. albidus* populations remained relatively stable, with no statistically significant change observed from the initial inoculation level of 6 log CFU/mL. This indicates that although moderate temperatures did not significantly hinder yeast survival, they also did not promote proliferation, possibly due to a balance between growth-promoting and stress-inducing factors. However, Lima et al. [23] do not agree with the current study, they reported that *C. laurentii* LS28 was able to survive better at the storage of 20°C than at 3°C in the apple. This disagreement may stem from strain-specific physiological differences, as well as variations in host fruit cultivar, storage atmosphere, or initial inoculum levels. Similarly, the numbers of *C. infirmo-miniatus* strain YY6 and *C. laurentii* strain HRA5 yeast inoculated in the wounds opened in Golden Delicious type apples increased 1.2 Log at 0°C within 10 days, and 1.4 log at 5, 10 and 20°C [10] indicating that cold storage may not necessarily suppress yeast growth entirely.

In this study, the initial number of yeast cells in the inoculum statistically influenced the survivability of the yeast in the apple wounds ($p<0.05$). Specifically, when the inoculum level was either 6 or 11 log CFU/mL, a reduction of approximately 1 to 2 log units was observed by the end of the 14-day storage period ($p<0.05$). However, when the initial concentration was 9 log CFU/mL, no statistically significant change was detected ($p>0.05$), indicating that this concentration may represent an optimal level for maintaining yeast viability during storage. This finding underscores the critical role of initial inoculum density in determining the competitive success of biocontrol agents like *C. albidus*. At lower concentrations (6 log CFU/mL), the yeast may be at a competitive disadvantage, especially when co-inoculated with a pathogen such as *P. expansum*, which was also present at a similar level in this study. In such scenarios, the limited resources and spatial constraints within apple wounds favor the organism with the faster growth rate or more aggressive colonization strategy. This aligns with previous findings highlighting the importance of initial population size in microbial competition and antagonism [24]. Interestingly, although a higher inoculum level (11 log CFU/mL) might initially seem advantageous, it did not support sustained survival. In fact, the yeast population declined significantly at this concentration. This could be attributed to nutrient depletion and limited space within the wound microenvironment, which may have created intense intraspecific competition among yeast cells. Particularly at higher storage temperatures, the metabolic demand may exceed the available resources,

leading to cell death or reduced proliferation. This suggests that over-inoculation may paradoxically compromise biocontrol persistence, especially under suboptimal storage conditions.

The observation that 9 log CFU/mL supported both stable survival and effective competition suggests a balance point where *C. albidus* has sufficient numbers to outcompete pathogens like *P. expansum*, without triggering excessive nutrient stress. This finding emphasizes the need for careful optimization of inoculum levels in practical biocontrol applications. It also reinforces the concept that biocontrol efficacy is a dynamic outcome shaped not only by microbial antagonism but also by environmental constraints such as temperature, RH, nutrient availability, and spatial limitations.

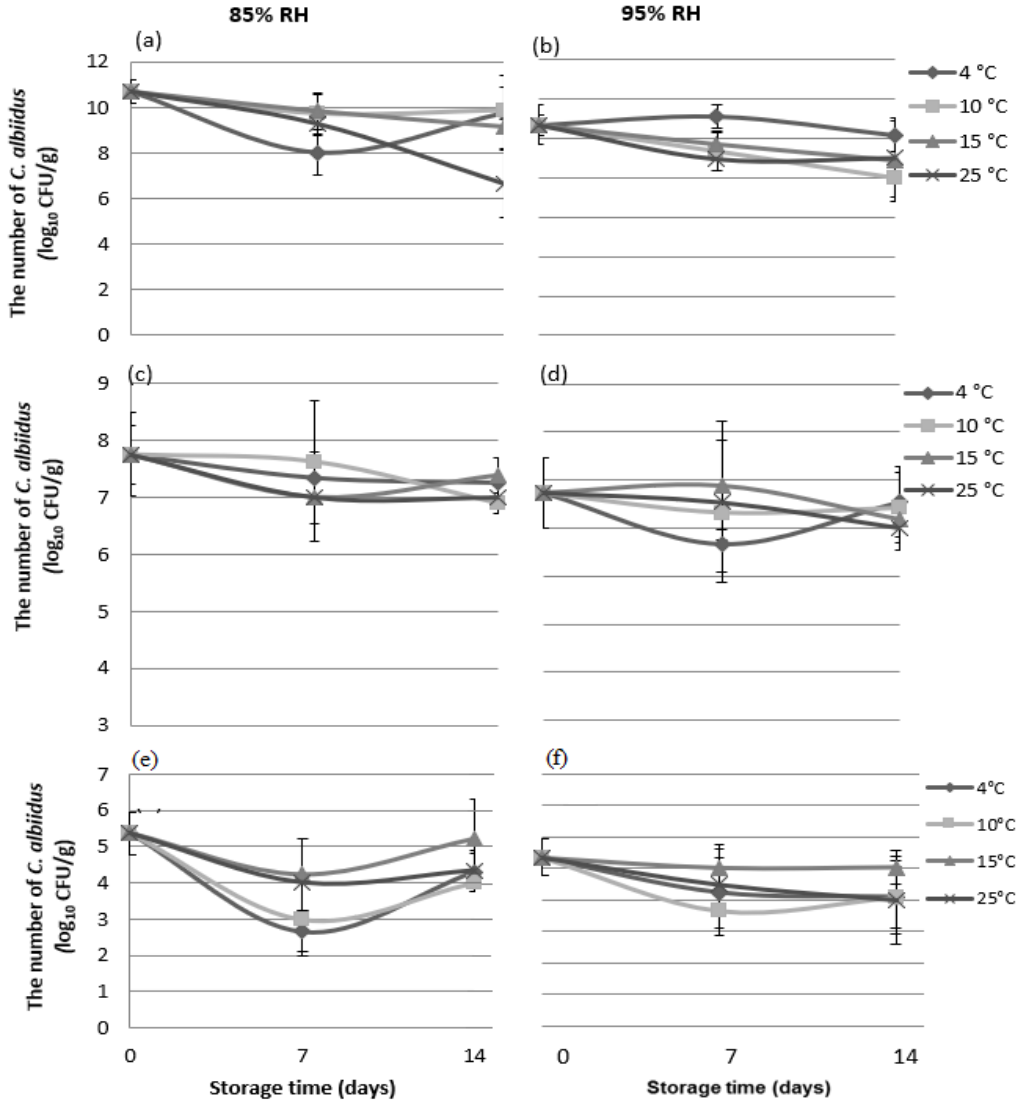

Figure 2. The survival of three different inocluation levels on the apples during 14 days of storage at 4, 10, 15 and 25°C at two different RH (85 and 95%). (a) 11 $\log_{10}$ CFU/mL, 85% RH, and (b) 11 $\log_{10}$ CFU/mL, 95% RH, (c) 9 $\log_{10}$ CFU/mL , 85% RH, (d) 9 $\log_{10}$ CFU/mL, 95% RH, (e) 6 $\log_{10}$ CFU/mL, 85% RH, (f) 6 $\log_{10}$ CFU/mL, 95% RH (mean (n = 3) ± standard deviation)

According to the analysis of variance, storage temperature, time, and yeast inoculum level -individually and in interaction- significantly affected the survival of *C. albidus* ($p<0.05$), highlighting the importance of optimizing these parameters for effective biocontrol (Table 1). In particular, temperature affects yeast metabolism and its competitive behavior within apple wounds, while the initial inoculum level plays a key role in the yeast's ability to establish itself and maintain viability over time. Interestingly, RH did not show a significant effect ($p>0.05$), either alone or in combination with other factors, suggesting that *C. albidus* may tolerate moderate RH fluctuations due to the inherently moist microenvironment of fruit tissue. These findings indicate that storage temperature and inoculum density are critical factors in ensuring the stability and performance of *C. albidus* during postharvest application, whereas RH within the tested range plays a less decisive role.

Table 1. Analysis of variance on the effect of different parameters and their interactions on the survival of *C. albidus* in apples (p-values for independent variables and interactions*).

Source	N_{parm}	DF	Sum of Squares	F Ratio	Prob>F
Trial	2	2	5.13142	3.4983	0.0330
Inoculum Level (IL)	2	2	895.16	610.2616	<0.0001
Temperature (T)	3	3	9.0709	4.1226	0.0078
Relative Humidity (RH)	1	1	0.88685	1.2092	0.2734
Storage Time (ST)	2	2	44.24535	30.1636	<0.0001
IL×T	6	6	15.67062	3.5611	0.0026
IL×RH	2	2	0.28668	0.1954	0.8227
IL×ST	4	4	9.98052	3.402	0.0109
T×RH	3	3	4.93022	2.2407	0.0863
T×ST	6	6	15.09707	3.4307	0.0035
RH×ST	2	2	0.90485	0.6169	0.5411
IL×T×RH	6	6	5.87084	1.3341	0.2461
IL×T×ST	12	12	11.10566	1.2619	0.2481
IL×RH×ST	4	4	1.3639	0.4649	0.7614
T×RH×ST	6	6	5.32049	1.2091	0.3053
IL×T×RH×ST	12	12	22.71482	2.5809	0.0040

*significant at p<0.05; significant at p<0.001.

Network Architecture Optimization

As presented in Table 2, the MSE and coefficient of determination (R^2) values varied across network architectures with hidden layers containing 2 to 8 nodes. These variations were expected due to the inherent randomness in data splitting and the stochastic initialization of network weights and biases. To minimize the influence of such randomness and improve reliability, each architecture was trained five times, and the average performance metrics were considered. Among the tested configurations, the architecture with six hidden nodes consistently demonstrated superior predictive accuracy, yielding the lowest MSE and highest R^2 values on average. This suggests that six nodes provided an optimal balance between under fitting and overfitting, capturing the underlying patterns in the dataset effectively without excessive model complexity. Simpler architectures may have lacked the capacity to represent the non-linear relationships in the growth dynamics of *C. albidus*, while more complex ones could have led to overfitting due to limited data. These results underscore the importance of systematically evaluating different network topologies and using repeated trials to identify robust and generalizable models, especially in biological systems where variability is high.

Model Performance

The network architecture with six hidden nodes yielded the lowest mean squared error (MSE=0.1401) and the highest coefficient of determination (R^2=0.9941) during initial training (Table 2), indicating it was the most accurate and well-performing model developed in this study. The high-test accuracy (R^2=0.99) further confirmed the model's ability to generalize well to unseen data, demonstrating strong predictive capability for *C. albidus* growth under varying conditions.

Table 2. The performance measures of the five-times trained network architectures had the various number of nodes in their hidden layers

	Trainings					
Nodes	1	2	3	4	5	Average
	MSE Values					
2	0.4661	0.4066	0.4644	0.4439	0.2596	0.4081
3	0.4829	0.4293	0.3596	0.3008	0.3547	0.3855
4	0.2225	0.6046	0.2323	0.4240	0.2837	0.3534
5	0.2439	0.2751	0.4355	0.2692	0.4434	0.3334
6	**0.1401**	0.1786	0.5159	0.3836	0.2518	**0.2940**
7	0.3032	0.3613	0.2454	0.4772	0.5061	0.3786
8	0.8843	0.2516	0.5847	0.3010	0.3116	0.4666
	R^2 values					
2	0.9843	0.9811	0.9883	0.9853	0.9888	0.9856
3	0.9751	0.9870	0.9922	0.9938	0.9911	0.9878
4	0.9869	0.9882	0.9939	0.9837	0.9866	0.9879
5	0.9925	0.9905	0.9849	0.9951	0.9879	0.9902
6	**0.9941**	0.9911	0.9900	0.9922	0.9916	**0.9918**
7	0.9948	0.9907	0.9930	0.9732	0.9885	0.9880
8	0.9631	0.9878	0.9727	0.9901	0.9880	0.9803

As illustrated in Figure 3, the optimal weights and biases were achieved at the 22nd epoch, corresponding to the point where the validation error reached its minimum. Although continued training reduced the training error further, the validation error began to increase, signaling the onset of overfitting where the model starts to memorize training data instead of learning generalizable patterns [25]. Recognizing this, training was stopped at the 22nd epoch, and the corresponding weights and biases were adopted as optimal. This outcome highlights the importance of early stopping as a regularization technique to enhance model generalization [26]. It also emphasizes that monitoring validation performance is essential for building reliable models in biological prediction tasks, where data can be noisy and complex [27]. The application of early stopping prevented overfitting effectively, thereby improving the robustness and predictive accuracy of the model.

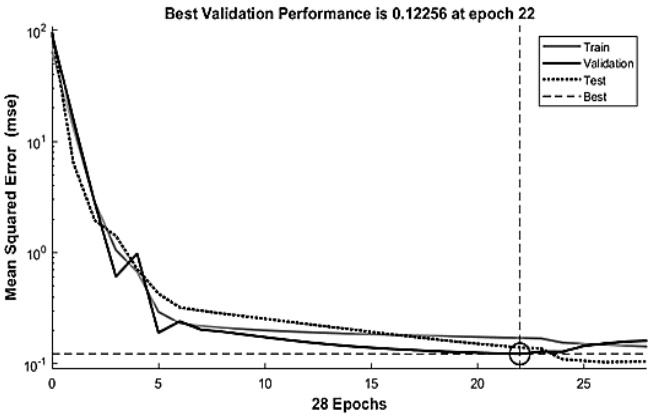

Figure 3. The fitting curves showing the consistency between the model predictions and the actual values

As illustrated in Figure 4, the fitting curves for the training, validation, and test datasets, along with the overall fitting curve, demonstrate the model's strong predictive performance. The dashed lines represent the ideal scenario, where the predicted values (Y) perfectly match the actual target values (T), while the solid lines indicate the actual fitting achieved by the model. The close alignment of these lines suggests a high degree of accuracy in capturing the underlying data patterns. Notably, the R^2 values for all individual datasets exceeded 0.99, indicating an exceptionally good fit between the model predictions and the experimental results. Such high R^2 values across all data splits confirm the robustness and consistency of the model, minimizing concerns of underfitting or overfitting. The particularly high accuracy on the test dataset (R^2=0.99) further supports the model's strong generalization capability, implying that it can reliably predict outcomes for new, unseen data. This is a crucial attribute in biological systems modeling, where variability is often high and data replication is limited. The results validate the effectiveness of the selected network architecture and training strategy and reinforce the model's potential utility as a predictive tool for future studies or applications involving *C. albidus* growth behavior.

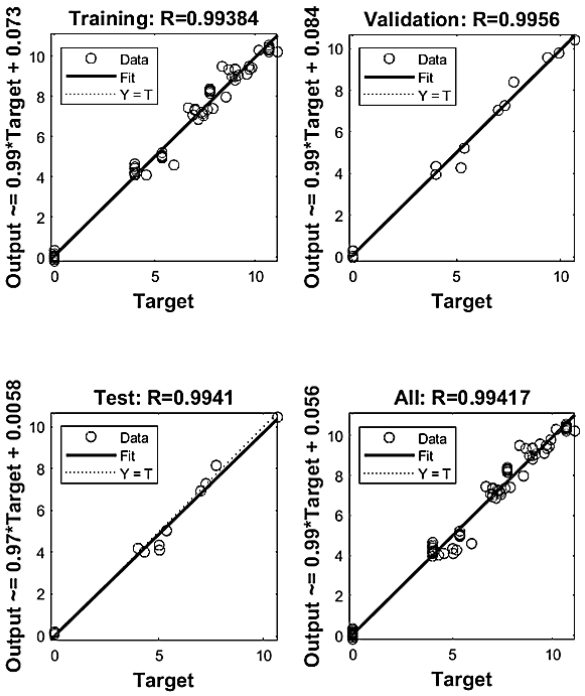

Figure 4. The performance measures (MSE) of the most accurate model in Table 2 across the epoch number. The gray, black and dotted lines represent the training, validation, and test sets errors, respectively.

Figure 5 shows the schematic diagram of the network architecture with six hidden nodes. The figure also provides the optimized weights and biases. The table columns with the *W* notation provide the weights. For instance, $W_{1,1-6}^{(1)}$ represents the weights for the relationship between the first parameter and the six hidden nodes. The biases (*b*) were provided in the table cells with the dashed borders. The diagram provides an algorithm and the optimized values for one to code a computer function. The function will take the four input parameters and calculate the number of *C. albidus* ($\log_{10}$ CFU/g) by using Equations 1, 2, and 3 with the optimized values provided in the diagram.

CONCLUSIONS

In addition to its lethal effect against pathogens, the most important factors in the commercialization of antagonistic yeasts are their ability to colonize fruit surfaces and to survive as long as possible under storage conditions. The survival ability of *C. albidus* in the apple wounds was significantly affected by temperature, time and inoculum level ($p<0.05$) but RH. Optimum survival of *C. albidus* was observed with their number was not significantly reduced when their inoculum number was 9 log CFU/mL at all storage temperature. Increase or decrease of inoculum level was not significantly improved the survival ($p>0.05$). The ANNs model developed and optimized in this study had a superior prediction performance with an R^2 of 0.99. The model predictions can help to understand the biocontrol mechanism and improve the efficiency of the biocontrol applications where *C. albidus* takes place.

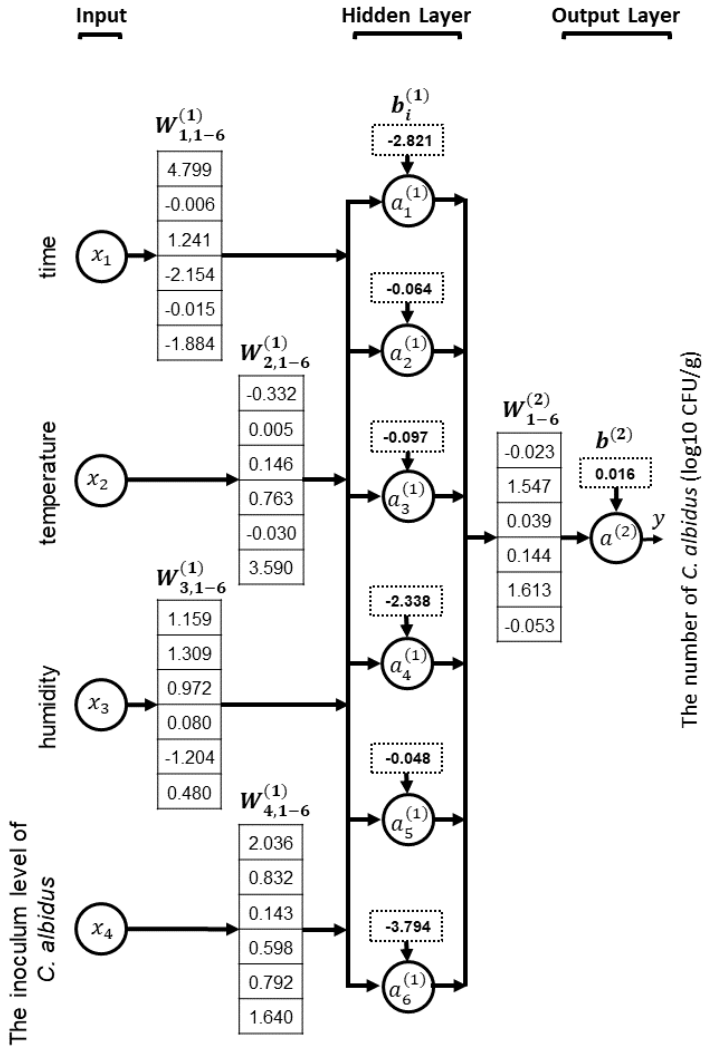

Figure 5. The algorithm of the ANN model with the optimized weights and biases

C. albidus is generally considered harmless saprophytic yeast; however, it has been occasionally reported to be associated with rare human infections in the literature [25]. Therefore, in cases where this biocontrol strategy is applied, it is recommended that apples are thoroughly washed under running water before consumption. Additionally, the use of edible fruit cleansers may further help reduce not only *C. albidus* but also other potential microbial contaminants. This precaution ensures the safety of the fruit for consumption and aligns with general food safety practices.

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