Improved Antibiotic Activity from Streptomyces monomycini strain RVE129 Using Classical and Statistical Design of Experiments

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Abstract

Streptomyces sp. has provided a wealth of bioactive secondary metabolites with interesting antimicrobial, antiviral, and anticancer activities. This study aimed to optimize antibiotic production medium and growth conditions of *Streptomyces monomycini* RVE129 isolated from rhizospher soil of Hawassa, Ethiopia, under shaking conditions by traditional and statistical methods. The classical one-factor-at-a-time (OFAT) approach and the statistical Plackett Burman (PB) design was used for the selection of the important media components; which were further optimized and identify their concentrations using Box-Behnken design for improved antibiotic production by *Streptomyces monomycini* RVE129. The antibacterial activity of obtained antibiotics by the strain in flask cultures was tested using the disc diffusion method with *S. aureus* as the test organism, and cell growth was measured as the weight of dry cells in a constant volume of culture broth. *Streptomyces monomycini* strain RVE129 was able to produce maximum antibiotics on a modified ISP4 medium consisting of 10 gL^{-1} starch 4 gL^{-1} yeast extract 1 gL^{-1} NaCl, 1 gL^{-1} $MgSO_4.7H_2O$, 2 gL^{-1} $(NH_4)_2SO_4$, and 1 gL^{-1} K_2HPO_4 in comparison to other tested media. The most efficient sources of carbon and nitrogen for the *S. monomycini* RVE129 strain were determined to be starch and soya bean meal. The optimum conditions for antibiotic production were observed at a temperature of 30°C and a pH of 7.5 on the 8th day of incubation under shaking conditions. Subsequently, media components such as soy bean meal, starch, and K_2HPO_4 were recognised as the most significantly affecting antibiotic production with a confidence level of above 95% by Plackett-Burman design. Further optimization of the concentration of three media factors by Box-Behnken (BB) design–RSM revealed optimal values of starch- 20 gL^{-1}, soybean meal- 7.5 gL^{-1}, and K2HPO4 1.25 gL^{-1} and there was nearly 21% increase in the yield of antibiotic production (35.67 mm) when compared to unoptimized medium (27 mm). Antibiotic yield was improved by optimizing the medium and culture conditions. To our knowledge, this is the first study on medium optimization for antibiotic production by *Streptomyces monomycini* RVE129.

1. Introduction

The genus *Streptomyces* constitutes the largest number of species and sources of most well-known antibiotics among the actinomycetes group. They have also been the source of novel bioactive natural compounds, the majority of which have significant uses as antibiotics, antifungal, anti-viral, antitumor, antiparasitic, antihelminthic agents [1, 2, 3, 4]. Hence, the exploration of new and novel bioactive metabolites produced by *Streptomyces* still continues and has attracted more interest in recent years for the production of novel antibiotics with unique mechanisms of action to treat various drug-resistant diseases and other disorders [2, 3]. Thus, *Streptomyces* sp. is considered a potential source in the search for novel antimicrobial compounds with clinical, pharmaceutical, and biotechnological aspects [6].

The capability of *Streptomyces* cultures to produce secondary metabolites is not a static phenomenon. It can be highly influenced by nutritional and cultural conditions in the cultivation medium [6, 7]. Hence, both nutritional and environmental factors of the culture conditions have great influences in the antibiotic

biosynthesis and production cost, it is critical to optimize the nutritional parameters of fermentation conditions to improve the biomass and antibiotic production by the potent *streptomyces* sp [4].

Developing a suitable cultivation condition for producer strains needs successive trials like selection of optimal medium, culture medium constituents such as carbon, nitrogen, and trace elements, optimization of various physical culture parameters, like incubation period, growth pH, and temperature [7].To enhance growth and antibiotic production by the potent *Streptomyces* sp, selection of carbon, nitrogen, and mineral sources as well as selection of suitable culture growth parameters can be carried out by using a conventional approach, the one-variable-at-a-time approach method [5]. Following that, Design-of-experiments (DOE) approach like the Plackett Burman design (PBD) and Response Surface Methodology (RSM) can be used to identify key medium components for their significant levels. The Plackett-Burman design is a known and frequently employed statistical technique for the screening of the production medium components in shake flask fermentation [8, 9]. Response surface methodology (RSM) can subsequently be used to optimize the important fermentation media parameters and their levels by applying mathematical and statistical tools [9, 10].

As a part of our search for novel antibiotic metabolites a potential strain *Streptomyces monomycini* RVE129 having a broad spectrum antibiotic activity was previously isolated from the central rift valley areas, Hawassa, Ethiopia [12]. This study was undertaken to select the optimal growth conditions for enhancing the production of biomass and antibiotics by *Streptomyces monomycini* strain RVE129 through medium and growth condition optimization using a one-variable-at-a-time and statistical tools that significantly improved antibiotic production of *S. monomycini* RVE129.

2. Materials And Methods

2.1. Test strains and their maintenance

An antibiotics producing strain previously isolated from the rhizosphere soil collected from the rift valley region, Hawassa, Ethiopia was used in this investigation [12]. The strain was identified and designated as *Streptomyces monomycini* RVE129. The strain was stored at 4°C as slant culture using tryptic soy agar medium and at − 20°C in the tryptic soy broth (TSB) containing (50%, v/v) glycerol in a freezer [13]. The test microorganism, *S. aureus* ATCC-259233 was supplied from the Ethiopian Health and Nutrition Research Institute (EHNRI) and maintained under refrigerated conditions.

2.2. Inoculum Preparation

To prepare spore suspension of fermentation inoculum of the strain a colony was transferred from seven-day-old culture grown on TSB medium plates by suspending with 10 ml of sterile normal saline and then the suspension of the strain used as seed culture [6]. Conical flasks with a volume of 250 mL containing 100 ml of TSB broth medium were used for the experiments. The medium was inoculated with 5.0 ml of spore suspension at a density of $1x10^8$spores/ml, at 150 rpm for 3 days at 30°C, and then used as fermentation seed stocks [6, 7].

2.3. Culture media selection for antibiotic synthesis and growth

The prepared inoculum of *Streptomyces* sp. was transferred separately into each eight different microbial growth media. The eight types of media used were: tryptone yeast extract broth (TYE), yeast extract-malt extract-dextrose (YMD), Oat meal broth (OM), Modified starch inorganic salts broth (SIS), glycerol-asparagine broth (GA), starch casein broth(SC), tyrosine broth (TB), and Glucose soybean meal broth (GSB), (with pH-7 ± 0.2) for the selection of suitable basal medium for production of antibiotics and growth of mycelia biomass and optimum medium was screened for further production of antimicrobial compound from RVE129. In 250 mL Erlenmeyer flasks Five ml (10% v/v) $1x10^8$spores/ml density was inoculated into 100 ml of various sterile medium and cultured for 8 days at 30°C in shaker at 150 rpm [1, 7, 15].

2.4. Determination of Antibiotic Activity

Fermentation broth was taken aseptically and each culture broth of *Streptomyces monomycini* RVE129 was centrifuged at 5,000 rpm for 20 min and followed by filtration to separate the cell free supernatant and the mycelia biomass to determine antibiotic activity and growth [12, 13]. The supernatant of culture broth was equally mixed with ethyl acetate in 1:1 proportion and shaken for 1 hr and extracted using rotary vacuum evaporator to recover a crude extract. Then, the extract was bio-assayed against *Staphylococcus aureus* ATCC 25923 by standard disc diffusion method [13]. An overnight culture suspension, 0.2 ml of (0.5 McFarland) of *S. aureus* ATCC 25923 containing 1.5×10^8 CFU/mL was uniformly and aseptically spread out on Mueller Hinton agar (MHA) plates. A 100 µl volume of antibiotic extract was put on sterile discs with a diameter of 6 mm and placed on the agar plates. The sterile discs (6.0 mm diameter) loaded with ethyl acetate were taken as a negative control and incubated for 24 h at 37°C. After incubation, the inhibition zone (ZI) diameter was measured and recorded.

2.5. Determination of Growth

The harvested mycelia in the above experiment were used to measure the growth of the strain. The mycelia were dried in an oven at 50°C overnight, and the strain growth was measured in gm/L of culture medium as dry cell weight [7, 15].

2.6. Optimization of nutritional conditions

Various carbon and nitrogen sources were supplemented to the basal medium to test their suitability for growth and antibiotic production by *S. monomycini* RVE129. Briefly, as described by [14], Carbon sources such as glucose, fructose, galactose, maltose, lactose, sucrose, cellobiose, mannose, mannitol, and glycerol were added separately into the medium at a rate of 1% (w/v) while other parameters remained constant. Similarly, nitrogen sources like ammonium sulphate, ammonium chloride, malt extract, soya bean meal, peptone, yeast extract, and casein were individually supplemented in the production media at a 0.3% (w/v) level while other constituents remained constant [15].

2.7. Optimization of growth conditions

To select the best incubation period for the growth and antibiotics production were determined by *S. monomycini* RVE129 strain was inoculated into 100 ml of the basal medium into each 250 ml conical flask and incubating for 1–14 days in a shaker at 150 rpm at 30°C using modified starch inorganic salts broth (SIS) medium at pH 7.5 according to Jose and Jebakumar, [13] with some modification. During fermentation, 5 ml culture samples were taken aseptically at 24 h intervals, and the pellets were collected from the broth culture by centrifugation.

Similarly, the optimum pH for maximum antibiotic and biomass production was examined by changing the pH (4–11) of the basal medium. A 100 ml Medium contained in a 250 ml Erlenmeyer flask was seeded with five ml of the spore suspension and incubated on a rotary shaker at 150 rpm, at 30°C for eight days to determine antibiotic activity and growth. The *S. monomycini* RVE129 strain was inoculated into modified SIS broth production medium and incubated for 8 days at various temperatures (20, 25, 30, 35, 40, and 45°C) in a shaking incubator (150 rpm) at pH 7.5. The antibiotic activity of crude extracts from mycelia-free culture filtrate was concentrated in a vacuum evaporator, and 50 µl was assayed to find the best incubation period, pH, and temperature for maximum antibiotic activity [13, 15, 22]. The dry cell weight of collected cells was reported as gm/L of culture media and used to determine growth.

2.8. Statistical optimization medium components

2.8.1 Plackett-Burman Design (PBD)

The most important goal of screening is to identify the major effects of significant nutritional factors. Based on the findings of OFT, soybean meal, and starch were found to be the excellent N and C sources for triggering the highest antibiotic activity used for screening purposes. Therefore, the already screened nitrogen, and carbon sources were used along with other constituents for media optimization experiments. The PBD screening aids in identifying the most crucial media components which have a significant impact on the production of bioactive metabolites from a vast pool of available candidates [9, 10, 13]. To identify the most important medium elements for antibiotic production by *Streptomyces monomycini* RVE129, a Plackett Burman design (PBD) was used (Table 1). The experiments were designed using Minitab 18.0 (Minitab Inc., PA, USA) software, which was also used to analyse the experimental data. This led to a design with 12 trials for eight variables. A total of eight independent medium components were examined in the experimental design, by displaying them at two levels, low (-1) and high (1) (Table 1). The details of the Plackett-Burman design are shown in Table 1. A trial is represented by a row, medium components within each experimental trail, the response value Y (antibacterial activity) for each experimental design is represented by columns was considered as response value and the following regression equation was obtained:

Antibiotic Activity (mm) = 20.438 + 6.108A + 4.662B + 0.563 C + 0.427D-4.398E + 0.582F + 0.9 38H + 1.103G.....Eq. 1

The factors that had confidence levels above 95% were expected to have a significant effect on the antibiotics production and were selected for further optimization.

Table 1
Plackett Burman experimental design determining high and Low levels of each variables.

Variables	Media components	levels	
		-1	1
A	Starch (g/L)	5	20
B	Soybean meal (g/L)	2.5	7.5
C	Yeast extract (g/L)	2	8
D	$CaCO_3$(g/L)	1	3
E	K_2HPO_4(g/L)	1	3
F	NaCl(g/L)	1	3
G	$FeSO_4.7H_2O$	0.0001	0.003
H	$ZnSO_4.7H_2O$	0.0001	0.003
Note: X_1-X_9 represent various impact factors; "1" and "-1" represent two different levels.			

2.8.2. Response Surface Methodology (RSM)

The PBD investigations were utilized to obtain the most important positively affecting media components, such as starch, soybean meal, and K_2HPO_4, for antibiotic production. They were then used to identify the optimal level of these components effecting improved antibiotic production by RSM in *S. monomycini* RVE129. In addition to enabling rapid screening of a large experimental domain, RSM optimization also takes into account the roles of each component [5, 6, 10]. The concentration of the selected components was optimized using the Box Behnken design (BBD) design matrix. As shown in Table 3, the three medium components (independent variables) were examined at three distinct concentrations such as less, moderate, and high, respectively. The observed value represented by antibacterial activity (mm) and the effect of the three variables on the antibiotic activity are also summarized in Table 4. Five replicas of the central point were used in a total of 17 trials, and the response values were the average of three replications (Table 4). Each response was used to fit a distinct second-order polynomial model after being measured for each trial. The experimental design and the results as well as the values expected by the fitted equation derived from BBD are shown in Table 4. Following the use of BBD, the regression shown in Eq. 2 below shows demonstrates an experimental association between the logarithmic values of antibiotic activity and the study factors.

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ij} X_i X_j + \Sigma\beta_{ii} X^2{}_i \quad (2)$$

Y is the predicted response (antibiotic activity), β0 is the constant term coefficient, βi is the linear coefficient, βij is the quadratic coefficient, βii is the interaction coefficient, and XiXj represents the independent variables.

Minitab 18.0 (Minitab Inc., State College, PA, USA) was used to perform a regression analysis on the collected data. An analysis of variance was used to determine the model's statistical significance (ANOVA). Model values, Fisher's F-test and significance probability P (F) were the essential calculations to determine the overall model significance. Regression models have a high degree of reliability when their F- and P-values are large. Employing the coefficient of determination (R^2) and adjusted R^2 were statistically confirming the accuracy of the polynomial model equation [9, 10]. The relationship between the responses and the experimental values of each independent variable was then depicted using three-dimensional response surface plots to illustrate the fitted polynomial equation [6, 13].

2.9. Experimental validation

By cultivating *S. monomycini* strain RVE129 in both unoptimized and optimized production media in shaking flasks, the combination of various optimized factors that produced the maximum response was experimentally validated. The upper organic layer of the fermented broths was dried for further examination after the cell-free supernatant was collected and extracted with an equal volume of ethyl acetate. The antibacterial activity was examined using extracted antibiotic.

3. Results

3.1. Optimal Nutrient Medium Selection

Different medium compositions, nutrition, and growing conditions all have an impact on a microorganism's potential to produce antimicrobial metabolites. In this work, the medium utilized for cultivation of the *S. monomycini* RVE129 strain, nutrient sources (carbon, nitrogen, and minerals), and culture conditions (incubation temperature, pH of production medium, and duration of fermentation) were studied for increased antibiotic production. Antibiotic activity of the strain was examined by growing it in various production media (Fig. 1). As shown in Fig. 1 among eight different liquid media tested, modified starch inorganic salts (SIS) broth medium showed maximum antibiotic production with the highest inhibition zone diameter (27.04 ± 0.26 mm), followed by Yeast extract malt extract dextrose broth medium (25.3 ± 0.54) then glucose soya bean meal broth (GSB) medium (23 ± 0 mm) against *S. aureus* by *Streptomyces monomycini* RVE129 strain (Fig. 1). Regarding cell growth, the highest biomass (3.8 ± 0.23 mg/ml) were obtained with modified starch inorganic salts broth (SIS), Starch casein broth (SC) (3.6 ± 0.12 mg/ml), and followed by the culture filtrate grown on yeast extract-malt extract-dextrose (YMD) broth (3.46 ± 0). Other medium investigated (tryptone yeast extract broth (TYE), Oat meal broth (OM), glycerol-asparagine broth (GA), and tyrosine broth (TB)) were found to be lower growth as well as the synthesis of the antibiotic compound. Modified starch inorganic salts broth (SIS) medium composed of starch 5 g, glucose 5 g, yeast extract 4 g, 5 NaCl 2g, $MgSO_4.7H_2O$ 1 g, and K_2HPO_4 1g, in 1.0 L distilled

water showed the highest antibiotic productivity therefore, it was selected for further experiments as the best basal medium for the antibiotic production as well as the growth of Streptomyces monomycini RVE129 in batch fermentation.

3.2. Optimization of nutritional conditions using OFAT

To enhance antimicrobial activity attempts were made under optimized nutritional conditions to culture media by growing *Streptomyces* sp. As presented in Fig. 2 different carbon sources on antibiotic production by *Streptomyces monomycini* RVE129 were investigated with Modified starch inorganic salts (SIS) broth selected as production medium. The strain was capable of producing antibiotics in all of the carbon sources tested, although the maximum biomass (3.7658 gm/ml) was recorded in a medium supplemented with starch (Fig. 2). Similarly, the medium treated with soluble starch as a carbon source produced the maximum antibiotic activity (zone of inhibition of 27.56 mm). The carbon sources like glucose, lactose, glycerol, mannose, and galactose, were recorded as comparatively remarkable antibiotic activity inhibition zones ranging from 16.95 to 25.3 mm (Fig. 2). However, fructose, and manitol, supplemented with the production medium also favoured the growth of the strain RVE129, the antibiotic activity recorded was lower ranging from (6.7 to 9.8 mm) when compared with starch (Fig. 2).

Similarly, different nitrogen sources supplemented in the media tested with *Streptomyces monomycini* RVE129 supported various levels of effect on antibiotic biosynthesis as well as biomass yields. As shown in Fig. 3, of all examined nitrogen sources higher antibiotic activity and good growth were recorded with tryptone (inhibition zone 24 ± 06 mm) followed by peptone (inhibition zone 21.18 ± 1.2 mm). However, the highest antibiotic activity was found to be with soybean meal which was shown as the suitable nitrogen source for maximum growth (3.65 gm/100ml) as well as antibiotic production (inhibition zone 27.1 ± 0.21) (Fig. 3). No antibiotic activity was recorded with urea.

3.3. Optimization of culture conditions

Antibiotic production and growth by many species of the genus *Streptomyces* were greatly influenced by optimal nutritional and cultural parameters. Suitability of incubation time for antibiotics production as well as growth was performed at fixed range of time (1–14) days by cultivating *Streptomyces monomycini* RVE129 in production medium in shake flask condition (Fig. 4). From data given in Fig. 4 it is clear that, the growth of the strain was started only after two days of incubation whereas little noticeable antibiotic production was begin on the 3rd day and then increased until it reached a maximum (27.64 mm zone of inhibition) on the 8th day of incubation. The antibiotic activity remained more or less stable until 10th day after while the mycelia biomass and antibiotic activity started to decrease gradually.

An experiment was carried out to find out the influence of pH on growth and antibiotic production by the strain RVE129 (Fig. 5). The findings observed in Figure (5) revealed that the antibiotic production was increased with increasing pH from 4.0 to 7.5, but maximum growth (3.78 gm/L) as well as highest antibiotic activity (27.66 mm zone of inhibition) was recorded at pH 7.5 (Fig. 5). Lower or higher pH values were unfavourable and caused a decline in both growth and level of antibiotic. The findings clearly

state that there is almost a positive correlation between pH and antibiotic production by *Streptomyces monomycini* RVE129.

The temperature of the incubation has an effect on biomass and antibiotic activity. The results of various incubation temperatures on the biomass and antibiotic biosynthesis by the *S. monomycini* RVE129 strain is indicated in Fig. 6. In this study, antibiotic activity was recorded at temperatures between 20 and 45°C. The optimum growth (3.76gm/L) as well as antibiotic production (zone of inhibition 27.3 mm) was recorded at 30°C (Fig. 6).

3.4. A statistical approach for optimization of the fermentation medium

3.4.1. Screening of significant medium ingredients by PBD

To identify the medium components that significantly affect the antibiotic activity by *S. monomycini* strain RVE129, the Plackett-Burman Design (PBD) was used with 12 trials. Table 2 presents the PBD experimental test for medium component screening. Based on the findings of the antibiotic assay, Table 2 shows the levels of selection and evaluation for each experimental component.

Table 2
Plackett-Burman design and experimental response obtained for *Streptomyces monomycini* RVE129.

Run	Variables								Antibiotic Activity (mm)
Order	A	B	C	D	E	E	F	G	
1	1	1	1	-1	1	1	-1	1	27.00 ± 1.54
2	-1	1	-1	-1	-1	1	1	1	25.00 ± 1.63
3	-1	-1	-1	-1	-1	-1	-1	-1	9.86 ± 2.11
4	1	1	-1	1	-1	-1	-1	1	33.82 ± 0.77
5	1	-1	1	-1	-1	-1	1	1	16.80 ± 0.00
6	-1	-1	-1	1	1	1	-1	1	6.83 ± 1.55
7	1	1	-1	1	1	-1	1	-1	29.30 ± 1.54
8	-1	1	1	-1	1	-1	-1	-1	17.36 ± 0.61
9	1	-1	1	1	-1	1	-1	-1	27.61 ± 0.00
10	1	-1	-1	-1	1	1	1	-1	14.63 ± 0.00
11	-1	1	1	1	-1	1	1	-1	26.30 ± 0.63
12	-1	-1	1	1	1	-1	1	1	6.87 ± 2.77

The effect, standard error, t-value, P value, and confidence level of each component are shown in Table 3. In comparison to other variables that were evaluated for their suitability to increased antibiotic activity of S. monomycini RVE129, three components (starch, soybean meal, and K2HPO4) were found to be the most significant in the production of antibiotics based on the low P-values ($p < 0.05$), which was evident from their confidence levels above 99%.

Table 3
Statistical analysis of the data generated by the PBD

Coded variable	Medium component	Effect	SE	t-Value	P value	Confidence level (%)
A	Starch	12.217	0.357	17.09	0.000	*
B	Soybean meal	9.323	0.357	13.04	0.001	*
C	Yeast extract	1.127	0.357	1.58	0.213	NS
D	$CaCO_3$	0.853	0.357	1.19	0.318	NS
E	K_2HPO_4	-8.797	0.357	-12.31	0.001	*
F	NaCl	1.163	0.357	1.63	0.202	NS
G	$FeSO_4.7H_2O$	1.877	0.357	2.63	0.079	NS
H	$ZnSO_4.7H_2O$	2.207	0.357	3.09	0.054	NS
Note: *; significant at the 0.01 level, NS; not significant at the 0.01 level						

It was further supported by the Pareto chart of the standardised effects (Fig. 7), which shows that the highest effects are shown for upper fields while the minimal impacts are shown for lower fields, with close to zero in the upper portion. The relationship between the t-value (effect) and ranks was shown using a horizontal reference line with the statistical significance (t = 3.18) (Fig. 5). For *S. aureus*, any effect that exceeds this reference line is regarded as significant, and vice versa. By using the response surface methodology, the medium ingredients starch, soybean meal, and K_2HPO_4 were further optimised to provide the best antibiotic activity from *S. monomycini* strain RVE129.

3.4.2. Optimization of Selected Medium Components by Box–Behnken Design

Using RSM with Box-Behnken design, the three selected variables such as starch, soybean meal, and K_2HPO_4, which influenced *S. monomycini* RVE129 ability to produce antibiotics, the optimal concentrations of media components were found in 17 experimental runs. Following a batch of experiments using the Box-Behnken design, the observed responses of the three variables to the antibiotic activity are summarised along with the predicted value (Table 4).

Table 4
Coded values (Low, moderate, and high) levels of the Box-Behnken design experimental variables for RSM

Variables	Range and level (g/L)		
Starch	+	0	−
Soybean meal	+	0	−
K_2HPO_4	+	0	−

The experimental BBD data (Table 5) and regression analysis (Table 6) were used to develop the quadratic polynomial equation (Eq. 3) that evaluates the relationship between the response and three variables.

Y (Antibiotic activity) = 27.11 + 0.497A + 0.61B-13.08 C- 0.0394A^2- 0.029 B^2 + 3.15 C^2 + 0.2318 A*B + 0.050 A*C-0.408 B*C..... (3)

Antibiotic activity is represented by the letter Y, whereas the codes for starch, soybean meal, and K_2HPO_4 are A, B, and C, respectively.

Table 5
Box-Behnken experimental design, experimental response, and predicted response (antibiotic activity)

Run Order	Factors			Antibiotic Activity(mm) ± SEM	
	Starch	soybean meal	K_2HPO_4	Observed	Predicted
1	0	0	0	25.27 ± 0.94	24.67
2	−	0	−	24.67 ± 1.54	25.16
3	0	+	−	35.33 ± 0.27	36.27
4	0	−	−	20.21 ± 00	20.11
5	0	0	0	24.56 ± 1.54	24.67
6	−	0	+	19.65 ± 0.81	19.22
7	0	0	0	23.82 ± 2.6	24.67
8	0	−	+	20 ± 2.6	20.12
9	0	0	0	23.82 ± 0.94	24.67
10	+	0	−	33.28 ± 0.81	33.41
11	−	−	0	19 ± 0.27	19.13
12	+	0	+	29.27 ± 1.54	29.3
13	0	0	0	25.27 ± 0.81	24.67
14	+	−	0	20 ± 0.32	20.22
15	−	+	0	19.65 ± 0.00	19.31
16	+	+	0	34.56 ± 0.32	35.05
17	0	+	+	32.23 ± 0.00	32.11
Mean ± SD where n = 3					

The response surface quadratic regression model was also statistically examined using an analysis of variance (ANOVA), and the findings are shown in Table 5. The model F value of 12.10 suggests that the proposed model is significant. The model variables with the codes A, B, C, AB, BC, and C2 are significant when the value of "prob F" is less than 0.05 (Table 5).

Table 6
Results of ANOVA for quadratic polynomial model and regression equation

Source	DF	SS	MS	F value	P value Probability > F
Model	9	459.36	51.04	12.10	0.002
A	1	145.01	145.01	34.39	0.001
B	1	202.00	202.00	47.90	0.000
C	1	20.23	20.23	4.80	0.045
A*B	1	59.68	59.68	14.15	0.007
A*C	1	0.59	0.59	0.14	0.021
B*C	1	2.91	2.91	0.69	0.432
A^2	1	1.28	1.28	0.30	0.599
B^2	1	0.49	0.49	0.12	0.743
C^2	1	28.00	28.00	6.64	0.037
Residual	7	2.62	0.73		
Lack-of-Fit	3	27.395	9.132	17.37	0.516
Pure Error	4	2.103	0.526		
Total	16	548.520			

Keys: SS = sum of squares DF = degree of freedom MS = mean square; P < 0.05 = significant, P > 0.05 = insignificant, R^2 = 0.9953, Adju. R^2 = 0.9823, Mean = 28.48, Coefficient of variation (CV) = 0.75%

The lower calculated F-value of (0.516), which shows that the lack-of-fit is insignificant in comparison to the pure error, shows that the statistical insignificance of the lack-of-fit value also supported the model equation, was sufficient to determine the antibiotic activity(Table 6). The coefficient of variation's result (CV % = 0.75) provided additional evidence of the model's accuracy and reliability. Figure 8 shows the interaction among the components. The second order polynomial model Eq. 2 might indicate 99.53% variation in the response, as shown by the determination coefficient of R^2 (0.9953) and adjusted coefficient of determination (0.9823), which can further demonstrate accuracy and reliability.

Response surface 3D plots showed the combined pair-wise of the three factors: starch, soybean meal, and K_2HPO_4, while the remaining components were maintained at the middle level (Fig. 8). The plots clearly show that higher starch and soybean meal concentrations and lower K_2HPO_4 concentrations favour higher antibacterial activity (Fig. 8A). With the increase in starch concentration from 15 to 20 gm/L (coded values, -1 to + 1), the antibacterial activity gradually increased to a maximum at a low concentration of KH_2PO_4 (coded values, + 1 to 0.0). (Fig. 8B). The same trend was observed for the

increasing concentration of soybean meal when its concentration increased from 2.5 to 10 gm/L (coded values, − 1 to + 1) (Fig. 8C). However, as the KH_2PO_4 level in the fermentation medium was increased, the antibacterial activity significantly decreased. Consequently, it is clear that the fermentation process was significantly impacted by the medium composition.

The model and regression equation performed validation testing in triplicate using the optimum (predicted) medium in shake flask fermentation. Maximum antibiotic activity was experimentally obtained, as predicted by a numerical optimization method was 35.67 mm, when the optimum values of independent components in the coded units were starch (20 gL^{-1}), soybean meal (7.5 gL^{-1}), and K_2HPO_4 (1 gL^{-1}), respectively. The maximal antibacterial activity against *S. aureus* was observed to be 31.49, indicating that the experimental and predicted values were in reasonable agreement. The antibacterial activity was increased from 27.0 to 35.67 mm (S. aureus) by optimising the medium components. This result revealed the suitability of the model for predicting the antibiotic production by *S. monomycini* strain RVE129.

4. Discussion

Many microorganisms have been evaluated for their optimal production of antimicrobial metabolites. The effectiveness of antibiotic production depends on the optimal accessibility of primary metabolites as precursors and which in turn guides the expression of antibiotic-producing genes to activate the necessary metabolic pathways [3]. Therefore, optimizations of culture conditions are a basic requirement for a better yield of secondary metabolites. Many studies have reported that improvement of antibiotic production as well as the growth of Streptomyces sp depends on the optimal nutritional and physical parameters of cultivation conditions [14, 17, 23].

The present work was carried out considering the above-mentioned facts, to investigate the influence of various culture medium, and components (carbon, nitrogen, and mineral sources) leading to an efficient yield of antibiotics by the *Streptomyces* RVE129. Incubation time, temperature, and pH of the culturing conditions were examined for their effects on biomass production and antibiotic activity. Selection of basal medium is an important step for better formulation and optimization of medium constituents to maximize antibiotic production by *Streptomyces* sp. Results showed that among the culture media tested, the modified starch inorganic salts (SIS) broth was the best medium for enhanced growth and production of antibiotic which was used as basal media to select appropriate carbon and nitrogen sources for *Streptomyces monomycini* RVE129 in batch fermentation. This may indicate that the constituents of SIS media favored antibiotics production and biomass of *Streptomyces monomycini* RVE129. Antimicrobial metabolite production and growth from *actinomycetes* sp. Nonomuraea sp. JAJ18 was found to be higher in modified starch inorganic salts (ISP4) broth fermentation medium [13]. Al-Ansar et al [17] also reported that increased antibiotic activity in the starch inorganic salts (ISP4) production medium from *Streptomyces* sp. AS11.

Carbon and nitrogen supplies are very important components of the culture growth media for increased production of bioactive metabolites from actinomycetes [18, 19]. Our findings confirmed the influence of various carbon sources on the antibiotics production by *S. monomycini* RVE129. This finding was in concordance with those obtained for *Streptomyces rochei* AK 39, which utilized starch as an efficient source of carbon for the production of antibiotics [20]. Similarly, various researchers reported starch as an effective source of carbon for increased antibiotics production [18, 19, 21]. According to Narayana and Vijaylakshmi [23], soybean meal the best organic nitrogen source for *S. albidoflavus'* for S. albidoflavus production of biomass and antibiotics.. Similar findings were obtained for soybean meal as the optimal source to produce the enhanced antibiotics by *Streptomyces rimosus* NRRL 2455 [6]. *Streptomyces sannanensis* strain SU118 [16], *Streptomyces Violates* [18], *S. tanashiensis* A2D [21], and *Streptomyces* sp. [24].

Culture parameters such as incubation temperature, time, and pH influence the production of bioactive metabolites by *Streptomyces* spp. [16]. At pH 7.5, *S. monomycini* RVE129 showed its maximal growth and antibiotic activity. This result suggests this strain could be placed in the neutrophilic range. Our results are in conformity to the previous reports of Muthukumar et al. [26] who observed optimum pH at 7.5 for biomass and antibiotic production of *Streptomyces aureus* BG03. Several researchers also reported initial pH of 7.5 was best for maximum antibiotic activity and growth for *Streptomyces* sp. [21, 27]. Temperature also has an influence on biomass and antibiotic production. *S. monomycini* RVE129 was grown and showed antimicrobial activity at temperatures ranging between 20–40°C, and the production of antibiotics and cell biomass was reported to be maximal at growth temperature of 30°C. This finding is in agreement with previously reported results on maximum antibiotic production by *Streptomyces sannanensis* strain SU118 [16], S. chilikensis ACITM-1 [21], and *Streptomyces* sp. KGG32 [27]. The antibiotic activity of strain *Streptomyces monomycini* RVE129 grown in a modified SIS broth medium was recorded after 3 days of growth and reached its maximum on the eighth day of incubation, after which it remained stable for 3 days, and then both the biomass and antibiotic activity started to decrease slightly on the eleventh day under optimized conditions. Similar findings were also obtained by Bundale et al [24], in the optimization of various growth conditions on the antibiotic production of *Streptomyces* spp. They reported that the antibiotic production was started on the third day of incubation while the highest antibiotic production by isolate R3 and Y8 of the genus *Streptomyces* was obtained on the eighth day.

It is vital to design an experiment employing a quality experimental method to achieve enhanced secondary metabolite production. Several researchers working on antibiotic discovery programmes that employed PBD and RSM as statistical tools to identify, modify, and improve influential medium components have documented the enhanced antibiotic production [5, 6, 9, 10, 13]. The Plackett-Burman design has demonstrated to be a significant tool for establishing the composition of the fermentation medium and the growth conditions in the several bioprocesses, including the production of antibiotics [8, 9]. RSM of Box-Behnken design can also be used to optimize the components of the production medium and the growth conditions to increase the production of secondary metabolites [9, 10, 13]. Plackett-Burman design and RSM were employed in this study to improve the fermentation parameters and the

antibiotics production from *S. monomycini* RVE129. The experiment results were validated to examine the reliability of the models in identifying optimum responses. The determination of the R^2 coefficient can be used to evaluate the validity of the model. The better the model predicts the response, the closer the R^2 value is to one [34]. The model R^2 of 0.9953 indicated that the model equation could represent 99.53% variation in the response. The measured R^2 value is comparable to other studies [5, 6, 9, 10, 13]. Antibiotic production was significantly higher on the optimized medium than on the unoptimized medium. This finding was found to be in strong agreement with the earlier results [9, 10, 13, 22]. The validation experiments demonstrated the agreement between the predicted and observed experimental results, and they also considered the accuracy and reliability of the statistical experiment for medium optimization. The current work is a great reminder that optimizing the medium and fermentation conditions using traditional and statistical methods continues to be highly helpful in enhancing the yield of antibiotic metabolites by *S. monomycini* RVE129.

5. Conclusion

The main focus of the study was to enhanced production of antibiotics by *S. monomycini* strain RVE129 as a function of the various nitrogen and carbon sources, their concentrations in the basal medium, and growth parameters. The results confirm the application of the conventional technique for selecting suitable nitrogen and carbon sources. As a result, it was observed that the PBD and RSM developed were highly efficient and reliable in identifying medium components for the production of antibiotics by *S. monomycini* RVE129. This is the first study to use *S. monomycini* strain RVE129 for the improvement of antibiotic activity using conventional and statistical experimental designs for medium and fermentation parameters. The antibiotic activity was improved from 27.0 to 35.67 mm (S. aureus) with an overall 21.30% increase in optimized medium as compared to unoptimized medium. Therefore, it can be concluded that managing various physical and nutritional components of the production medium through classical and statistical experimentation could maximize antibiotic production of *S. monomycini* RVE129. This will be helpful for further formulation of a large-scale fermentation for enhancing antibiotic production from the *Streptomyces monomycin* strain RVE129.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and material

All the data analyzed during this study are included in this published article.

Conflicts of interest/Competing interest

The authors declare that they have no competing interests related to this work.

Authors' contributions

SM and DM executed, planed and coordinated the study, confirmed the results, reviewed the manuscript, FE designed and conducted experiments and wrote initial manuscript, BT, manipulated, analysed and interpreted statistical data. All the authors read and approved the final manuscript.

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Figures

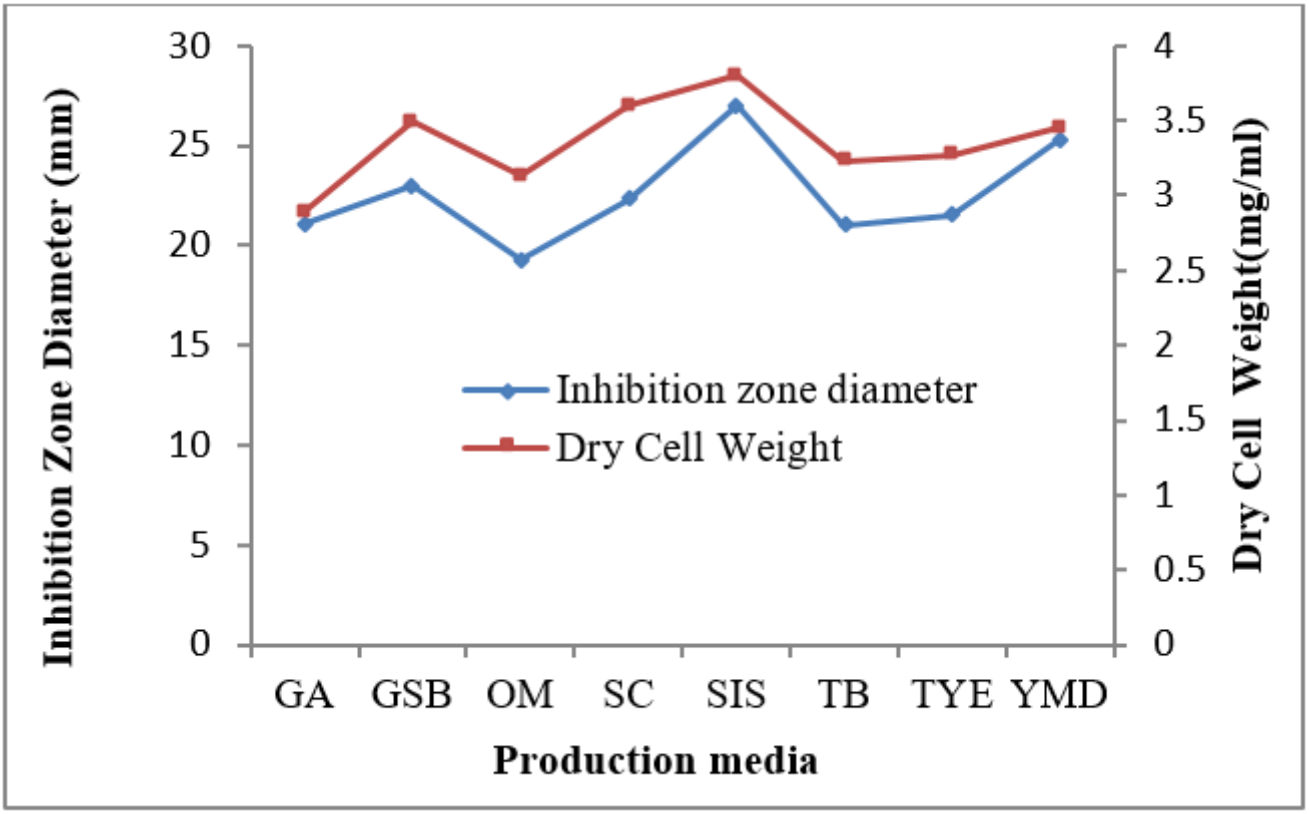

Figure 1

Influence of various culture media on antibiotic production by *Streptomyces monomycini* RVE129

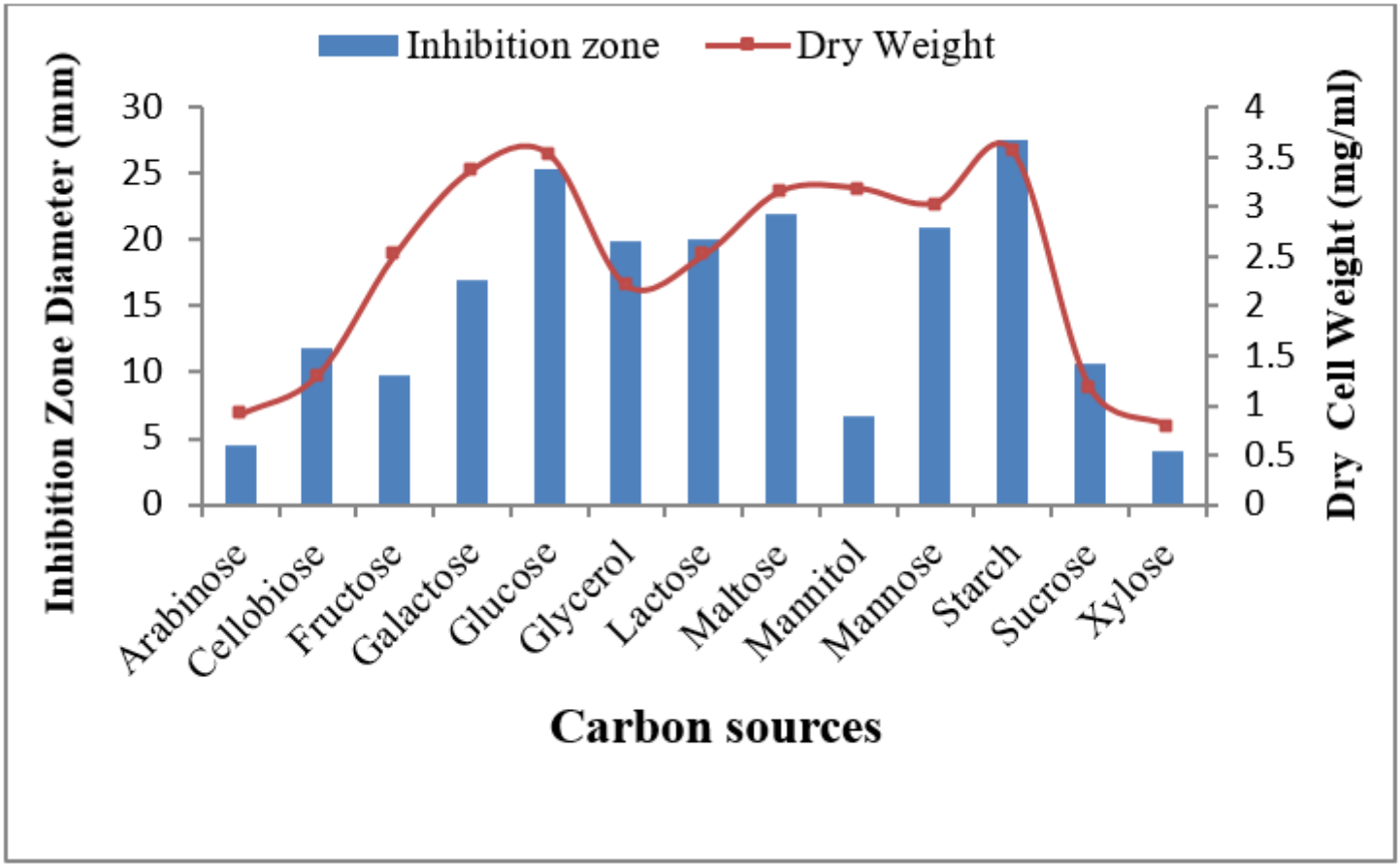

Figure 2

The impact of various carbon source for the production of antibiotics using *Streptomyces monomycini* RVE129

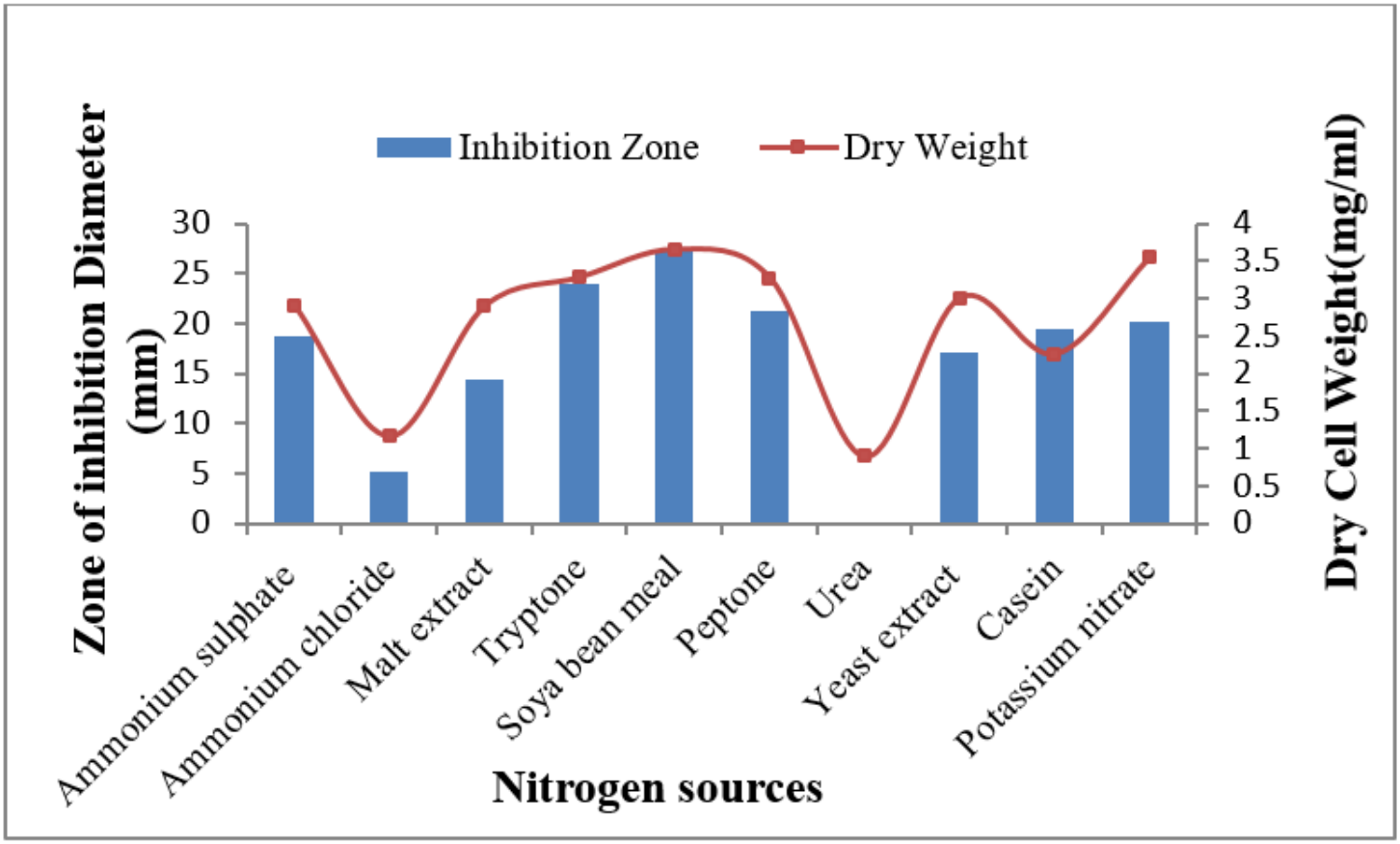

Figure 3

Influence of various nitrogen sources on biomass and antibiotic activity of

Streptomyces monomycini RVE129

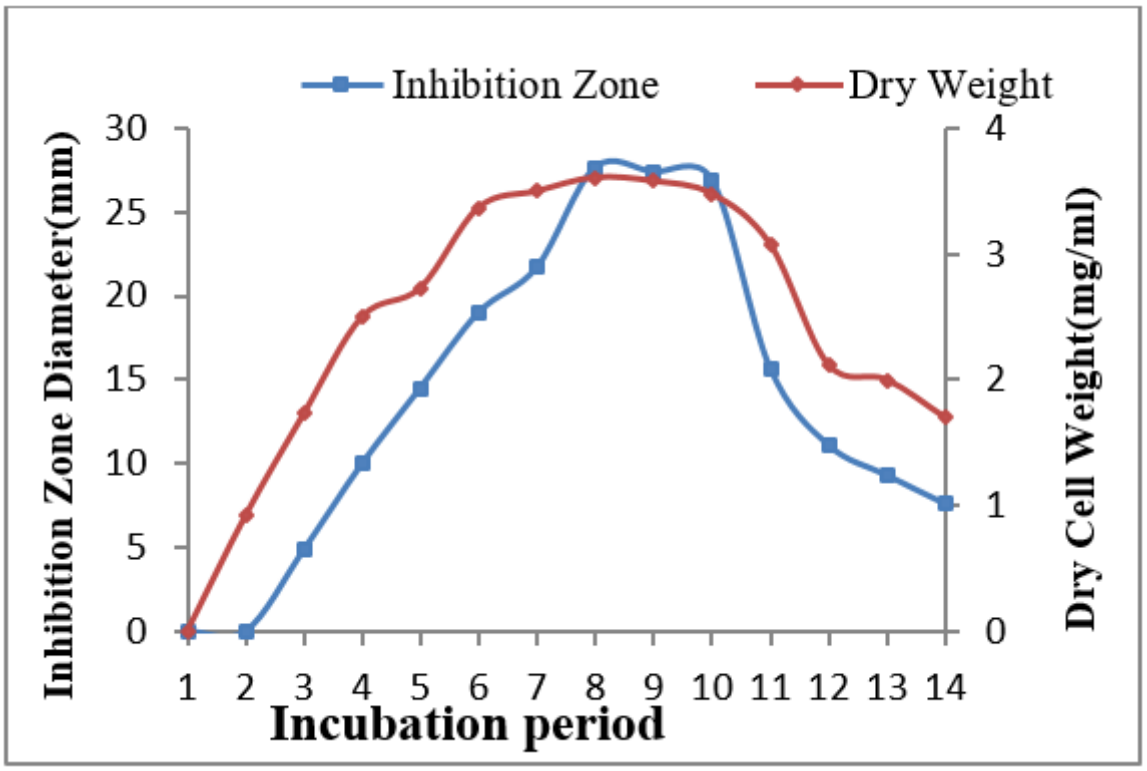

Figure 4

The impact of incubation period on the production of biomass and antibiotics by *Streptomyces monomycini* RVE129

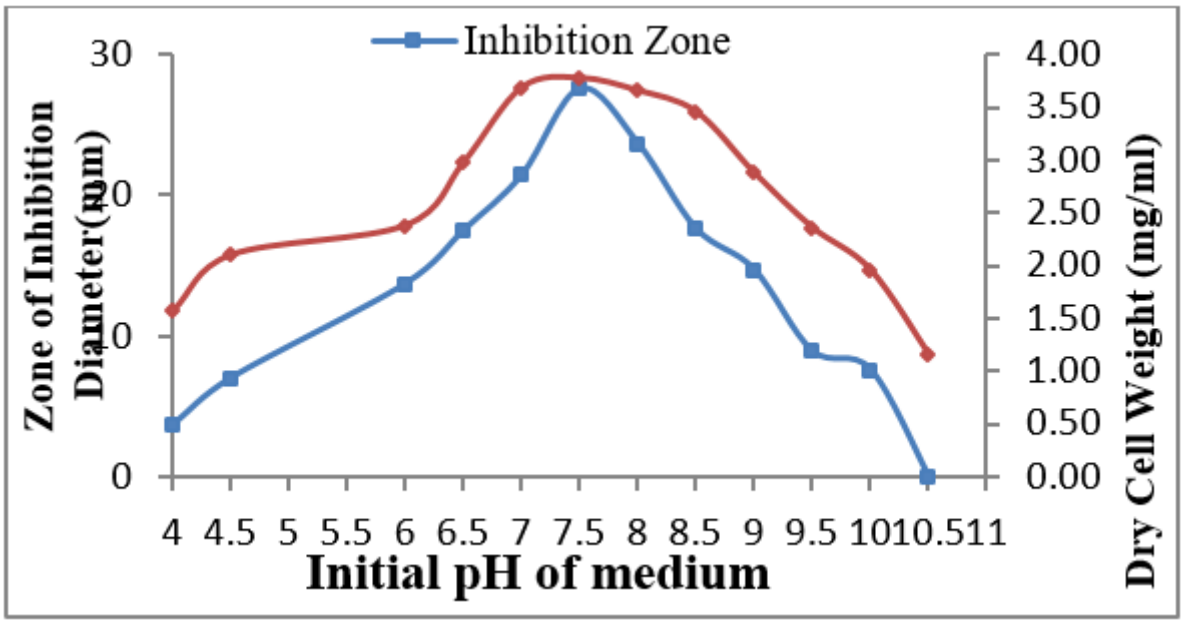

Figure 5

The impact of pH on the production biomass and antibiotic by *Streptomyces monomycini* RVE129

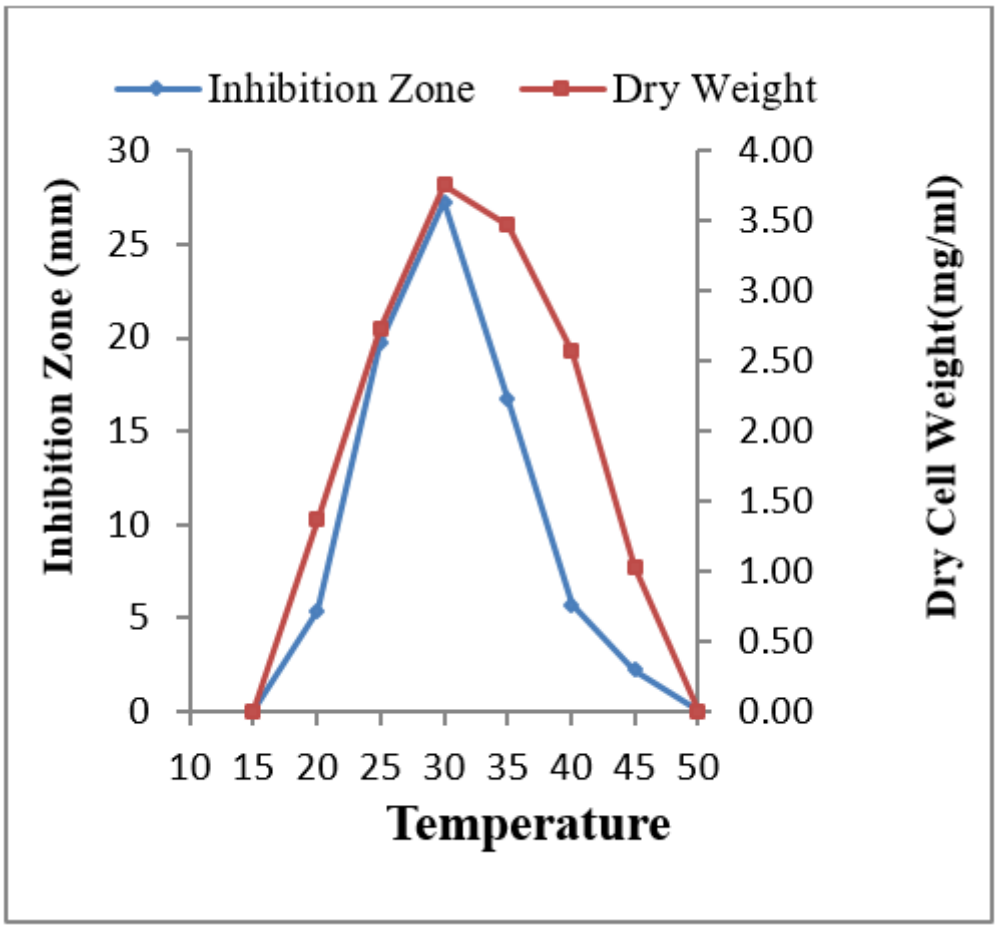

Figure 6

The impact of incubation temperature on the production of biomass and antibiotics by *Streptomyces monomycini* RVE129

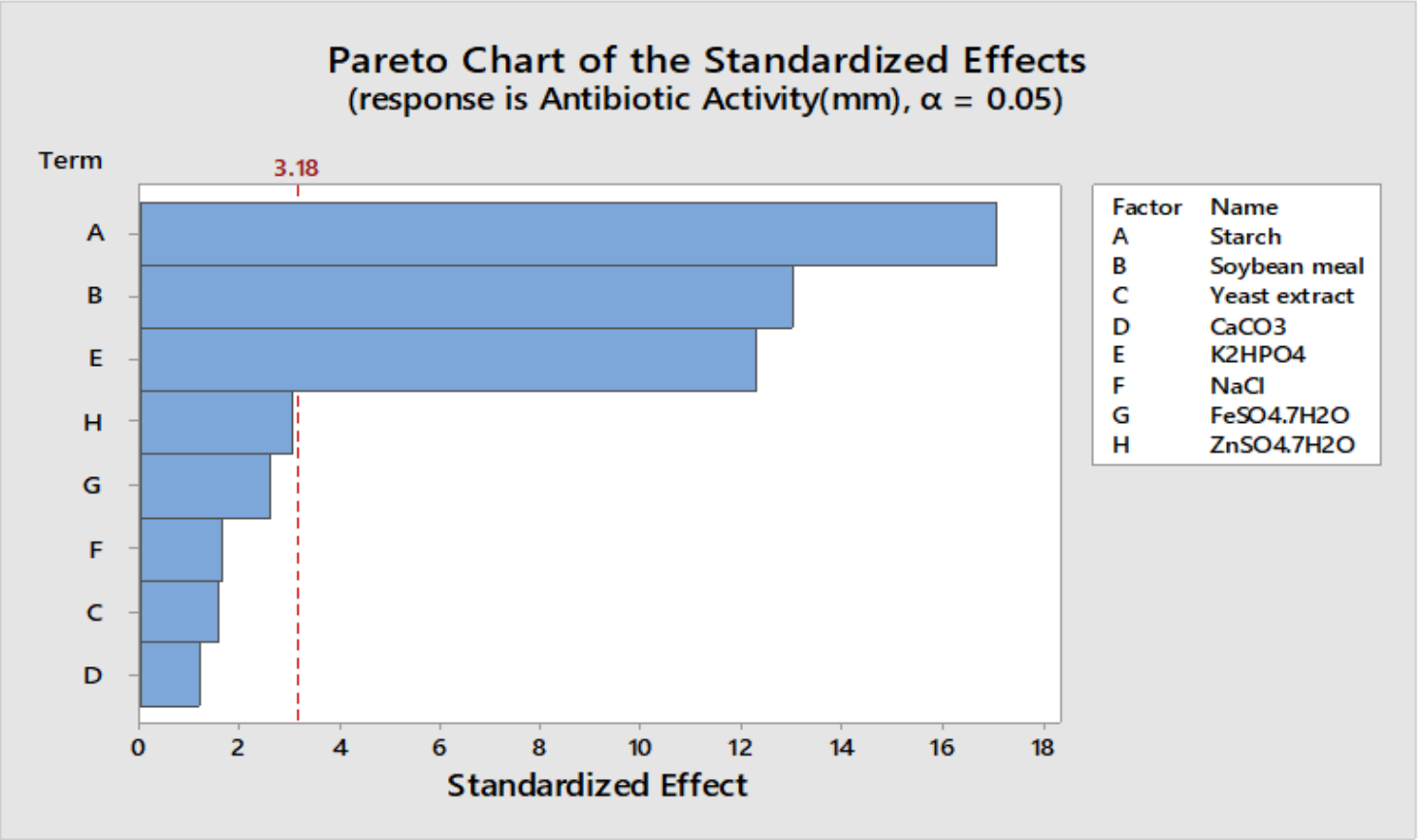

Figure 7

A Pareto chart depicts the influences of variables on antibiotic activity of *S. monomycini* RVE129 studied in the PlackettBurman design

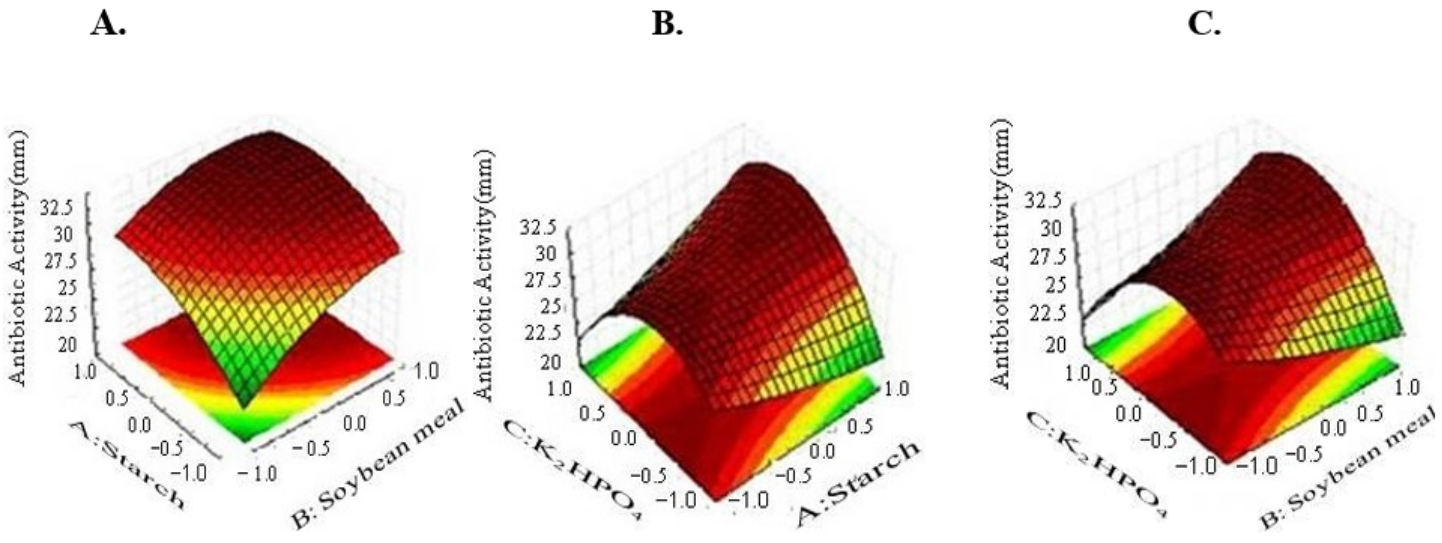

Figure 8

3D response surface plots exhibited the interaction between individual and combined influences of variables on antibiotic activity of *S. monomycini* RVE129: (A) the interaction between starch and soybean meal on antibiotic activity, (B), the interaction between starch and KH_2PO_4 on antibiotic activity (C) the interaction between starch and KH_2PO_4 on antibiotic activity.