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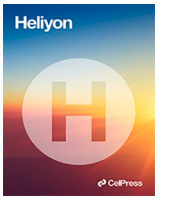

Advanced modeling and optimizing for surface sterilization process of grape vine (*Vitis vinifera*) root stock 3309C through response surface, artificial neural network, and genetic algorithm techniques

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A R T I C L E I N F O

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A B S T R A C T

In vitro, sterilization is one of the key components for proceeding with plant tissue cultures. Since the effectiveness of sterilization has a direct impact on the culture's final outcomes, there is a crucial need for optimization of the sterilization process. However, compared with traditional optimizing methods, the use of computational approaches through artificial intelligence-based process modeling and optimization algorithms provides a precise optimal condition for *in vitro* culturing. This study aimed to optimise *in vitro* sterilization of grape rootstock 3309C using RSM, ANN, and genetic algorithm (GA) techniques. In this context, two output responses, namely, Clean Culture and Explant Viability, were optimised using the models developed by RSM and ANN, followed by a GA, to obtain a globally optimal solution. The most influential independent factors, such as $HgCl_2$, NaOCl, $AgNO_3$, and immersion time, were considered input variables. The significance of the developed models was investigated with statistical and non-statistical techniques and was optimised to determine the significance of selected inputs. The optimal clean culture of 91%, and the explant viability of 89% can be obtained from 1.62% NaOCl at a 13.96 min immersion time, according to MLP-NSGAII. Sensitivity analysis revealed that the clean culture and explant viability were less sensitive to $AgNO_3$ and more sensitive to immersion time. Results showed that the differences between the GA predicted and validation data were significant after the performance validation of predicted and optimised sterilising agents with immersion time combinations were tested. In general, GA, a potent methodology, may open the door to the development of new computational methods in plant tissue culture.

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1. Introduction

Plant tissue culture is a well-acknowledged biotechnological approach for crop improvement, the synthesis of phytochemicals, and the *in vitro* conservation and propagation of numerous plant species [1]. It involves hypersensitive, accurate, multiple variables and processes that have been included while in view of the extended array of plant tissue culture-based techniques, viz., somatic embryogenesis, protoplast fusion, haploid production, hairy root culture, virus elimination, *in vitro* conservation, Agrobacterium-mediated genetic transformation, and very recently *in vitro* plant tissue culture has been playing a crucial role in genome editing technologies like CRISPR [2–6].

Successful culture commencement demands the removal of all contagious pathogens, including micro-arthropods like mites and thrips that can detrimentally affect the explant's growth and developmental process the reason that the *in vitro* culture environment offers suitable nutrients and culture conditions for a broad range of microorganisms [7]. The microbes present in explants emancipate plant metabolites and other complex substances that negatively affect the plant tissues and amend the constituents and conditions of the culture medium [8]. The contamination colonizes the media at a quick growth rate and in combination with the nutrient's availability, and the explant eventually dies in the following days. Disinfection competence be subject to the contamination types like epi phytic and endophytic or latent and chronic microbial contaminations, the explant features like kind, maturity, proportions, part of the explant, explant collection season and time, biological and physiological makeup of the donor plant, along with the culture conditions, coupled with the sterilization techniques [9,10]. As a result, numerous substances were utilized to create aseptic culture, including fungicides, antibiotics, hydrogen peroxide (H_2O_2), mercuric chloride ($HgCl_2$), calcium hypochlorite $Ca(OCl)_2$, and silver nanoparticles [11]. However, sodium hypochlorite (NaOCl) is the chemical disinfection alternative that is given the most thought due to its wide antibacterial spectrum, quick bactericidal activity, solubility in water, and general stability. Additionally, the explant's future development pathway may be influenced by the disinfection process, and the explant's health is the primary variable that has a significant impact on its capacity for regeneration.

Utilising statistical models like response surface methodology (RSM) and neuro-fuzzy logic are different techniques for enhancing *in vitro* shoot growth by altering mineral nutrients. [12]; Gago et al., 2011; [13]. RSM is an approach in statistical for modelling and analysing reactions affected by several factors, with the primary goal of optimizing the response. A first-order RSM model (multiple regression) is used when the response is a linear function of the components. If the response surface has curvature, a higher-degree polynomial, such as a second-order polynomial, should be employed. The parameters of the polynomials are determined using the Least Squares method. Response Surface Methods are designs and models that enable continuous factor optimization [14]. RSM has been utilized in plant sciences to optimise the synthesis of intermediate metabolites, enzyme processes [15], and the growth medium [16].

Recently plant tissue culture is recognising the benefits of the application of artificial intelligence and optimization algorithms in spite of their complexity in genetic programming and modelling [5,17]. Radial Basis Function (RBF), Generalized Regression Neural Network (GRNN), Probabilistic Neural Network (PNN), Neuro-Fuzzy Logic (NFL), Support Vector Machine (SVM), and Multilayer Perceptron (MLP) are some of the most widely used artificial neural networks (ANNs) for modeling and protocol optimization in plant tissue culture. (Hesami et al., 2020 [18];). To achieve the optimum results during surface sterilization, the kind, and concentration of sterilant and immersion duration must be optimised for each species and explant. It is expensive and time-consuming to optimise this process, and some disinfectants might be harmful to human health or the environment. A hybrid AI-OA could be a trustworthy and practical statistical tool for forecasting and optimizing this stage to help solve the problem [19,20]. [19] indicated that MLP models with various neurons in the hidden layer and activations of both linear and sigmoid functions can accurately predict sterilization efficacy.

The commercial fruit crop, grape (*Vitis vinifera* L.) originated in warm and temperate regions of the world. In line with a distinction in usage for fresh consumption (table grapes) or winemaking, grape varieties moved primarily westward from the cradle of domestication6 due to human migration and maritime trade (wine grapes) [21,22]. Numerous non-linear biological processes that are only observed in plant tissue culture can be characterized by using appropriate methodologies for modeling and augmenting possible prediction of *in vitro* growth kinetics [12,23].

The RSM is a statistics-based optimization and design technique with two sets of variables known as independent and dependent. RSM analyses and presents the relationship between these two sets of variables. Among the different RSM classes, Box- Behnken Design (BBD) is the best model for both linear and non-linear relationships between the independent and dependent variables/responses. In ANN, the techniques Non-Linear Programming (NLP) method is more accurate than other ways, however, the process of solving the problem is time-consuming and complicated. Among the specific settings that the GA can be potentially put into operation, are optimization of different *in vitro* culture stages, optimization of the MLP neural network weights, and optimization of the relevant parameter in the present study to establish the clean culture and for explant viability.

One of the most remarkable challenges confronting the advancement of *in vitro* culture is modelling the various processes of plant tissue development. This increase is a result of the physical complexity of plant tissue culture as well as the time and expense required for analysing various steps involved *in vitro* culture. Even while many biological events are easily visible at various phases of *in vitro* growth, they are all non-linear, non-deterministic, and affected by a variety of additional factors as well. Traditional statistics-based optimization is difficult to perform since it would require an excessive number of treatments due to the complicated interplay of numerous components. [24]; Hesami et al., 2020). In order to optimise protocols with fewer treatments, the deployment of the relevant AI models can be seen as a valuable and precise way to simulate and anticipate various growth and developmental processes under *in vitro* circumstances. Conventional optimization approaches focus on one specific Pareto-optimal solution at a time, transforming the multi-objective optimization problem into a single-objective problem by using multi-criterion decision-making techniques. This

strategy requires repeated application in order to find a variety of solutions during every test run. As the first evolutionary multi-objective optimization algorithm, the Non-dominated Sorting Genetic Algorithm-II (NSGA-II) seeks the domain of solutions for finding Pareto-optimal solutions in a multi-objective framework.

Multi-objective functions can be used to assess the effectiveness of plant tissue culture techniques for optimization complications. The inputs must be optimised by numerous trials and errors. Genetic algorithms (GA) have been employed recently in studies to lower computing loads [24,25]. The most well-known optimization method, GA, helps to achieve the best results with the least amount of computation. On the other hand, plant tissue culture challenges must take into account multiple restrictions in order to meet diverse objective functions. The common GA, however, is a single-objective algorithm that cannot simultaneously optimise several functions. In accordance with this investigation, our efforts were focused on determining the optimal non-linear MLP-NSGAII modelling and optimization approach for sterilants and immersion time. In order to obtain the best efficiency and the ideal concentrations of sterilants as well as immersion times throughout the *in vitro* sterilization process, creating a strong association between the MLP model and NSGAII is our top goal. The main goal of this work was to model and optimise the ideal sterilant concentrations and immersion times for sterilising grape nodal explants.

The goal of this study was to use Response Surface Methodology (RSM) and Artificial Neural Network (ANN) for estimating and optimizing the ideal doses of sterilising agents and impregnation time for disinfection of nodal explants of grapes root stock 3309C.

2. Materials and methods

2.1. Plant materials

The nodal explants of grape rootstock "3309C" were collected from Castel Winery vineyard, Ziway, Ethiopia. The Castel winery vineyard is located in the town of Ziway with an elevation of around 1600 m above sea level, with a sub-humid to sub-arid climate. The nodal segments were used as explants from mature field-grown mother plants from the collection site. The healthy and young sprouts from the selected mother plants were selected for explant collection. The explants were collected during the early morning (7–9 a.m.) and the explants were stored in sterile water in tissue culture jars in the cooler box during transportation from the field to the laboratory. The nodal explants were cleaned with running tap water for 30 min before being rinsed with a liquid soap solution. Furthermore, the laminar airflow chamber was sterilized using UV radiation. Besides, the explants were sterilized for 1 min in 70% aqueous ethanol, immersed in various concentrations and types of sterilising agents for varying periods, and washed three times in double distilled water. The segments were then inoculated on a 200-ml tissue culture jar with 30 ml MS basal media.

2.2. Media and culture conditions

[26] (MS) medium with 0.8% agar (Duchefa Biochemie, Netherlands) and 3% sucrose were employed as a basal media in this experiment. Before autoclaving at 121 °C for 20 min, the pH of the medium was adjusted to 5.8 with 1 N KOH or 1 N HCl. All cultures were kept at 26 ± 2 °C for 16 h with a light intensity of 50 μmol m^{-2} s^{-1}

2.3. Experimental design

The trials were carried out using a completely randomized design (CRD, based on computational optimization techniques, the influence of sterilant and impregnation times on *in vitro* sterilization of rootstock (3309C) nodal explants was examined.

2.4. Response surface methodology

In order to determine the individual impact of the selected parameters on the clean culture and explant viability, a one-variable-at-a-time (OVAT) approach was executed [27]. In such a way, different concentrations of NaOCl (0.1, 0.5, 1, 1.5, 2.0, and 2.5%), impregnation times (5, 10, 15, 20, 25, and 30 min), concentrations of $HgCl_2$ (0.1, 0.25, 0.5, 0.75, 1.0, and 1.25%), and concentrations of $AgNO_3$ (0.1, 0.25, 0.5, 0.75, 1.0, and 1.25%) were examined via OVAT. During the OVAT test on one parameter, other parameters were kept constant. Accordingly, 15 min for impregnation time, and 1% for the concentration of $HgCl_2$ and $AgNO_3$, respectively, were fixed as constants while varying the NaOCl concentration. When applying the OVAT approach for impregnation time, the concentrations of $HgCl_2$, $AgNO_3$, and NaOCl were fixed at 1%, 1%, and 1.5%, respectively. Due to the preliminary impact of $HgCl_2$, the concentrations of $AgNO_3$ and NaOCl were fixed at 1.5 and 1%, respectively, with 15 min for impregnation time. Similarly, yet another independent factor, $AgNO_3$, was examined by keeping other parameters constant, such as NaOCl and $HgCl_2$, at 1.5 and 1%, respectively, for 15 min of impregnation time. Based on the results obtained, the limit values were determined for each independent variable. Accordingly, the interaction limits were selected for further response surface analysis and optimization. The response surface method is a well-known statistical approach that can be widely applied to obtain models of correlation, interaction effects, and optimization. In the present study, the chosen four parameters, viz., impregnation time and concentration for NaOCl, $HgCl_2$, and $AgNO_3$, were statistically correlated using the RSM approach. For that, the Box-Behnken design (BBD) was executed to test the different combinations of the parameters (Dimitriadou et al., 2022). The experiments were conducted based on a completely randomised design (CRD) using a factorial arrangement with 29 combinations of treatments. The effect of sterilising agents and impregnation times on *in vitro* sterilization of grape rootstock was evaluated by determining the percent clean culture and percent explant viability after three weeks of inoculation. Design-Expert® software was used for generating BBD combinations, modelling, and optimization. In general, the model

can be generated by the software as presented in equation (1) [28].

$$y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i X_j \tag{1}$$

Whereas, y is known as the response (in the present study, clean culture/explant viability). βi, βij, and βii are the corresponding coefficients. β_0 is constant. xi refers to the independent variable. ANOVA (analysis of variance) was applied to analyze the appropriateness of the model. Followed by 3-D response surface plots were generated to visualize the effects of the independent variables on the response.

2.5. Artificial neural network (ANN)

The artificial neural network is a biologically inspired module of one of the important aspects of artificial intelligence called machine learning (Chen et al., 2021). In the world of medicine, the deep learning methodology is effective for the process of disease detection and categorization. The disease Covid-19 and brain tumors were identified using Deep Convolutional Neural Network (DCNN) [29,30]. ANN functions are synonyms for the brain of a human being. In the brain, sensory processing occurs through the adjustment of neurons in response to a specific synoptic signal. Similarly, ANN processes information by sending it through a network of interconnected nodes called neurons. As the signal travels through these nodes, adjustments are made to the weights and biases associated with these networks. The adjustments will proceed until a desirable outcome or output is attained. In this study, the ANN model was constructed using the experimental conditions and responses obtained from RSM-based optimization. The NNTOOL (Neural Network Toolbox) of MATLAB software (R2013a version) was used to deploy the ANN model. To construct the ANN model, the number of input nodes in the input layer was adjusted to the total number of experimental variables used (number of input nodes = 4), and they correspond to NaOCl concentration, impregnation time, $HgCl_2$ concentration, and $AgNO_3$ concentration. Concurrently, the number of nodes in the output layer was set to the sum of dependent variables (number of output nodes = 2), and they correspond to clean culture % and explant viability %. The network type and learning rule adapted to construct the ANN model were feed-forward back--propagation and the Levenberg-Marquardt algorithm, respectively [31]. Additionally, transfer functions such as tansig (tangent sigmoidal transfer function) and purelin (linear transfer function) are used to activate the neurons of the input and output layers. To construct a desirable ANN model, the optimal number of neurons required in the hidden layer was determined by constructing an ANN architecture with different numbers of hidden layer neurons ranging from 1 to 25. The mean square error (MSE) obtained during the validation of the network performance was used as a criterion to select the optimal number of neurons required in the hidden layer. Prior to setting the sample input and target data, normalization of the data was carried out to hasten the training process using equation (2) [32].

$$\text{Normalization} = \frac{2 * \left(x_A - x_{A,min}\right)}{x_{A,max} - x_{A,min}} - 1 \tag{2}$$

where xA, xA, min, and xA, max are the experimental data and the maximum and minimum values of the experimental data. After constructing the network, network training was carried out using the input and target data. Once the training was completed, the output predicted by the ANN was subjected to denormalization using equation (2). Then, performance metrics such as correlation coefficient (R) [equation (3)], coefficient of determination (R^2) [equation (4)], adjusted coefficient of determination (adj. R^2) [equation (5)], mean square error (MSE) [equation (6)], root mean square error (RMSE) [equation (7)], mean absolute error (MAE) [equation (8)], standard error of prediction (SEP) [equation (9)], and absolute average deviation (ADD) [equation (10)] were calculated for both ANN and RSM predicted outcomes using the following expressions:

$$\text{R} = \frac{\sum_{i=1}^{n}\left(t_i - t_{i,avg}\right) * \left(o_i - o_{i,avg}\right)}{\sqrt{\left[\sum_{i=1}^{n}\left(t_i - t_{i,avg}\right)^2 * \sum_{i=1}^{n}\left(o_i - o_{i,avg}\right)^2\right]}} \tag{3}$$

$$\text{R}^2 = 1 - \frac{\sum_{i=1}^{n}\left(o_i - t_i\right)^2}{\sum_{i=1}^{n}\left(t_i - o_{i,avg}\right)^2} \tag{4}$$

$$\text{Adj} - \text{R}^2 = 1 - \left[\frac{\left(1 - R^2\right) * N - 1}{N - k - 1}\right] \tag{5}$$

$$\text{MSE} = \frac{1}{n}\sum_{i=1}^{n}\left(t_i - o_i\right)^2 \tag{6}$$

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(t_i - o_i\right)^2} \tag{7}$$

$$\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}|t_i - o_i| \tag{8}$$

$$\mathrm{SEP} = \frac{RMSE}{o_{i,avg}} * 100 \tag{9}$$

$$\mathrm{AAD} = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{o_i - t_i}{o_i}\right| \tag{10}$$

where oi is the observed experimental data, ti is the predicted data from RSM and ANN, oi,avg is the average value of the observed experimental data, and ti,avg is the average of the predicted values, N/n is the number of experimental runs, and k is the sum of the experimental variables used in this study [33]. In addition, a separate set of six experimental combinations that were not part of RSM-based experiments was carried out to validate the predictions made by both RSM and ANN.

2.6. Multi-objective genetic algorithm

The genetic algorithm (GA) is a metaheuristic search algorithm developed akin to the natural selection theory of Darwin and widely used to solve problems involved in constrained and unconstrained optimization [34]. The simultaneous optimization of both clean culture % and explant viability percent falls into the domain of multi-objective optimization. In GA, an optimization procedure called non-dominated sorting genetic algorithm II (NSGA-II) was widely employed to solve multi-objective optimization problems [35]. While employing NSGA-II for solving multi-objective optimization problems, a set of equally good solutions called a pareto solution will be obtained rather than a single solution. The regression equations obtained at the end of the RSM-based optimization were used as

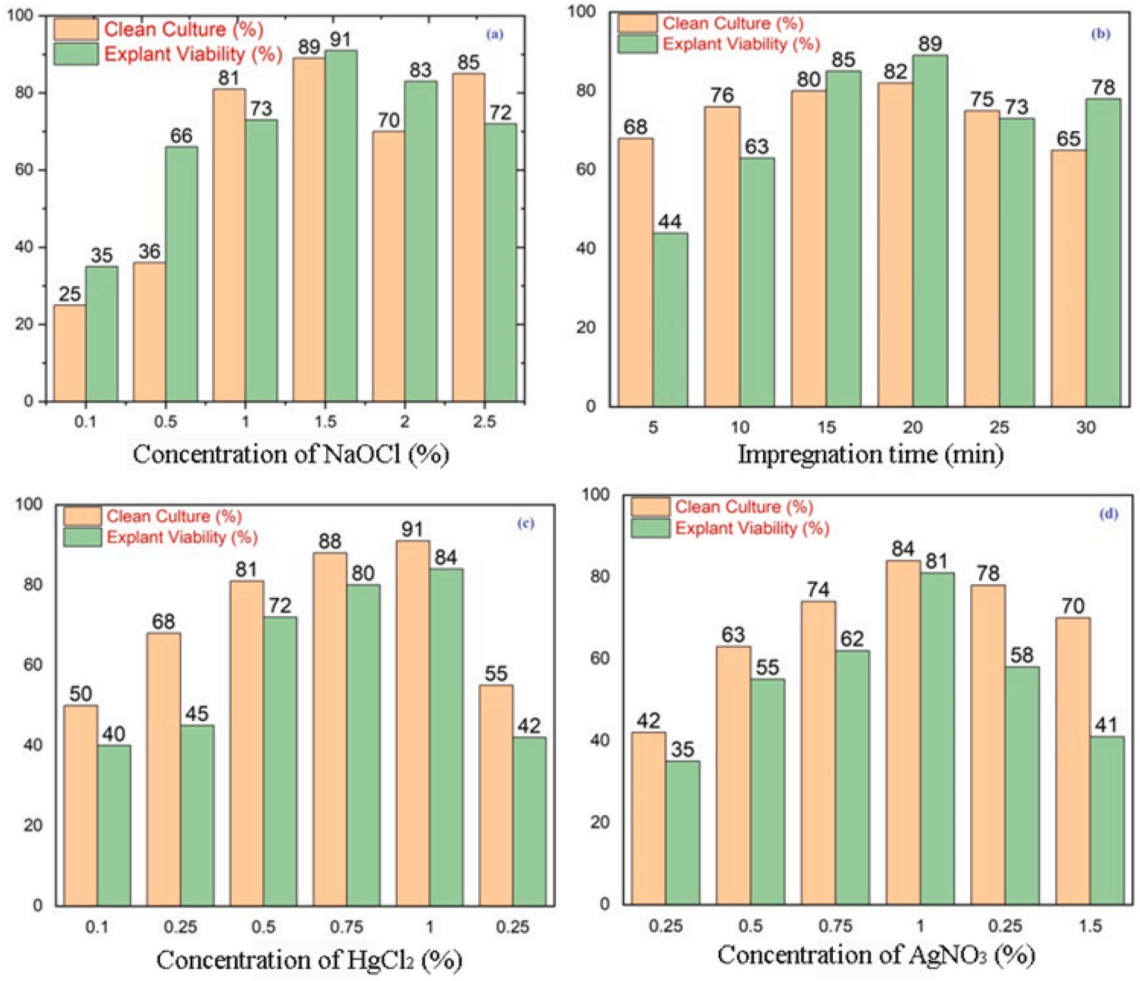

Fig. 1. Results obtained from OVAT approach for NaOCl, impregnation time, $HgCl_2$, and $AgNO_3$ on clean culture and explant viability.

a fitness function to run multi-objective GA. The 'gamultiobj' function of the optimization toolbox of MATLAB software (R2013a version) was used to implement multi-objective GA in this study. The parameters used to run multi-objective GA include population size = 80, crossover fraction = 0.75, mutation rate = 1.0, and the maximum time allowed for iteration = 120 min.

3. Results

The success of *in vitro*-based plant biotechnology requires contamination-free culture. Aseptic initiation is a particularly difficult step, especially in woody species. Meanwhile, over-sterilization may be harmful to plant tissue. With the recent rise of machine learning algorithms in plant tissue culture, an advanced interpretive tool for the combinational effect of influential factors for such *in vitro*-based steps is proposed.

3.1. Response surface plots, interaction analysis, and modelling

Prior to carrying out the response surface analysis, the selected independent parameters were undertaken for the determination of limits that are to be studied for the interaction effect and optimization. Accordingly, the OVAT method was executed for each parameter, as illustrated in Fig. 1. From the results, the mid-value of the selected parameters was found to be 1.5% for NaOCl, 20 min for impregnation time, and 1% for $HgCl_2$ and $AgNO_3$. Using these outcomes, the BBD of the experiment (as presented in Table 1) was developed using Design-Expert® software. According to the experimental investigation, the clean culture and explant viability were determined after three weeks of inoculation and analyzed through the RSM. Further, the models were developed for clean culture [Equation (11)] and explant viability [Equation (12)] with respect to the selected parameters were given below.

$$\begin{aligned}\text{CleanCulture}(\%) = &-85.04167 + 50.83333(\text{A}) + 3.54167(\text{B}) + 133(\text{C}) + 114(\text{D}) + 0.2(\text{A})(\text{B}) - 10.5(\text{A})(\text{C}) + 1(\text{A})(\text{D}) - 2.13 \\ &\times 10^{-16}(\text{B})(\text{C}) + 1.25(\text{B})(\text{D}) - 4(\text{C})(\text{D}) - 15.95833(\text{A})^2 - -0.134583(\text{B})^2 - 79.83333(\text{C})^2 - 67.83333(\text{D})^2\end{aligned} \tag{11}$$

$$\begin{aligned}\text{Explantviability}(\%) = &-74.3 + 28.55(\text{A}) + 4.54(\text{B}) + 126.43333(\text{C}) + 108.46667(\text{D}) + 0.2(\text{A})(\text{B}) + 0.375(\text{A})(\text{C}) - 8.5(\text{A})(\text{D}) \\ &+ 6(\text{B})(\text{C}) - 0.35(\text{B})(\text{D}) + 5.63 \times 10^{-16}(\text{C})(\text{D}) - 12.55833(\text{A})^2 - -0.128083(\text{BTime})^2 - 64.23333(\text{C})^2 \\ &- 52.23333\end{aligned} \tag{12}$$

Using the aid of the RSM-BBD, the importance of the independent process parameters, like the concentration of NaOCl, $HgCl_2$, $AgNO_3$, and impregnation time, was examined on clean culture and explant viability. Fig. 2(a–f) shows the interaction upshots (3-D response surface) of different combinations of two selected parameters on clean culture and explant viability, respectively. The outcome with respect to interaction effects for all chosen combinations on the development of clean culture followed an increasing trend up to an optimal level, then showed a decline in response except at the optimal point. This optimal value can be statistically determined by solving the model developed by RSM. Equation (11) depicts the correlation between clean culture and the chosen parameters. Statistical analysis using the ANOVA has been provided in Table 1 for the model correlation to clean culture.

From the model, the mutual interaction of NaOCl and $HgCl_2$ concentration showed an undesirable effect on the development of clean culture with a coefficient value of 10.5. Similarly, the mutual interaction of the concentrations of $HgCl_2$ and $AgNO_3$ had a negative impact on the clean culture, with a coefficient value of 4. Among the selected parameters, impregnation time and concentration of $HgCl_2$ showed no interaction effect since the mutual interaction had an ignorable value. As seen in Table 2, the p-value of the

Table 1
Statistical analysis using ANOVA for the model development on clean culture.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model (Significant)	5251.84	14	375.13	562.7	<0.0001
A: Conc. of NaOCl	0.0833	1	0.0833	0.125	0.7289
B: Impregnation Time	102.08	1	102.08	153.12	<0.0001
C: Conc. of $HgCl_2$	126.75	1	126.75	190.12	<0.0001
D: Conc. of $AgNO_3$	10.08	1	10.08	15.13	0.0016
AB	16	1	16	24	0.0002
AC	110.25	1	110.25	165.37	<0.0001
AD	1	1	1	1.5	0.2409
BC	0	1	0	0	1
BD	156.25	1	156.25	234.37	<0.0001
CD	4	1	4	6	0.0281
A^2	1651.9	1	1651.9	2477.85	<0.0001
B^2	1174.88	1	1174.88	1762.31	<0.0001
C^2	2583.8	1	2583.8	3875.69	<0.0001
D^2	1865.42	1	1865.42	2798.12	<0.0001
Residual	9.33	14	0.6667		
Lack of Fit (Not Significant)	7.33	10	0.7333	1.47	0.3797
Pure Error	2	4	0.5		
Cor Total	5261.17	28			

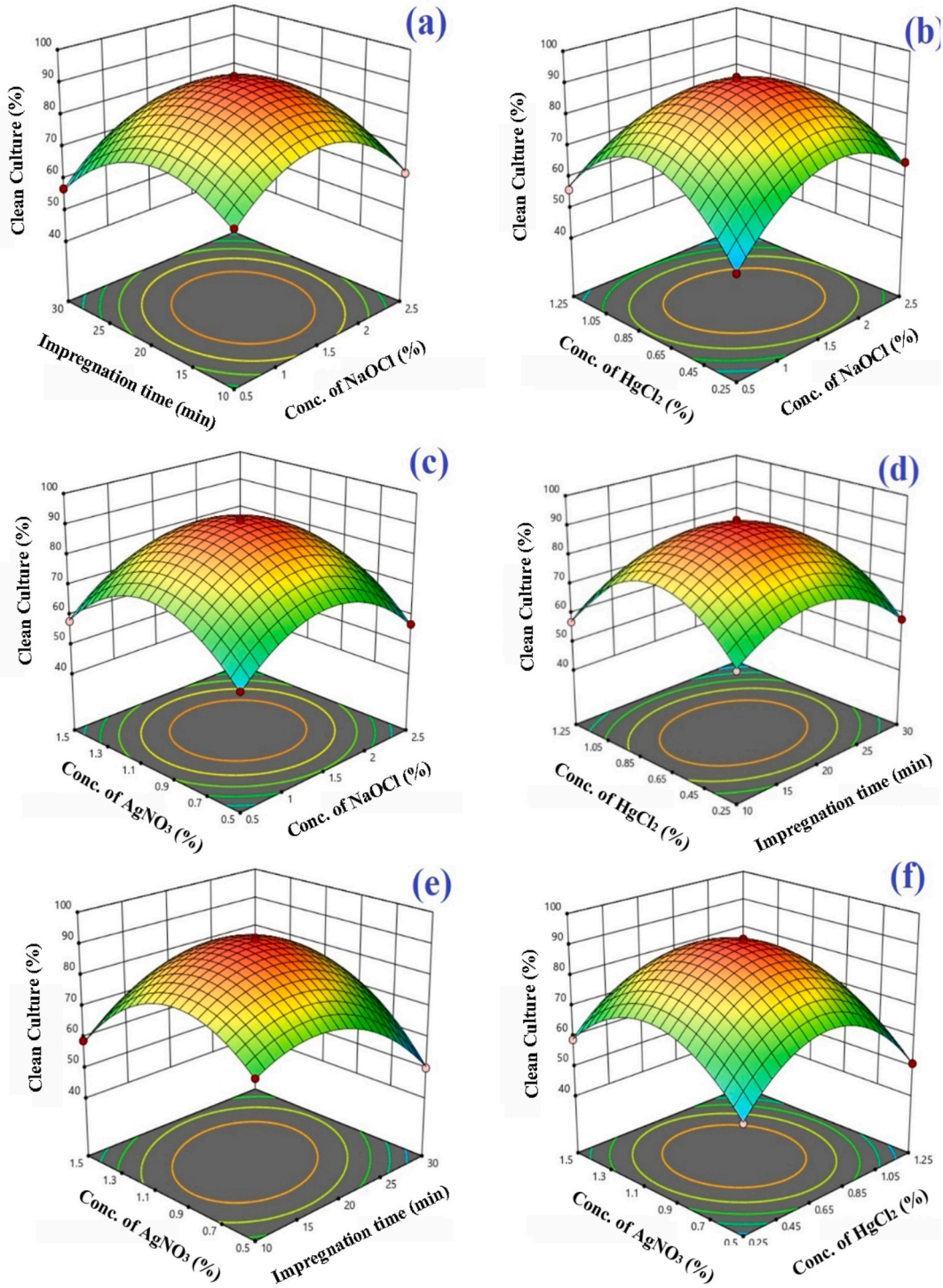

Fig. 2. The developed 3-D interactive plots for illustrating the clean culture as response with respect to different combinations of selected parameters (a) Interaction effect of impregnation time and NaOCl concentration, (b) Interaction effect of $HgCl_2$ concentration and NaOCl concentration (c) Interaction effect of $AgNO_3$ concentration and NaOCl concentration (d) Interaction effect of $HgCl_2$ concentration and impregnation time (e) Interaction effect of $AgNO_3$ concentration and impregnation time (f) Interaction effect of $AgNO_3$ concentration and $HgCl_2$ concentration.

model has been found to be less than 0.001, which indicates that the model had a substantial effect on the experimental output. The F-value of the acquired model was 562.70, which implied the model was significant. Fig. 3(a–f) illustrates the output of explant viability for different combinations of selected parameters. The outcome with respect to interaction effects for all chosen combinations on the

Table 2
Statistical analysis using ANOVA for the model development on explant viability.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model (Significant)	3619.6	14	258.54	728.78	<0.0001
A: Conc. of NaOCl	48	1	48	135.3	<0.0001
B: Impregnation Time	96.33	1	96.33	271.54	<0.0001
C: Conc. of $HgCl_2$	96.33	1	96.33	271.54	<0.0001
D: Conc. of $AgNO_3$	3	1	3	8.46	0.0115
AB	56.25	1	56.25	158.56	<0.0001
AC	72.25	1	72.25	203.66	<0.0001
AD	36	1	36	101.48	<0.0001
BC	12.25	1	12.25	34.53	<0.0001
BD	0	1	0	0	1
CD	64	1	64	180.4	<0.0001
A^2	1023	1	1023	2883.61	<0.0001
B^2	1064.1	1	1064.13	2999.56	<0.0001
C^2	1672.7	1	1672.67	4714.91	<0.0001
D^2	1106.1	1	1106.08	3117.8	<0.0001
Residual	4.97	14	0.3548		
Lack of Fit (Not Significant)	4.17	10	0.4167	2.08	0.2498
Pure Error	0.8	4	0.2		
Cor Total	3624.6	28			

explant viability also flowed an upsurge trending up to an optimal level; further, it showed a decline in response except at the optimal point. Equation (12) gives the correlation between explant viability and the chosen parameters. A statistical analysis using the ANOVA has been provided in Table 2. From the model, the mutual interaction of NaOCl and $AgNO_3$ concentration was found to have an undesirable effect on the development of explant viability with a coefficient value of 8.5. Similarly, the mutual interaction of the concentrations of $HgCl_2$ and $AgNO_3$ had a significant negative impact on the explant viability, with a coefficient value, 16. It was observed that impregnation time and concentration of $AgNO_3$ had no interaction effect that could be ignored.

3.2. ANN-based modelling and comparison with RSM

ANN-based models are widely employed as a non-statistical strategy to relate independent variables (input) with dependent variables (target). The ANN makes use of a known set of experimental combinations and their corresponding outcomes to construct a model that can predict the outcomes of unknown experimental combinations. The models' accuracy for the two examined parameters was demonstrated by a high coefficient of determination between observed and predicted values for both the training and testing processes. In plant tissue culture, ANN has demonstrated high efficacy in numerous investigations. [24,25,36]; Jamshidi et al., 2016) In this study, the ANN model was constructed, relating the input variables such as $HgCl_2$ concentration, impregnation time, $HgCl_2$ concentration, and $AgNO_3$ concentration with their responses like clean culture % and explant viability %. Prior to the network's construction, the dataset was separated into training (70% of the data), validation (15% of the data), and testing (15% of the data) categories. After constructing the network, training of the model was carried out with different numbers of neurons in the hidden layer (1–25). Among these training phases, the minimum validation MSE of 0.015201 was obtained when training the network with 4 neurons in the hidden layer (Figs. 4 and 5). In addition to MSE, the highest regressions obtained during the training, validation, and testing phases are 0.98758, 0.9832, and 0.98922, respectively. Similarly, the overall regression was found to be 0.98676, which indicates a good correlation between the actual data and the predicted data. Hence, ANN was successfully implemented to predict the sterilization efficiency of the input variables. The regression plots associated with the training, validation, and testing of ANN are depicted in Fig. 6. While constructing the successful ANN-based models [equations 13–16], the weight and bias values were adjusted to the following values:

$$\begin{aligned} iw\{1,1\} = [&-5.7046\ 4.0522\ 2.0669\ 3.2041;\ 1.3577\ 1.8533\ 8.5268\ -3.3571;\ -4.4294\ 0.94039\ -3.9394\ -2.0477;\ -2.9136 \\ &-2.0558\ -2.5812\ -6.1561] \end{aligned} \tag{13}$$

$$iw\{2,1\} = [1.0439\ -1.3256\ -1.2383\ 1.1145;\ 1.2035\ -1.1932\ -1.1585\ 1.1165] \tag{14}$$

$$b\{1\} = [6.4006;\ -5.3315;\ -2.8773;\ 2.7252] \tag{15}$$

$$b\{2\} = [-2.9121;\ -2.8326] \tag{16}$$

The clean culture percentage and explant viability percentage predicted by both RSM and ANN models for all 29 experimental runs are tabulated in Table 3. When comparing the predictions, it was found that both RSM and ANN-predicted values were close enough to the observed clean culture percentage and explant viability percentage. When comparing the models, the RSM-based optimization model predicted the outcome more accurately than the ANN-based model. Hence, the residuals of RSM-predicted values are found closer to the value of 0. A strong fluctuation in the residuals of the ANN-predicted values was observed, as can be seen in Figs. 7 and 8. In addition to this, the performance metrics of the RSM-based prediction model are relatively better than those of the ANN-based

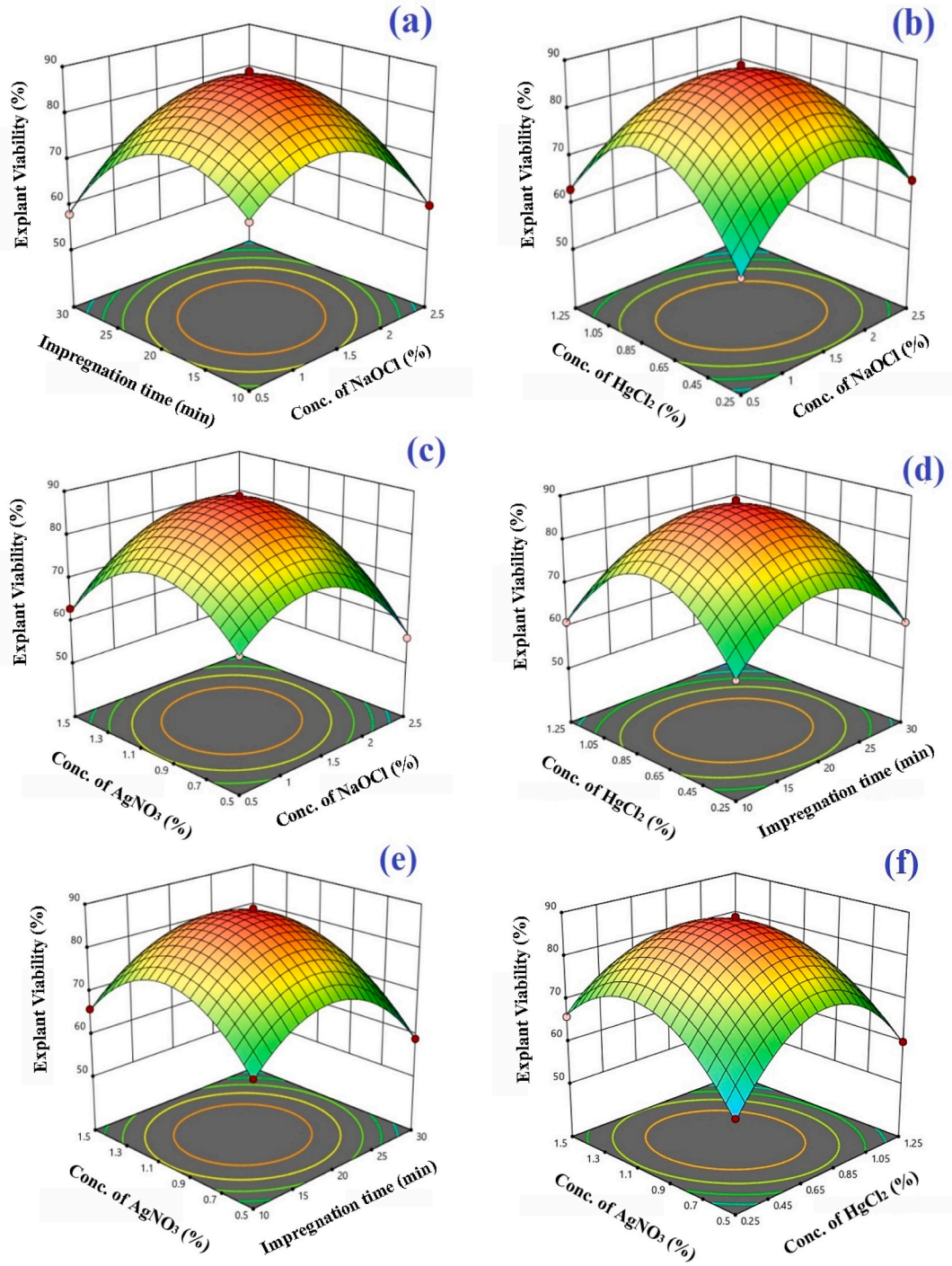

Fig. 3. The developed 3-D interactive plots for illustrating the explant viability as a response with respect to different combinations of selected parameters (a) Interaction effect of impregnation time and NaOCl concentration, (b) Interaction effect of $HgCl_2$ concentration and NaOCl concentration (c) Interaction effect of $AgNO_3$ concentration and NaOCl concentration (d) Interaction effect of $HgCl_2$ concentration and impregnation time (e) Interaction effect of $AgNO_3$ concentration and impregnation time (f) Interaction effect of $AgNO_3$ concentration and $HgCl_2$ concentration.

model (Table 4). Finally, the validation experiments performed with a set of separate experimental combinations yielded results that were close to the ANN-predicted clean culture percentage and explant viability percentage (Table 5). Therefore, ANN can be used to model surface sterilization for plant tissue culture.

In recent times, an increasing number of studies utilize ANN-based methods to model a variety of processes, including the

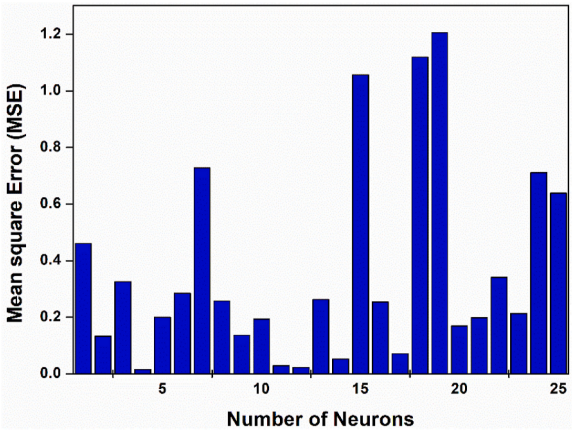

Fig. 4. Mean square error vs number of hidden layer neurons plot.

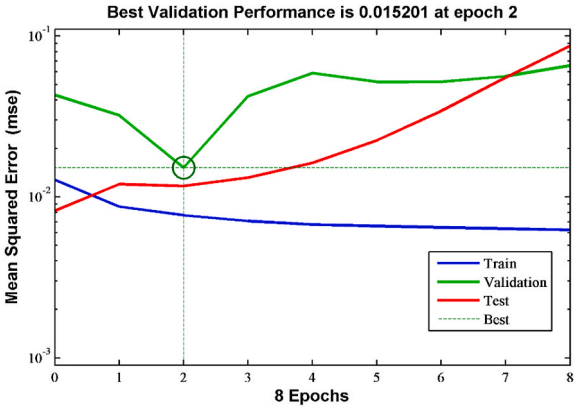

Fig. 5. The performance plot of the constructed ANN model.

procedures for tissue culturing. For instance Ref. [37], have used a multi-layer perceptron to model the *in vitro* sterilization of Pistacia vera L. explants. The RMSE and R^2 values obtained by them are found to be quite inferior to the performance metrics observed in this study. In another study by Ref. [38]; ANN was used to model the disinfection and scarification of cannabis seeds for *in vitro* propagation. The R^2 values for the training and testing datasets were found to be 0.938 and 0.918, respectively. When compared to this study, both RMSE and R^2 values obtained by Ref. [38] were significantly lower. Therefore, the model developed in this study is more robust in performance than previously reported ANN models for sterilization of explants for plant tissue culture.

3.3. *Multi-objective GA – RSM hybrid model*

Despite the better prediction efficiency, the RSM-based model fails to provide global optimal solutions. Hence, RSM-based models are always combined with GA to yield the global optimum. In this study, multi-objective GA was implemented due to the simultaneous optimization of clean culture percent and explant viability percent. After running GA, a set of 21 Pareto solutions was obtained, which are depicted in Fig. 9 and Table 4. Among these solutions, solution number 1 shows the maximum clean culture of 91.293667%. Similarly, the maximum explant viability of 88.558319% was observed in solutions 2 and 19. But the best solution should have both response variables at reasonably high values. Solution number 4 has both clean culture and explant viability at reasonably high values of 91.280797% and 88.507402%, respectively. A confirmatory experiment over the experimental conditions in solution number 4 yields a clean culture of 91% and 89%, respectively. Fig. 10 depicts the growth of the plant, which had been carried out according to optimised sterilising conditions acquired by GA.

4. Discussion

While optimizing with different sophisticated techniques, such as statistical (RSM), non-statistical (ANN), and multi-objective GA, solution number 4 from Table 6 was found to be a globally optimal solution. By allowing for the construction of AI models from experimental and observed data and by also enhancing decision-makers responses to complex systems in plant tissue culture, AI

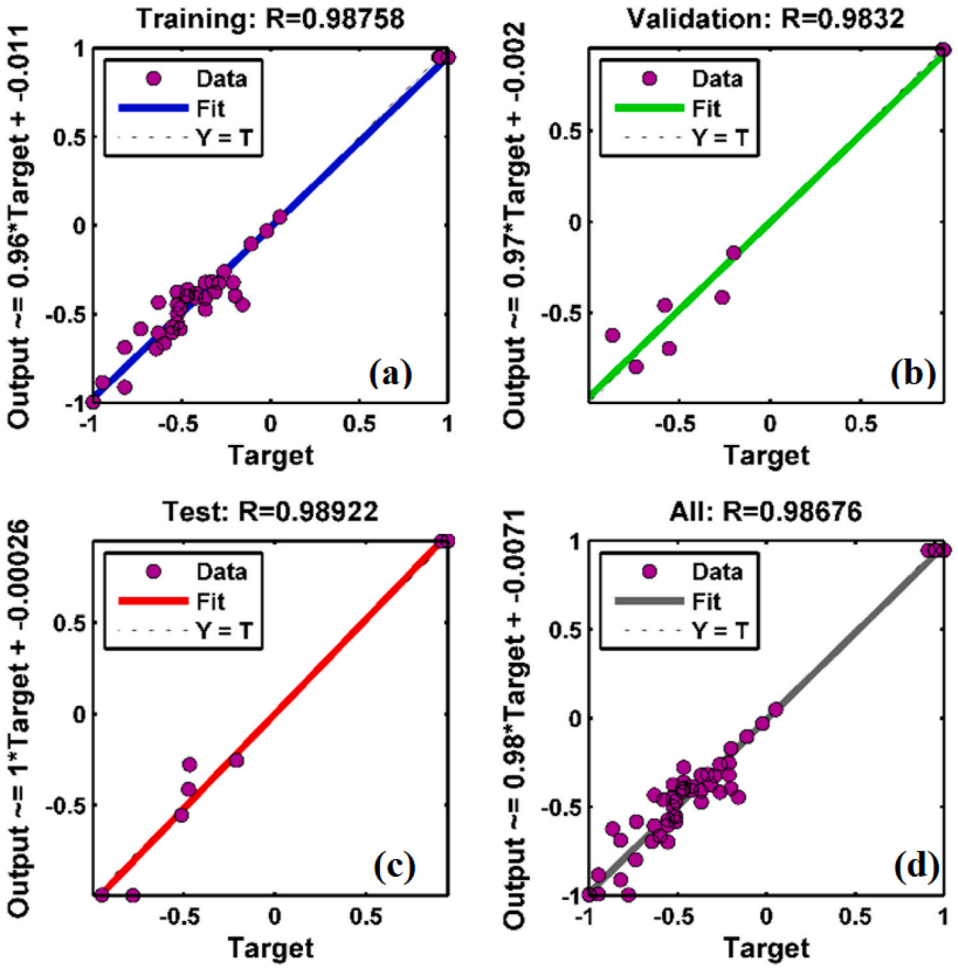

Fig. 6. The regression plots of the constructed ANN model (a) training, (b) validation, (c) test, and (d) overall performance.

models have been noticed to be highly relevant as accurate methods to help manage such issues and challenges. It can be challenging for optimization using conventional methods, where single variables are often examined sequentially in isolation, due to the intricacy of plant tissue culture techniques and the interactive complexity of the variables. For Instance, the two critical concerns in the medical industry are considering analysing data and a precise diagnosis. However, analysing pulmonary abnormalities is time-consuming and may depend on the doctors' diagnostic competence and clinical expertise [39]. Pre-processing is done on the pulmonary incoming sound patterns in order to successfully extract the key elements required for future processing. With the help of the collected features, data augmentation is done to improve detection performance overall by reducing overfitting challenges [40]. Although RSM can detect curvature in the response in addition to linear effects, it cannot combine nominal data, such as cultivars; as a result, it creates separate individual models for each cultivar, making the data analysis in plant tissue culture studies more time-consuming and difficult. (Hesami et al., 2020).

In this line [41], have observed that the apical tips treatment of adult male pistachio by increasing NaOCl concentration from 5 to 20% (v/v) for 20 min resulted in better decontamination, however, the proportion of survived explants was negatively affected. In addition, the outcome of this study supports the results documented by Ref. [42]. In this line, the lowest contamination and browning response with the highest percentage of callus induction and growth were obtained in Melia azedarach L. culture with benomyl (a systemic fungicide) pre-treatment (2 g L^{-1}) for 2 h, 7% H_2O_2 for 10 min, and 2% (w/v) NaOCl for 12 min. As a result, variable but convergent chemical levels are generally recommended for effective decontamination.

In theory, regardless of the potential induced damage, a more concentrated disinfectant, escorted or not with increased exposure time, will ensure the eradication of more microorganisms. This is evident, for example, during *in vitro* sterilization of chrysanthemum using various concentrations of six sterilising agents and impregnation times [20]. However, this notion was refuted here because more emphasis on treatment does not always result in better asepsis. Disinfection treatment, combined with other variables such as explant orientation (vertical, inverted vertical, horizontal, and abaxial or adaxial face), can have a major impact on tissue response. Hot water and plant preservative mixture (PPM), a broad-spectrum biocide/fungicide for plant tissue culture, improves bud germination and differentiation in ginger (*Zingiber officinale*) explants by 4–50%. As previously stated, a variety of surface disinfectants with varying

Table 3
Comparison of RSM and ANN-predicted percentage of clean culture and percentage of explant viability.

Run. No	NaOCl (%)	Impregnation time (min)	$HgCl_2$ (%)	$AgNO_3$ (%)	Observed clean culture %	RSM-predicted clean culture %	ANN-predicted clean culture %	Observed explant viability %	RSM-predicted explant viability %	ANN-predicted explant viability %
1	0.5	20	1.25	1	56	57	54.55	63	63	61.03
2	2.5	20	0.75	1.5	59	59.58	60.58	64	64.08	62.86
3	0.5	20	0.75	1.5	58	58.42	59.02	63	62.08	62.21
4	1.5	10	1.25	1	57	57.25	55.92	61	61.08	62.42
5	2.5	30	0.75	1	60	60.75	60.35	62	61.75	62.73
6	2.5	10	0.75	1	62	62.58	62.39	60	59.92	59.57
7	1.5	30	0.75	1.5	65	64.83	60.58	60	60	62.87
8	1.5	20	0.25	0.5	55	55.42	53.85	58	57.42	58.51
9	1.5	30	1.25	1	52	51.42	47.11	52	51.92	51.16
10	2.5	20	0.75	0.5	57	56.75	53.81	56	57.08	54.86
11	1.5	20	0.75	1	91	91	90.79	88	88.2	88.03
12	1.5	10	0.75	1.5	59	58.17	63.26	66	65.67	65.17
13	1.5	20	0.75	1	90	91	90.79	88	88.2	88.03
14	0.5	20	0.25	1	53	53	56.39	60	60.17	61.55
15	0.5	10	0.75	1	67	66.42	67.18	71	71.42	70.93
16	1.5	20	0.75	1	91	91	90.79	88	88.2	88.03
17	1.5	20	1.25	0.5	51	50.92	54.05	60	59.75	60.53
18	2.5	20	0.25	1	65	63.67	65.66	65	64.67	62.09
19	1.5	20	0.25	1.5	59	59.25	61.40	66	66.42	63.91
20	1.5	10	0.25	1	63	63.75	62.22	63	63.25	63.90
21	2.5	20	1.25	1	47	46.67	47.10	51	50.5	51.15
22	1.5	20	1.25	1.5	51	50.75	49.01	52	52.75	53.20
23	1.5	20	0.75	1	92	91	90.79	89	88.2	88.03
24	1.5	10	0.75	0.5	69	68.83	68.84	65	64.67	65.06
25	0.5	20	0.75	0.5	58	57.58	56.37	67	67.08	61.53
26	1.5	30	0.25	1	58	57.92	57.02	61	61.08	62.14
27	1.5	30	0.75	0.5	50	50.5	55.47	59	59	61.27
28	1.5	20	0.75	1	91	91	90.79	88	88.2	88.03
29	0.5	30	0.75	1	57	56.58	56.62	58	58.25	61.77

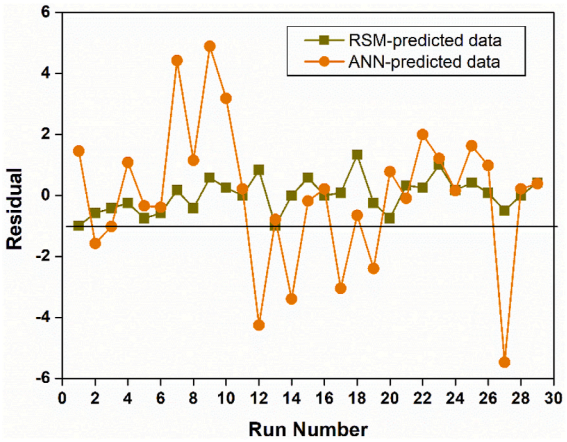

Fig. 7. Residual plot for percentage of clean culture.

degrees of effectiveness have been used in plant tissue culture, frequently in conjunction with a few drops of a wetting agent (e.g., Tween 20). Nonetheless, NaOCl is the most widely used compound [43]. The germicidal effect of NaOCl was attributed primarily to hypochlorous acid (HOCl) in diluted solution and to its high pH (12.5–13.5) and hypochlorite ion (ClO) oxidizing agent in concentrated form [44]. Similar to our findings [45], demonstrated that for the surface sterilization of *Pinellia ternata* (Thunb.) Breit, $HgCl_2$ was superior to NaClO and H_2O_2. Most people think that $HgCl_2$ is a potent disinfectant. Living materials should not lose biological activity by sterilization procedures, and only contaminants should be removed; explants should be surface sterilized only by treatment with the disinfectant solution at appropriate concentrations for a specified period [46].

Our results showed that 0.7% $AgNO_3$ showed the maximum explant viability and clean culture. In many crop plants, including the Brassica species, the *Capsicum* species, and *Coffea canephora*, $AgNO_3$ is known to increase *in vitro* regeneration [47,48]. The sterilization technique is the most significant step for establishing successful *in vitro* plant regeneration in different crop species. [49–51]; Gu

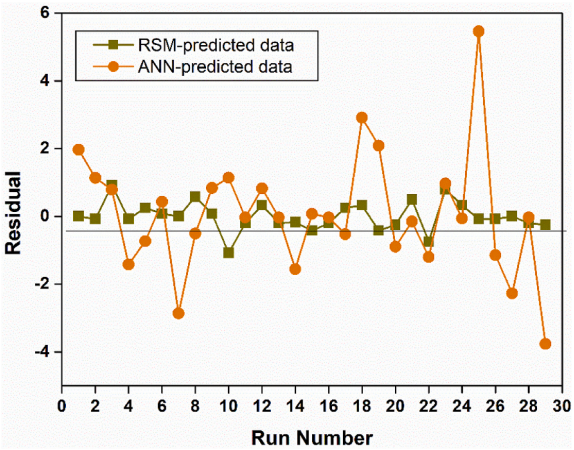

Fig. 8. Residual plot for percentage of explant viability.

Table 4
Performance measures of RSM and ANN-prediction models.

	Clean culture %		Explant viability %	
Metrics	RSM	ANN	RSM	ANN
R	0.9991	0.9859	0.9993	0.9877
R^2	0.9982	0.9718	0.9986	0.9746
Adj-R^2	0.9979	0.9671	0.9984	0.9704
MSE	0.3212	5.0975	0.1710	3.0681
RMSE	0.5667	2.2578	0.4135	1.7516
MAE	0.4479	1.6417	0.3072	1.2364
SEP	0.8917	3.5526	0.6507	2.7562
AAD (%)	0.0154	0.0288	0.0049	0.0200

Table 5
Validation dataset used to test RSM and ANN models.

Run. No	NaOCl (%)	Impregnation time (min)	$HgCl_2$ (%)	$AgNO_3$ (%)	Observed clean culture (%)	RSM-predicted clean culture (%)	ANN-predicted clean culture (%)	Observed explant viability (%)	RSM-predicted explant viability (%)	ANN-predicted explant viability (%)
1	2.5	30	1.25	0.5	46.25	8.67	52.48	42.28	24.30	58.58
2	2.5	20	0.75	1.5	52.88	59.58	47.15	53.42	64.08	51.22
3	0.5	30	1.25	1.5	42.15	27.33	47.59	49.87	22.30	51.65
4	1.5	10	0.25	0.5	49.76	51.13	47.20	48.92	45.69	51.13
5	2.5	30	1.25	1	78.44	32.29	86.90	81.65	36.86	84.90
6	0.5	10	0.25	1.5	48.31	22.67	56.63	60.77	40.63	61.78

et al., 2022). Traditional optimization techniques focus on one specific Pareto-optimal solution at a time, transforming the multi-objective optimization problem into a single-objective problem by using multi-criterion decision-making techniques. This strategy requires repeated application in order to find a variety of solutions during every computational run. The complexity of the network is influenced by the number of neurons, and the network is simpler when there are fewer neurons. It is emphasised that underfitting occurs when a network is too basic, while overfitting occurs when a network is too complicated. This current investigation is a case study of the efficacy of these tools and the successful implementation of a multi-objective genetic algorithm that can be extended to any nonlinear multivariate process.

5. Conclusion

By considering various constraints, problems involving plant tissue culture must satisfy various conflicting objective functions. Therefore, using a multiobjective algorithm in the optimization process is absolutely necessary. As a case study, this study has introduced MLP-NSGAII as a new computational tool for prediction and optimization of sterilization techniques in grape root stock 3309C as an initial step for efficient *in vitro* plant regeneration. Based on the results, MLPNSGAII can more quickly and accurately identify interaction effects for a large number of experiments than with traditional statistical analysis. Finally, MLP-NSGAII can be

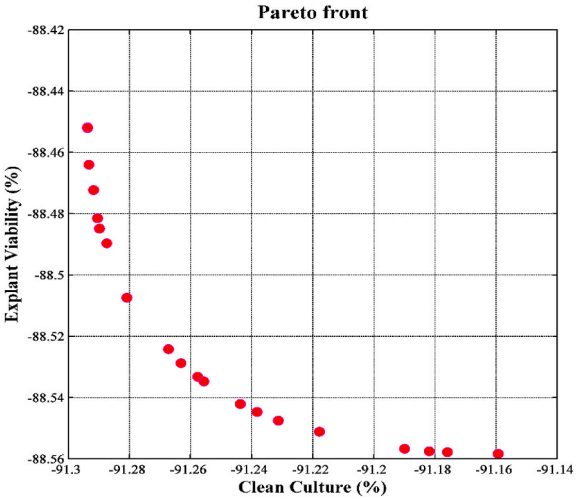

Fig. 9. The pareto chart depicts the percentage of clean culture *vs* percentage of explant viability.

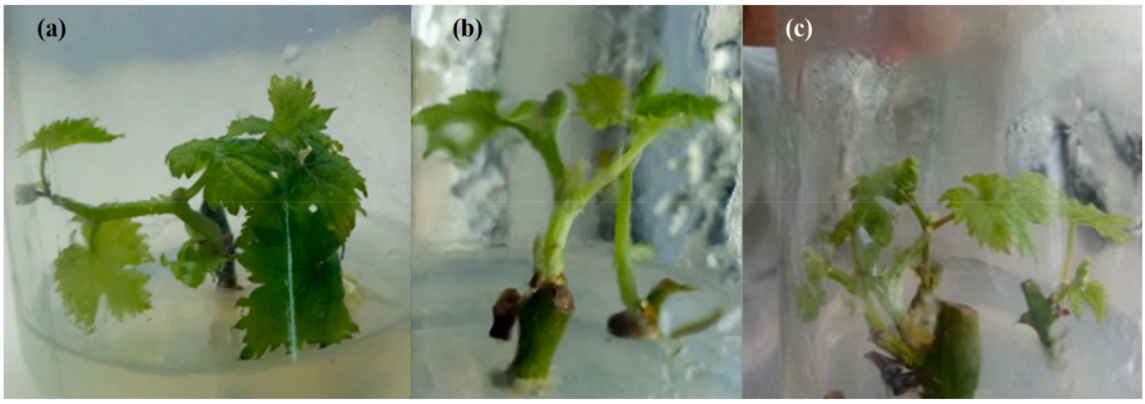

Fig. 10. Effect of sterilization procedure of nodal explants of the grape root stock 3309C (a). Shoot initiation after three weeks of inoculation from the viable nodal explants from the optimised sterilization procedure (1.5099% of NaOCl, 0.7085% $HgCl_2$ and 1.0054% of $AgNo_3$). (b) Shoot initiation after a week of second subculture in the optimised disinfection protocol. (c) Explants with chlorosis in higher concentration of $HgCl_2$ (0.72%).

Table 6
The pareto solutions obtained from multi-objective GA.

Solution No.	NaOCl (%)	Impregnation time (min)	$HgCl_2$ (%)	$AgNO_3$ (%)	Clean culture %	Explant viability %
1	1.5099	18.9501	0.7085	1.0051	91.2937	88.4518
2	1.4181	18.8522	0.7133	1.0106	91.1594	88.5583
3	1.4657	18.9177	0.7107	1.0066	91.2631	88.5287
4	1.4813	18.9155	0.7101	1.0065	91.2808	88.5074
5	1.5012	18.9058	0.7102	1.0074	91.2917	88.4723
6	1.4927	18.8867	0.7104	1.0090	91.2874	88.4897
7	1.4940	18.9406	0.7092	1.0055	91.2898	88.4849
8	1.4628	18.9437	0.7095	1.0090	91.2576	88.5332
9	1.4262	18.8543	0.7129	1.0101	91.1819	88.5575
10	1.4408	18.8852	0.7101	1.0080	91.2178	88.5511
11	1.4509	18.8932	0.7115	1.0088	91.2382	88.5447
12	1.4688	18.9110	0.7095	1.0055	91.2672	88.5242
13	1.5045	18.9475	0.7091	1.0056	91.2932	88.4640
14	1.4541	18.9103	0.7114	1.0094	91.2437	88.5421
15	1.4240	18.8706	0.7132	1.0106	91.1760	88.5578
16	1.4610	18.8742	0.7107	1.0077	91.2556	88.5347
17	1.4963	18.9069	0.7096	1.0068	91.2904	88.4815
18	1.4474	18.8989	0.7118	1.0095	91.2312	88.5475
19	1.4181	18.8522	0.7133	1.0106	91.1594	88.5583
20	1.4292	18.8715	0.7129	1.0101	91.1899	88.5567
21	1.5099	18.9501	0.7085	1.0054	91.2937	88.4520

acknowledged as a potent technique for application in various *in vitro* culture fields. Neural modelling may be used in the future to mechanize and automate plant breeding through *in vitro* cultures and to separate plant tissues based on quality, even under aseptic conditions. ANNs have a strong potential for modelling studies in plant *in vitro* cultures and can be useful as a prognostic tool as well. Additionally, ANNs are more advantageous than other strategies in hybrid models and are very flexible.

Author contribution statement

Habtamu Dagne: Performed the experiments.
S. Venkatesa Prabhu: Analyzed and interpreted the data.
Hemalatha Palanivel: Conceived and designed the experiments; Wrote the paper.
Alazar Yeshitila: Analyzed and interpreted the data.
Solomon Benor: Contributed reagents, materials, analysis tools or data.
Solomon Abera: Conceived and designed the experiments.
Adugna Abdi: Conceived and designed the experiments.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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