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A Study of the Use of Deep Artificial Neural Network in the Optimization of the Production of Antifungal Exochitinase Compared with the Response Surface Methodology

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Abstract

This study aimed the using of deep artificial neural network in the optimization of exochitinase production as an alternative method to response surface methodology. The isolated fungus *Alternaria* sp. strain Sha that was identified by its morphological features then by the 18S rDNA technique, was used for the production of the enzyme by solid state fermentation. A Plackett–Burman design was constructed to screen the effect of seven independent variables on the enzyme production. The overall enzyme production increased from 3.4 to 28.931U/g dry substrate by approximately 8.5 folds with the coefficient of determination (R^2) value being 0.996 using deep artificial neural network in comparison with the R^2 value 0.76 using response surface methodology. It was clear that the deep artificial neural network was more accurate than the response surface methodology in predicting the enzyme activity. Finally, the produced exochitinase showed antifungal activity against the resistant controllable soil-borne fungus *Rhizoctonia solani.*

Keywords: Exochitinase; *Alternaria* sp., Solid state fermentation, Deep artificial neural network, Response surface methodology, Antifungal.

1. Introduction

Chitin is a linear polymer of N-acetylglucosamine, with β-1,4-glycosidic bonds (Blaak *et al*., 1993). It is the second most abundant biopolymer after cellulose in nature that exists as part of fungi, yeasts, nematodes, shrimps, and other invertebrates (Young *et al*., 2005).

Chitinases are chitinolytic enzymes that are capable of degrading the chitin chain by different mechanisms. The degradation can be done by endochitinases randomly within the chain or by exochitinase that degrades the chain from the terminal end leading to the production of diacetylchitobiose or N-acetylglucosamine units (Sahai and Manocha, 1993). Chitinases gained importance due to its agricultural, industrial, and medical applications (Hamid *et al*., 2013).

The microorganisms are considered the main source for exochitinase production. The upsurge in the exochitinase applications urged the efforts to maximize the microbial production of the enzyme to satisfy the industrial needs (Chavan and Deshpande, 2013).

The fermentation conditions have a crucial impact on microorganisms' growth and their metabolic products as well as the production cost (Shivalee *et al*., 2018). The optimization of the fermentation conditions was initially performed using the classical one-variable-at-a-time approach because of its simplicity and ease. However, this method has several limitations as it is time-consuming and involves a great deal of experiments that may increase the production cost, in addition to the loss of the interactive effects between various parameters. Consequently, statistical models have been employed in fermentation technology to overcome these limitations (Desai *et al*., 2008).

Response surface methodology (RSM) is a statistical technique that was originally described by Box and Wilson, (1951) and has been successfully applied in microbiological and biotechnological fields (Astray *et al*., 2016). As a matter of fact, it is the most popular technique used in optimizing the production of microbial enzymes, and has been used occasionally for maximizing the yield of microbial exochitinase (Kumar *et al*., 2012 and Awad *et al*., 2017).

Over the last two decades, artificial neural networks (ANNs) were utilized in different science aspects for modeling and optimization processes. ANN has several advantages as it can use data with noise and can modulate incomplete and highly non-linear behaviors (Astray *et al*., 2016, de Araujo Padilha *et al*., 2017 and Cui *et al*., 2018). In addition to the various advantages of the ANN, the introduction of deep learning has led to more accurate results (Serwa, 2017). ANN has been previously used in the optimization of the production of some microbial

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enzymes such as L- asparaginase (Gurunathan and Sahadevan, 2011), α-galactosidase (Edupuganti *et al*., 2014), α-amylase (Mishra *et al*., 2016) and protease (Ramkumar *et al*., 2018).

In the current study a comparitive study between RSM and deep artificial neural network (DANN) was performed to predict the optimum conditions for exochitinase production by solid state fermentation (SSF). Additionally, the antifungal activity of the produced exochitinase against phytopathogenic fungi was examined.

2. Materials and Methods

2.1. Materials

Wheat bran and sugarcane bagasse were purchased from the local market. Chitin, 4-NitrophenylN-acetyl-β-D-glucosaminide, 4-Nitrophenol and Yeast extract were purchased from Sigma-Aldrich, Saint Louis, USA. Potato dextrose agar (PDA) medium was purchased from Merck, Darmstadt, Germany. All other chemicals were of analytical or HPLC grade.

2.2. Microorganism and Identification

The fungus used in the current study was previously isolated from waste of marine organisms (shrimp and fish) collected from the local seafood market. Its morphological features were studied by field emission high resolution scanning electron microscope (quanta 250). Molecular identification of the strain was carried out in Sigma Scientific Services Co. by the 18S rDNA technique.

2.3. Exochitinase Production

2.3.1. Inoculum Preparation

An inoculum suspension was obtained by scrapping the fungal potato dextrose agar (PDA) slants with 10mL sterile distilled water containing 0.1 % (w/v) tween 80 (Sridevi and Reddy, 2016).

2.3.2. Fermentation Process

The fermentation process was performed in 250mL Erlenmeyer flask containing 5g of wheat bran and 50mg of colloidal chitin moistened with 10mL of a basal salt solution composed of (g %) yeast extract, 1.85; K_2HPO_4, 0.15; $MgSO_4$, 0.01; KCl, 0.2 and $FeSO_4.7H_2O$, 0.01, inoculated with 2mL of inoculum suspension and incubated at 30°C for seven days. The concentration of the salts was constructed on the base of previous studies on the production of chitin and chitosan hydrolyzing enzymes (Hosny *et al*., 2010 and Hashem *et al*., 2018).

At the end of fermentation, 50mL of distilled water was added and mixed in a rotary shaker at 150rpm for one hour. The mixture was centrifuged at 4°C for twenty minutes at 5000 rpm, and the clear supernatant was used as the crude enzyme solution (Sridevi and Reddy, 2016).

2.4. Enzyme Assay

The exochitinase activity was determined using 0.1 % of the synthetic substrate 4-NitrophenylN-acetyl-β-D-glucosaminide in 0.2M acetate buffer pH 5 (Rustiguel *et al*., 2012). The reaction was performed by mixing 50µL of the clear extract with 50µL of the substrate solution and the reaction mixture was incubated at 30°C for fifteen minutes. At the end of the assay time, 1mL of 1M NaOH was added to stop the reaction and the yellow color developed was quantified at 410nm. One unit of exochitinase was defined as the amount of enzyme that released 1µmol of p-Nitro-phenol (equivalent to 1µmolof N-acetylglucosamine) per minute under the assay conditions.

2.5. Optimization of the Enzyme Production

Initially, the effects of varying the fermentation period in addition to the effects of adding various salt solutions (tap water, SR salt solution [consisting of (g %) $MgSO_4$, 0.012; KH_2PO_4, 0.015; $NH_4H_2PO_4$, 0.05; peptone, 0.02 and yeast extract, 0.45], Khanna salt solution [consisting of (mg %) NH_4NO_3, 100; KH_2PO_4, 65; $MgSO_4.7H_2O$, 18.1; KCl, 4.9; $MnSO_4.H_2O$, 0.69; $ZnSO_4.H_2O$, 0.35; $FeCl_3.6H_2O$, 0.33; $CuSO_4.5H_2O$, 0.31 and yeast extract, 100]) were examined in a medium containing 5g wheat bran and 50mg colloidal chitin (Rustiguel *et al*., 2012).

The Plackett–Burman experiment (Plackett and Burman, 1946) was performed in order to identify the variables that influence the exochitinase production. Seven independent variables (temperature, incubation period, volume of moistening agent, age of fungus, concentration of chitin, addition of shrimp shells and sugarcane bagasse) were examined in eight trials by which each variable was tested at two levels (a high +ve and a low –ve levels). Plackett–Burman experimental design based on the first order linear model:

$$Y=B_o+\sum B_iX_i \quad \text{Eq. (1)}$$

Where Y is the response (exochitinase production), B_o is the model intercept, and B_i is the variable estimation. The main effect of each variable was determined by the following equation:

$$E_{(Xi)}=2(\Sigma M_i^+-M_i^-)/N \quad \text{Eq. (2)}$$

Where $E_{(Xi)}$ is the effect of the tested variable. M_i^+ and M_i^- represent exochitinase production from the trials performed at high and low concentrations of the independent variable (Xi), respectively, and N is the number of trials.

2.5.1. Modeling Procedure for Response Surface Methodology

RSM using Box-Behnken design (Box and Behnken, 1960) was performed to optimize exochitinase production. The three most significant independent variables extracted from Plackett–Burman design were examined at three different levels, low (-), high (+) and control (0). For predicting the optimal point, a second order polynomial function in the form of the three factors was fitted to correlate the relationship between the independent variables and the response as follows:

$$Y=ß_0+ß_1X_1+ß_2X_2+ß_3X_3+ß_{11}X_1^2+ß_{22}X_2^2+ß_{33}X_3^2+ß_{12}X_1X_2+ß_{13}X_1X_3+ß_{23}X_2X_3 \quad \text{Eq. (3)}$$

Where Y is the predicted response (exochitinase production), and X_1, X_2 and X_3 are the most significant independent variables; $ß_0$ is the intercept regression coefficient, $ß_1$, $ß_2$, $ß_3$ are linear coefficients, $ß_{11}$, $ß_{22}$, $ß_{33}$ are quadratic coefficients, and $ß_{12}$, $ß_{13}$, $ß_{23}$ are cross product coefficients.

All experiments were performed in triplicates and the average of the results was reported. The results were statistically analyzed using the analysis of variance (ANOVA) techniques, in which p value of ≤ 0.05 was regarded as significant. The statistical software SPSS (version 16.0) was used for the regression analysis of the experimental data obtained.

2.5.2. Modeling Procedure for Artificial Neural Network

The data sets presented to the ANN model were performed in the same order as in the Box-Behnken design. Multi-layer perceptron (MLP) is the most used architecture in ANN models, which was composed of three different kinds of layers: the input, the output and the hidden layers. In the case of DANN (Figure 1), the number of hidden layers is larger than the traditional ANN(Serwa, 2017).

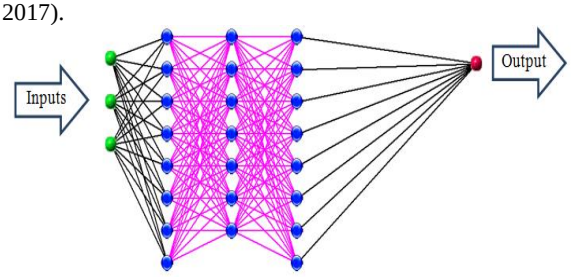


Figure 1. MLP architecture with deep learning.

The back propagation neural networks (BPNN) algorithm is used to adjust the weights of the network. The target output is known from training data which is the exochitinase activity (experimental work results).

The used software that applied the ANN model by DANN has been reported by Serwa, (2017). The relationship between the experimental and the predicted results was expressed by the root mean square error (RMSE). The accuracy of the predicted model was expressed as the overall average accuracy %.

$$RMSE=\sqrt{\frac{\sum_{i=1}^{N}(Xpred-X)^2}{N}} \quad \text{Eq. (4)}$$

Accuracy % = 100-[Absolute error/X*100] Eq. (5)

Overall average accuracy % = ∑ Accuracy %/N Eq. (6)

in which X is the experimental results.

2.6. Some Properties of the Crude Exochitinase

2.6.1. Effect of pH

The effect of different pH's on the activity and stability of the enzyme were examined in the pH range of 4 to 7 and 4 to 5, respectively using 0.2M acetate buffer.

2.6.2. Effect of temperature

The optimum temperatures for the enzyme activity in-addition to its thermostability, were investigated in the range from 30°C to 60°C, and from 35°C to 60°C, respectively.

2.6.3. Effect of Substrate Concentrations

The effect of different substrate concentrations (0.05 to 3mg/ml), was studied at the optimal pH and temperature of the tested enzyme.

2.7. Antifungal Activity of the Produced Exochitinase

The well diffusion method according to Neto *et al*., (2016) was used to examine the antifungal activity of the produced exochitinase against the phytopathogenic fungi *Rhizoctonia solani* and *Dothideomycetes* sp. The experiment was performed in a petri dish containing 20mL of potato dextrose agar inoculated with 0.2ml of the fungal strains spore suspension. Wells of 7mm in diameter were made in the agar plate with a sterile glass Pasteur pipette and 0.1mL of the produced enzyme (45U/mL) was added into the wells. The plates were then incubated at 30ºC for forty-eight hours.

3. Results

3.1. Identification of the Fungal Exochitinase Producer Strain

The fungal isolate used in this study was previously isolated from the waste of marine organisms and screened for its chitinolytic activity (unpublished data). Morphological features of the isolate in terms of sporangia were examined under scanning electron microscope (Figure 2). The molecular identification of the isolate was performed using the 18S rDNA technique and the evolutionary history was inferred using the Maximum Likelihood method. The tree with the highest log likelihood (-1003.8762) is shown in Figure 3. The phylogentic analysis of the isolated fungus revealed that the strain was 100 % identical to other *Alternaria* sp. The data of the 18S rDNA partial sequence was submitted to NCBI under the name *Alternaria* sp. strain Sha and received the accession number of MK139827.

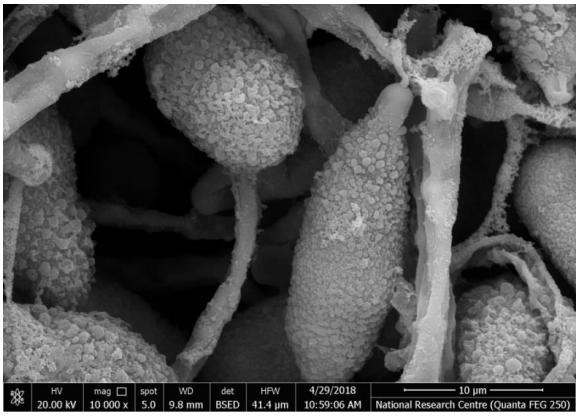


Figure 2. Scanning Electron Micrograph of the fungal isolate.

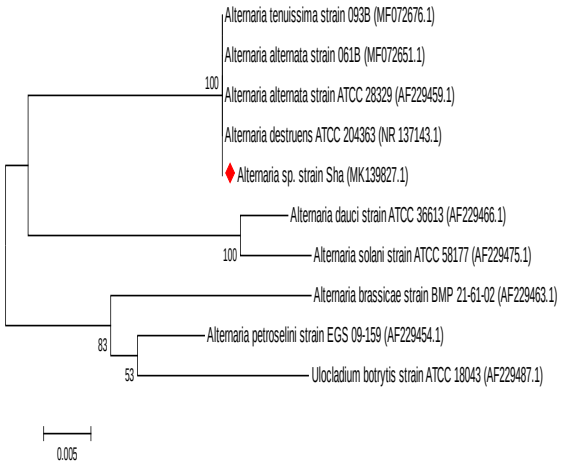


Figure 3. The Phylogenetic tree by the Maximum Likelihood method.

3.2. Optimization of the Enzyme Production by SSF

The production of exochitinase using 5g of wheat bran and 50mg of colloidal chitin as a substrate at different incubation periods indicated that the used strain produced 3.4U/g dry substrate (g ds) after seven days and increased to reach 10.1U/g ds after fourteen days of incubation.

However, a further increase in incubation period leads to a decrease in the enzyme production (Figure 4).

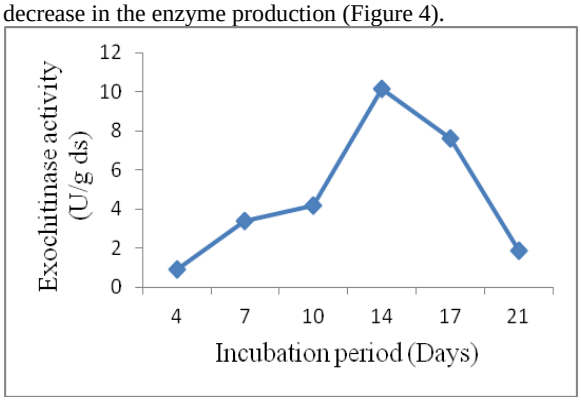


Figure 4. Production of exochitinase at different incubation periods.

The effect of various salt solutions on moistening the solid substrate (5g wheat bran and 50mg chitin) indicated that a 29.1 % increase in the activity was achieved using the SR salt solution compared with the basal salt solution (Table 1).

Table 1. The effect of different salt solutions on the enzyme production

Salt solution	Exochitinase activity (U/g ds)
Basal salt solution	10.147
Khanna salt solution	10.777
SR salt solution	13.102
Tap water	8.924

3.2.1. Selection of the Variables that Influence the Enzyme Production by Plackett–Burman Design

The Plackett–Burman design was applied to identify the variables that influence exochitinase production by SSF in which seven independent variables were studied in eight trials (Table 2). A wide variation in the exochitinase productivity ranging from 2.901 to 16.521U/g ds was observed. A maximum exochitinase activity of 16.521U/g ds was achieved in the third trial in which the optimized medium contained (g/flask) wheat bran; 5g, sugarcane bagasse; 1g and chitin; 75mg moistened by 5mL SR salt solution, then inoculated by 2mL of spore suspension of a five-day-old fungus, and incubated for sixteen days at 27°C.

The multiple regression analysis of the data (Table 3) clarified that all of the examined variables significantly influence the exochitinase production. The first order equation describing the relation between the seven variables and the enzyme activity, obtained after applying the regression analysis of the experimental results, was as follows:

$$Y=29.952 -1.031X_1+0.291X_2-0.273X_3+ 0.288X_4+ 0.043X_5+ 0.978X_6+ 6.441X_7 \quad \text{Eq. (7)}$$

Y is the exochitinase activity. X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are temperature, fermentation period, age of the fungus, volume of the moistening agent, addition of chitin, addition of shrimp shells, and addition of sugarcane bagasse respectively.

The R^2 value for the applied model was 0.9919 and the analysis of variance (ANOVA) indicated that the model terms used were significant since the F value was 278.42 with Prob>F value of 1.69E-15 (less than 0.05).

The main effect of each variable was calculated and represented graphically in figure 5; it was evident that the temperature and the volume of the salt solution have negative values while the other variables have positive values. So the variables which represented positive effects on the enzyme production were set at high levels (+ve), while the variables that exerted negative effects were maintained at low levels (-ve) in the further experimental optimization.

Table 2. Plackett – Burman design and the observed results.

Trials	Temperature (°C)	Incubation period (day)	Volume of moistening agent (mL)	Age of the fungus (days)	Chitin (mg/flask)	Shrimp (g/flask)	Sugarcane bagasse (g/flask)	Exochitinase activity (U/g ds)
1	(27) -	(12) -	(5) -	(9) +	(75) +	(1) +	(0) -	11.043
2	(33) +	(12) -	(5) -	(5) -	(25) -	(1) +	(1) +	8.004
3	(27) -	(16) +	(5) -	(5) -	(75) +	(0) -	(1) +	16.521
4	(33) +	(16) +	(5) -	(9) +	(25) -	(0) -	(0) -	2.901
5	(27) -	(12) -	(15) +	(9) +	(25) -	(0) -	(1) +	11.630
6	(33) +	(12) -	(15) +	(5) -	(75) +	(0) -	(0) -	0
7	(27) -	(16) +	(15) +	(5) -	(25) -	(1) +	(0) -	6.181
8	(33) +	(16) +	(15) +	(9) +	(75) +	(1) +	(1) +	9.736

Table 3. Statistical analysis of Plackett- Burman design showing coefficient values, t-and *P*- values for each variable on exochitinase production.

Variables	Exochitinase analysis			
	Coefficient	*t*-statistics	*P*-value	Confidence level (%)
Intercept	29.952			
Temperature (°C)	-1.031	-27.9452	5.23E-15	100
Incubation time (day)	0.291	5.266116	7.68E-05	99.99
Volume of moistening agent (ml)	-0.273	-12.3397	1.37E-09	100
Age of the fungus (days)	0.288	5.203531	8.7E-05	99.99
Chitin (mg/flask)	0.043	9.697626	4.2E-08	100
Shrimp shell (g/flask)	0.978	4.419094	0.00043	99.96
Sugarcane bagasse (g/flask)	6.441	29.10956	2.75E-15	100

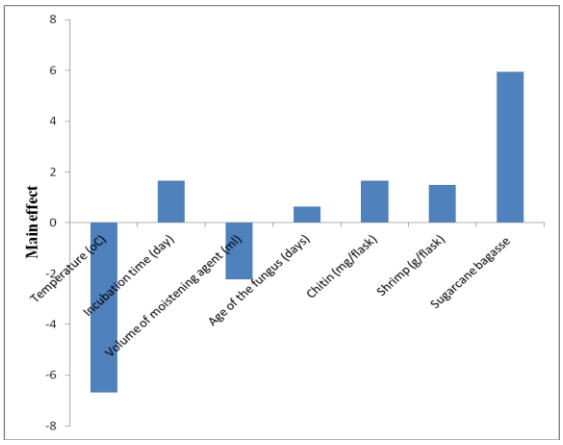


Figure 5. Main effects of the selected independent variables on exochitinase production according to the results of the Plackett – Burman experiment.

3.2.2. Response Surface Methodology Model

RSM using Box-Behnken design was applied in order to reach the optimum concentration of the variables (temperature, addition of sugarcane bagasse, and the volume of moistening agent) that exert the highest main effect on exochitinase production. The results (Table 4) showed an increase in exochitinase production to reach 28.931U/g ds using a medium composed of (g/flask) wheat bran, 5; sugarcane bagasse, 1.5 and colloidal chitin, 0.075, moistened with 5mL of SR salt solution, and incubated at 25°C for sixteen days.

The ANOVA (Table 5) revealed the significance of the model since the model terms had values of Prob> F equal to 7.01 E-07, but the R^2 value of that model was 0.76.

The second order polynomial equation of Box-Behnken model obtained after applying the regression analysis of the experimental results was as follows:

$$Y= 371.167-24.289X_1+1.507X_2-8.967X_3+0.433X_1^2+26.77X_2^2-0.612X_3^2-2.323X_1X_2+0.555X_1X_3+1.062X_2X_3 \quad \text{Eq. (8)}$$

Where Y was the response (exochitinase production) and X_1, X_2, and X_3 were the coded values of the tested variables (temperature, addition of sugarcane bagasse and the volume of moistening agent) respectively.

In order to investigate the interaction between the experimental variables, the three dimensional response surface plots were constructed by representing the regression equation. Figure 6 a-c represents the response surface plots of temperature and the addition of sugarcane bagasse, temperature, and the volume of moistening agent, the addition of sugarcane bagasse, and the volume of moistening agent respectively while keeping the other variables at the zero level.

The competence of the constructed model was confirmed by performing an experiment under the optimized conditions. The exochitinase production was 26.259U/g ds which is in accordance with the predicted value 26.486U/g ds, confirming the validation of the model.

Table 4. Examined concentration of the key variables and results of Box-Behnken Design experiments with the predicted values by RSM and ANN.

	Independent variable					
Trials	X1Temperature (oC)	X2 Sugarcane bagasse (g/flask)	X3 Volume of moistening agent(mL)	Observed exochitinase (U/g ds)	Predictedexochitinase by RSM(U/g ds)	Predictedexochitinase by ANN(U/g ds)
1	25(-)	0.5(-)	5(0)	23.790	25.055	23.225
2	29(+)	0.5(-)	5(0)	25.952	28.050	26.522
3	25(-)	1.5(+)	5(0)	28.931	26.486	29.430
4	29(+)	1.5(+)	5(0)	21.465	20.187	20.883
5	25(-)	1(0)	3(-)	15.737	17.999	13.465
6	29(+)	1(0)	3(-)	8.503	11.491	7.692
7	25(-)	1(0)	7(+)	17.725	15.675	17.844
8	29(+)	1(0)	7(+)	20.333	18.045	21.229
9	27(0)	0.5(-)	3(-)	24.915	19.838	25.306
10	27 (0)	1.5(+)	3(-)	20.444	16.179	17.385
11	27(0)	0.5(-)	7(+)	21.510	23.224	20.821
12	27(0)	1.5(+)	7(+)	21.589	23.813	17.891
13	27(0)	1(0)	5(0)	18.071	16.521	15.966

Table 5. Analysis of Box-Behnken Design for exochitinase production.

Term	Regression coefficient	Standard error	t- test	P-value
Intercept	371.167	216.115	1.717	0.097
X_1	-24.289	15.530	-1.564	0.129
X_2	1.507	25.512	0.059	0.953
X_3	-8.967	6.574	-1.364	0.183
X_1^2	0.433	0.286	1.511	0.142
X_2^2	26.770	4.582	5.842	2.457 E-06
X_3^2	-0.612	0.286	-2.138	0.041
X_1X_2	-2.323	0.866	-2.683	0.012
X_1X_3	0.555	0.216	2.563	0.016
X_2X_3	1.062	0.866	1.227	0.230

F value = 10.197; P>F= 7.01 E-07; R^2=0.760; R =0.872; Adjusted R^2=0.685

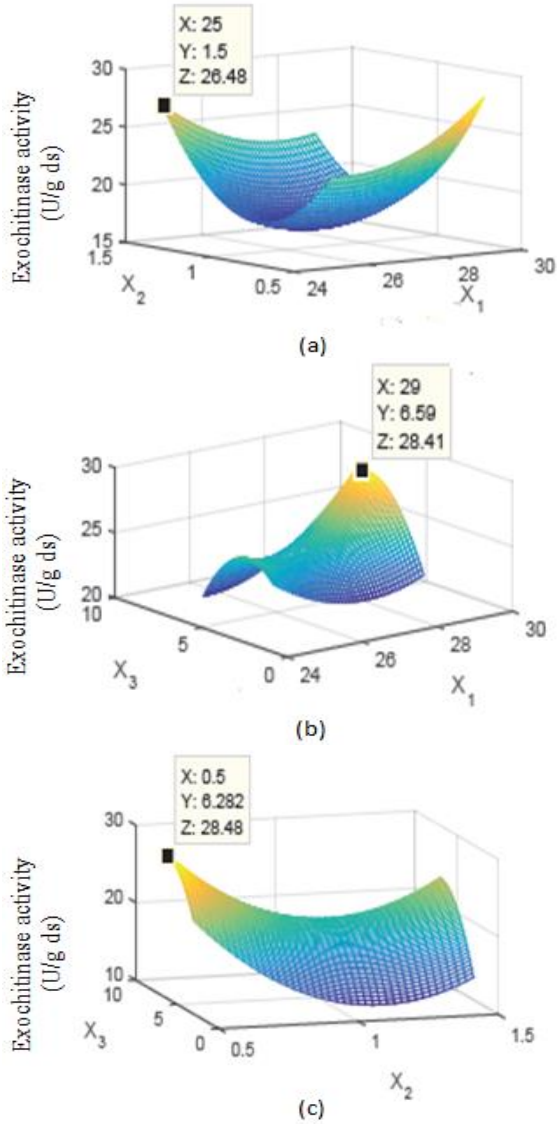


Figure 6. Response surface plot of exochitinase production

(a) Showing the interactive effects of $X_1$and X_2at X_3 = 0.

(b) Showing the Interactive effects of X_1 and X_3 at X_2= 0.

(c) Showing the Interactive effects of X_2 and X_3 at X_1= 0.

in which X_1 is the temperatures (25- 29°C), X_2 is the additions of sugarcane bagasse (0.5-1.5g/flask), and X_3 is the volumes of moistening agent (3-7mL).

3.2.3. Artificial Neural Network Model

The applied neural network was selected based on the fit for validation phase. The predicted results for exochitinase production using DANN are shown in table (4). The analysis of the data indicates that the predicted model was well-fitted since the R^2 value was 0.996. Also, the analysis of the data indicates that there was a small error between experimental and predicted values since the root mean square error was 0.67, and the overall average accuracy % was 96.3 %.

Some Properties of the Crude Exochitinase

The profile of the produced exochitinase at different pHs using a 0.2M acetate buffer and different temperatures is shown in figure 7. The results indicate that the enzyme was optimally active at pH 4.5 and 55°C using a 0.001g/mLsubstrate concentration. By incubation of the enzyme for different periods (up to 2h) without its substrate at pH ranging from 4 to 5 using a 0.2M acetate buffer, the enzyme retained 100 % of its activity for two hours at pH 4.5 and 5, but it lost 11.7 % of its activity after ninety minutes at pH 4. The thermal stability of the enzyme without the substrate at different temperatures ranging from 40 to 60°C was determined. The enzyme had a half-life time higher than two hours at temperature range from 40 to 55°C (figure 8).

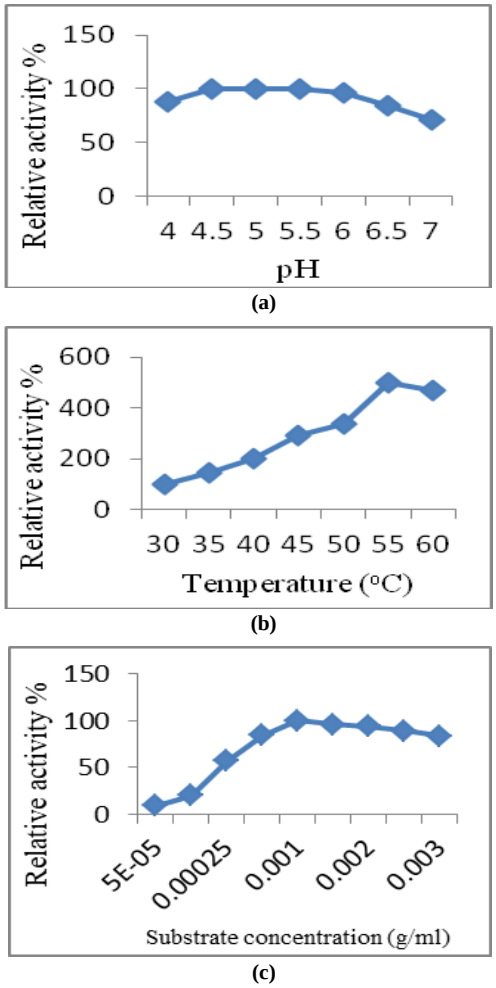
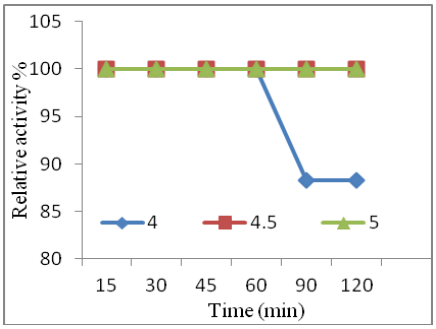
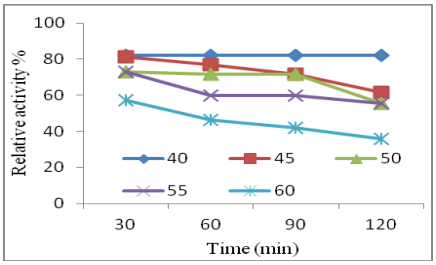


Figure 7. Effect of (A) the reaction pH (control is pH 5) (B) the reaction temperature (30°C is the control) (C) the substrate concentration (0.001g/mL is the control) on the activity of the crude exochitinase (the absence of error bars indicates that the errors are smaller than the symbols).



A

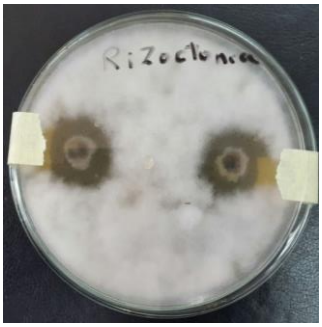


B

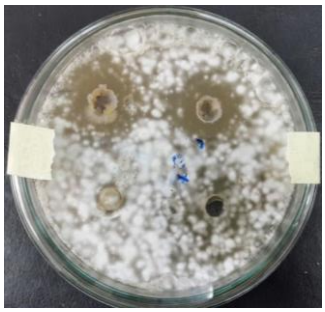
Figure 8. (A) The pH stability of the crude exochitinase at different time intervals. (B) The temperature stability of the crude exochitinase at different time intervals. The control in both experiments, is the activity of the enzyme at zero time (the absence of error bars indicates that the errors are smaller than the symbols).

3.3. Antifungal Activity of the Produced Exochitinase

The produced exochitinase showed antifungal activity against the phytopathogenic fungi *Rhizoctonia solani* and *Dothideomycetes* sp. as shown in figure 9.



A



B

Figure 9. Antifungal activity of the produced exochitinase against A: *Rhizoctonia solani* B: *Dothideomycetes* sp. Strains.

4. Discussion

Exochitinases are chitinases capable of the hydrolysis of chitin from its terminal end; it has gained a great attention due to its various industrial, medical and agricultural applications (Sahaiand Manocha, 1993). Microorganisms are the highest chitinase producers as reported by Sridevi and Reddy, (2016). The environmental and the nutritional conditions required by the microorganisms have a crucial impact on the cost of the production process; for the use of agro-industrial residues is an economic approach (Sudhkar and Nagarajan, 2010 and Narendrakumar *et al*., 2015). Various agro-industrial residues as rice bran (Sudhakar and Nagarajan, 2010), wheat bran (De Freitas Soares *et al*., 2015), and sugarcane bagasse (Sudhakar and Nagarajan, 2011) have been used previously for the production of fungal chitinases. In this study the *Alternaria* sp. Sha strain isolated from marine organism wastes was used for the production of the enzyme by SSF using 5g of wheat bran supplemented with 1 % chitin as a substrate and moistened with 10mL of SR salt solution; the produced enzyme has an activity of 13.102U /g ds after a fourteen-day fermentation period. De Freitas Soares *et al*., (2015) reported the production of chitinase with the maximum activity of 6.2U/g ds by SSF of the same substrate using the fungus *Monacrosporium thaumasium*. The isolated microorganism was initially identified according to its cultural and morphological features then by the 18S rDNA technique. According to the 18S rDNA sequence, the evolutionary history of the isolated strain was inferred using the Maximum Likelihood method. The phylogenetic tree with the highest log likelihood indicated a 100 % similarity of the isolate with other *Alternaria* sp. strains. Evolutionary analyses have been conducted in MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0) (Tamura *et al*., 2013). The production of chitinases using *Alternaria* sp. has been reported by other researchers (Abbasi *et al*., 2017; El-Shora *et al*., 2017; Ghanem *et al*., 2011).

The optimization of the nutritional and cultural conditions of the fermentation process is necessary to increase the yield of the enzyme production that consequently will decrease the cost. RSM is a popular statistical technique that has been used for the optimization of the production of various enzymes (Hashem *et al*., 2018). In the current study, sequential optimization of the exochitinase production was carried out in two phases. Firstly, seven variables were examined using the Plackett–Burman design in order to verify the significant factors. The results demonstrated a wide variation in the exochitinase productivity ranging from 2.901 to 16.521U/g ds. This reflects the importance of the initial enzyme production screening using statistical strategy for the selection of the fermentation medium components and the culture conditions that influence productivity. By calculating the main effect value of the examined variables, it was clear that the temperature, the addition of sugarcane bagasse and the volume of the moistening agent were the variables that exerted the highest main effect respectively and consequently were subjected to a further step of optimization. Additionally, from the calculated value of the main effect, it was evident that the temperature and the volume of the salt solution had

negative values. This confirms that positive effects on production were achieved when the temperature and the volume of the salt solution were adjusted to their low (-1) level values, while the opposite effect was shown with the other variables. The R^2value for the current applied model was 0.9919, suggesting that the variation of 99.19 % was due to the independent variables, while there was only a 0.81 % chance that the response was not due to the experimental model variables. The R^2 value (> o.9) indicates the accuracy of the model since it measures the degree of response exerted by the experimental variables (Edwards *et al*., 2008).

In the second step, the Box-Behnken design was applied to optimize the most significant variables. The optimization process increased the exochitinase productivity to reach 28.931U/g ds. Although the ANOVA results showed a high F value (10.197) and a low P value (7.01 E-07) of the model indicating the significant and the high potentiality impact of the model terms used in this design, but the R^2 value of the model was 0.76. RSM has been used successfully in the optimization of exochitinases (Kumar *et al*., 2012; Awad *et al*., 2017), but in this study, the R^2 value of the applied model indicated the lack of fitness of the model since it was less than 0.9. Therefore, the use of ANN has been examined in this study as an alternative tool in predicting the experimental results.

ANN has been considered as one of the most important methods used in modeling and optimization processes that have been applied in various fields (Astray *et al*., 2016; de Araujo Padilha *et al*., 2017; Cui *et al*., 2018). DANN is characterized by a larger number of hidden layers which is higher than the traditional ANN, and so the weight updates gradually moves toward the optimum solution with more accuracy (Serwa, 2017). In this study, the DANN module with three hidden layers (8, 7 and 8 neurons) was applied, and the analysis of the data presented better results than RSM in terms of the R^2 value which was 0.996.

The influence of pH on the enzyme activity was estimated using a 0.2M acetate buffer, and the results showed that the highest enzyme activity was achieved at acidic pHs ranging from 4.5 to 5, and the enzyme retained 100 % of its activity up to two hours in this range of pH. The former results agree with De Freitas Soares *et al*., (2015) who reported the production of fungal chitinase with the highest activity at pH 5.5.

In agriculture, the use of biocontrol agents as an alternative tool against pathogenic fungi can overcome the harmful effects of the current used strategies (A Veliz *et al*., 2017; Honari *et al*., 2018). *Rhizoctonia solani* is an important soil-borne fungus that causes difficult controllable diseases worldwide (Lahlali and Hijri, 2010; Woodhall *et al*., 2007; Yandigeri *et al*., 2015). In this study, the produced exochitinase showed antifungal activity against *Rhizoctonia solani.* The antifungal activity of chitinases against pathogenic fungi has been previously reported by Jankiewicz and Brzezinska, (2016), Velusamy and Kim, (2011) and Yandigeri *et al*., (2015).

5. Conclusion

DANN can be applied for the optimization of microbial enzymes as an alternative method to RSM. In the current study, DANN presented more accuracy in the prediction of the selected model with an R^2 value of 0.996 in comparison with the R^2 value of 0.76 using RSM. The produced exochitinase exhibited an antifungal activity against *Rhizoctonia solani.*

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