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BIOSYNTHESIS OF ERGOT ALKALOIDS FROM *PENICILLIUM COMMUNE* USING RESPONSE SURFACE METHODOLOGY (RSM)

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Abstract

The present study employed the response surface methodology (RSM), a statistical technique, for the identification, screening and optimization of fermentation factors to produce ergot alkaloids under laboratory conditions by *Penicillium commune.* The static surface culture fermentation technique helped to enhance the production of ergot alkaloids. In the first step Plackett-Burman design (PBD) was used to evaluate the effect of ten factors, including nine ingredients of fermentation medium and one process parameter. It was found that sucrose, yeast extract and $FeSO_4.7H_2O$ played the pivotal role in enhancing the yield of ergot alkaloids. In the second step, the effect of concentration levels of sucrose, yeast extract and $FeSO_4.7H_2O$ was further optimized using Box-Behnken design (BBD) under the same fermentation conditions. The optimized concentrations of sucrose, yeast extract and $FeSO_4.7H_2O$ were 41%, 39% and 0.11% respectively, which significantly enhanced the yield of ergot alkaloids.

Key words: Ergot alkaloids, Response surface methodology, *Penicillium commune*, Static surface culture fermentation, Plackett-Burman design, Box-Behnken design.

Introduction

Secondary metabolites produced in plants, animals and fungi in the form of alkaloids are amongst the major class of naturally occurring compounds (Polak & Rompala, 2007). Alkaloids in microbes, such as fungi, were initially recognized in *Claviceps purpurea*, which is the causative agent of ergot of rye. The genus *Claviceps* forms sclerotia on the rye grains which produce ergot alkaloids by turning whole kernel of rye into the dark-coloured sclerotia (Burfening, 1973). Sclerotia of different species of genus *Claviceps* are able to produce various commercially important ergot alkaloids. Besides the sclerotia of *Claviceps*, other fungi such as *Balansia*, *Epichole*, *Penicillium* and *Aspergillus* and several higher plants also harbor some quantity of ergot alkaloids (Zafar *et al.*, 2010).

Ergot alkaloids are usually classified as clavine, ergoamide and ergopeptine alkaloids. Human intoxications and ergot poisoning have been observed in farm (stallion, goat, cow, lamb, and sheep) and wild animals (hog, buffalo and duiker) have been reported in literature, and all of these are adversely affected by ergot alkaloids (Mavungu *et al.*, 2011).Their effects on CNS are due to the structural similarity of ergot alkaloids with nor-adrenaline, dopamine and serotonin (Katzung, 2009). Ergot alkaloids sometimes react positively on the target cells as agonists and sometimes they behave poisonous and act as antagonists (Sinz, 2008). Various substituents of organic compounds when attached to the carboxyl end of ergot alkaloids, they enhance their physiochemical, physiological and pharmacological properties (Flieger *et al.*, 1997).

The study of various types of ergot alkaloids is significant mainly due to their useful and positive results. They are helpful in curing animal and plant diseases despite their poisonous and hazardous effects in contaminated foods and feeds (Neilsen *et al.*, 2014). Historically, midwives employed these ergot alkaloids in very less amount to the pregnant women to induce labor pains. In United States and Europe, ergot alkaloids were used to control the post-partum hemorrhage as well. Moreover, the peptide alkaloids, such as ergotamine, dihydroergotamine and bromocriptine, are important for the treatment of health problems, such as cardiac, liver, kidney, coronary artery and hypertension. These therapeutic agents are quite potent for the treatment of severe to moderate migraine, hyperprolactinemis and pituitary prolactinoma diseases (Lüllmann *et al.*, 2000; Katzung & Julius, 2001; King & Herndon, 2005).

The biosynthesis of ergot alkaloids is being carried out commercially for the production of a variety of significant drugs. Many fermentation processes are in use for the production of alkaloids, such as solid state fermentation (SSF), submerged fermentation (SMF) and surface culture fermentation (SCF). The laboratory and industrial synthesis of ergot alkaloids can be stimulated and enhanced adding various organic and inorganic compounds in the fermentation medium. The traditional approach used for the optimization of fermentation process includes various parameters; usually one factor is employed at one time. However, each variable optimization method in a single experiment is not only monotonous as well as time-consuming, and thus may lead to misunderstanding and misinterpretations of results, especially when the concerted effects among different factors are overlooked (Wenster-Botz, 2000). Hence, this one factor at a time (OFAT) technique has been unsuccessful to recognize the factor which has most favorable response (Deepak *et al.*, 2008).

The increasing use of statistical and mathematical modeling permits quick screening of many fermentation factors and their interactions to obtain the maximum yield of the product. Statistical procedures exhibit the significance of each factor in any fermentation experiment. In the present study, response surface methodology (RSM) comprising of mathematical and

statistical procedures, used for the development of empirical formula and process modeling. RSM is used as a powerful tool for optimizing fermentation factors for the synthesis of therapeutically important drugs and enzymes (Khurana *et al.,* 2007).

The use of response surface methodology (RSM) tools makes the fermentation process less expensive and sustainable by reducing the cost of unnecessary wastage of resources. Plackett-Burman design (PBD) and Box-Behnken design (BBD) models are quite significant tools to identify the variables which can control and enhance the yield of commercially important products. The increasing demand of ergot alkaloids as pharmaceutical and therapeutic agents is compelling to develop a cost-effective process for the biosynthesis of ergot alkaloids for commercial use. Hence, the present study was designed and conducted to enhance the yield of ergot alkaloids by employing such useful statistical tools to optimize the fermentation process.

Materials and Methods

Response surface methodology: Two statistical designs of response surface methodology (RSM) were employed in this study. First, Plackett-Burman design (PBD) was employed for the identification and screening of significant fermentation factors and Box-Behnken design (BBD) to optimize and determined the combined interaction effects of selected factors, respectively. Both the designs were employed for the better intensification of mycelium and enhanced production of ergot alkaloids by *Penicillium commune*. This technique was done after Venil and Lakshmanaperumalsamy (2009a) and Nelofar *et al.* (2011). Three major steps were taken for statistical optimization, such as selecting the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response or adequacy of the statistical model.

Maintenance of *Penicillium commune*: *Penicillium commune* strain was grown on malt extract agar (MEA) medium slants. The slants were prepared by dissolving malt extract (2 g) and agar (2 g) in 100 ml of distilled water in 250 ml Erlenmeyer flask. The medium was sterilized in autoclave at 121°C under 15 lb for 15 minutes. A loop full of pure and mature culture was streaked aseptically to the slants containing 7 ml of MEA medium. Inoculated slants were kept in incubator for 5 days at 25°C. The slants having full growth were stored at 4°C for further analytical studies.

Preparation of inoculum for screening purpose: Spore suspensions of *Penicillium commune* were prepared by adding 5 ml distilled water in a fully grown slant. The colonies of *Penicillium commune* were scrapped using inoculating loop under aseptic conditions. The slant was agitated for 2-3 minutes to break the hyphae from mycelial mass. Hemocytometer was used to keep the spore count at 10^7spores/ml.

Fermentation technique: A static surface culture fermentation technique was employed for the production of ergot alkaloids.

Response surface methodology experimental designs: For this purpose, Plackett-Burman design (PBD) and Box-Behnken design (BBD) were used.

Screening of fermentation variables by Plackett-Burman design (PBD): The PBD model identified the potent physico-chemical factors significant for the maximum ergot alkaloid yield.PBD identified "n" factors in "n+1" experimental runs (Plackett & Burman, 1946). The factors selected in the present investigation were sucrose, yeast extract, succinic acid, asparagine, tryptophan, KH_2PO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$ and pH in the fermentation medium. All the factors were described as mathematical factors and studied at two levels, as low (-1) and high (+1) levels for mycelial growth and ergot alkaloids production (Table 1). The PBD experimental design for screening and identification of important factors is given in Table 2.

In the first step, factors impacting the yield of ergot alkaloids were investigated at high (+1) and low level (-1), during twelve experiments as shown in Table 2. All the experimental runs were completed in the groups of three and the standard yield was measured. Impact of each fermentation factor on ergot alkaloids yield was obtained using the formula mentioned below:

$$Y = \beta_o + \sum \beta_{ni}$$

Here, Y is the response (ergot alkaloids), β_o is the intercept and β_{ni} is the linear coefficient of independent factors. The pH of fermentation media was adjusted at 5.0 using 0.1 N HCl and ammonia solution. After sterilization at 121°C under 15 lb for 15 min, the fermentation media was incubated at 25°C for 21 days.

Optimization of fermentation factors using Box-Behnken design (BBD): After screening, using PBD model, three factors/variables were selected for the further optimization and estimating their combined interaction effect on the production of ergot alkaloids. For this purpose, Box-Behnken design (BBD) model was used (Box & Behnken, 1960).The experiments were consisted of 13 runs. The selected factors were studied at low (-1), medium (0) and high (+1) levels as shown in Table 3. All the experiments were done in triplicate and the average response of ergot alkaloids yield was taken as Y. The formula is as follows:

$$Y = \beta_o + \sum \beta_{ii} + X \sum \beta_{ii} \text{ x } i^2 + \sum \beta_{ij} n_i n_j$$

Table 1. Plackett-Burman design for experimental range and level for the screening of fermentation factors.

Fermentation factor	Level& Range	
	-1	+1
Sucrose, n1	5	35
Yeast Extract, n2	5	30
Succinic acid, n3	0.1	1
Asparagine, n4	0.1	1
Tryptophan, n5	0.1	1
KH_2PO_4, n6	0.1	1
$MgSO_4$. $7H_2O$, n7	0.25	0.625
$FeSO_4 7H_2O$, n8	0.01	0.1
$ZnSO_4 7H_2O$, n9	0.02	0.2
pH, n10	3	5

n1, n2, n10 are the independent factors of fermentation medium

Table 2. Screening of fermentation factors using PBD model.

Runs	Variables (x)									
	n1	n2	n3	n4	n5	n6	n7	n8	n9	n10
	Sucrose	Yeast extract	Succinic acid	Asparagine	Tryptophan	$MgSO_4$	KH_2PO_4	$ZnSO_4$	$FeSO_4$	pH
1.	35	5	0.1	0.1	1	0.625	0.1	0.2	0.1	5
2.	35	30	0.1	0.1	0.1	0.625	1	0.02	0.1	5
3.	5	5	0.1	0.1	0.1	0.25	0.1	0.02	0.01	3
4.	5	30	0.1	1	1	0.25	0.1	0.02	0.1	5
5.	35	5	0.1	1	0.1	0.25	1	0.2	0.01	3
6.	35	5	1	1	1	0.25	1	0.02	0.1	3
7.	5	5	1	0.1	1	0.625	1	0.02	0.01	3
8.	5	30	0.1	1	1	0.625	1	0.2	0.01	3
9.	35	30	1	0.1	1	0.25	0.1	0.2	0.01	5
10.	5	30	1	0.1	0.1	0.25	1	0.2	0.1	3
11.	5	5	1	1	0.1	0.625	0.1	0.2	0.1	5
12.	35	30	1	1	0.1	0.625	0.1	0.02	0.01	5

n1, n2, n10 are the independent factors of fermentation medium

Table 3. BBD Experimental design of range and level for optimization studies.

Factor/Component	Level& Range		
	-1	0	+1
Sucrose, n1	5	23	41
Yeast Extract, n2	5	22	39
$FeSO_4.7H_2O$, n3	0.01	0.06	0.11

n1, n2 and n3 are the independent factors of fermentation medium

Table 4. Experimental design for optimization of significant variables.

Runs	Variables		
	n1	n2	n3
	Sucrose	Yeast Extract	$FeSO_4$
1.	41	5	0.06
2.	41	39	0.06
3.	41	22	0.01
4.	41	22	0.11
5.	5	5	0.06
6.	5	39	0.06
7.	5	22	0.01
8.	5	22	0.11
9.	23	5	0.01
10.	23	39	0.01
11.	23	5	0.11
12.	23	39	0.11
13.	23	22	0.06

n1, n2 and n3 are the independent factors of fermentation medium

Table 5. PBD model for the screening of variables for ergot alkaloids production.

Run	Yield of ergot alkaloids (mg/100 ml)
1.	10.98 ± 0.01
2.	14.76 ± 0.01* (sucrose, yeast extract and $FeSO_4$)
3.	0.36 ± 0.02
4.	5.50 ± 0.03
5.	9.96 ± 0.05
6.	12.99 ± 0.01
7.	0.64 ± 0.06
8.	5.63 ± 0.03
9.	10.54 ± 0.01
10.	5.95 ± 0.02
11.	0.44 ± 0.02
12.	12.38 ± 0.01

"±" indicates the standard deviation among three replicates

In this equation, "ninj" are the contributory factors which control the yield response Y; β_o is the intercept coefficient; β_{ii} is the linear coefficient; β_{ii} x i^2 is the quadratic coefficient and β_{ij} is the interaction of fermentation factors; n1, n2 and n3 are the fermentation factors that were selected using PBD. Statistical model for the optimization of selected factors/variables on ergot alkaloids yield is shown in Table 4. The pH was adjusted at 5using0.1 N HCl and ammonia solution. Flasks were sterilized under 15lb/cm^2at 121°C for 15 min and incubated at 25°C for 21 days.

Statistical analysis: In this study, Fisher's test (F-test), t-test, *p* test (probability), correlation coefficient (R), and determination coefficient (R^2) were performed. For each individual factor, 3-D plots and RSM curves were obtained using STATISTICA version 7 (Stat-Ease Inc., Minneapolis, USA) software.

Results and Discussion

Screening of fermentation factors using Plackett-Burman design (PBD): In the first step, PBD model was used for screening of individual factors including sucrose, yeast extract, succinic acid, asparagine, tryptophan, KH_2PO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$ and pH to achieve the maximum yield of ergot alkaloids. Maximum yield of ergot alkaloids, i.e. 14.76 mg/100 ml was found from extracellular extract of run No. 2 (14.76 mg/100 ml) while the lowest yield was found in run No. 3(0.36 mg/100 ml) (Table 5).

The difference in ergot alkaloids yield was calculated by using the following equation:

$$Y = \beta_o + \sum \beta_{Xi}$$

Here, values of individual factors having probability (*p*-value <0.05 at 90% confidence level) were identified as significant variables that can influence the synthesis and yield of ergot alkaloids. The analysis of variance of PBD is presented in Table 6, which clearly shows that sucrose, yeast extract and $FeSO_4.7H_2O$enhanced the production of ergot alkaloids. These results also indicated

that PBD model is the efficient statistical tool to identify key factors affecting ergot alkaloids production in extracellular extracts. PBD screening is also presented in Pareto charts to represent the essential factors for the production of ergot alkaloids (Fig. 1). The values indicated in Pareto chart also represented the significance of sucrose, yeast extract and $FeSO_4.7H_2O$in the production of ergot alkaloids.

Box-Behnken design (BBD): Box-Behnken design (BBD) was used to optimize and determine combined interaction effect of the selected factors including sucrose, yeast extract and $FeSO_4$. This model was comprised of 13 experimental runs by varying the values of sucrose, yeast extract and $FeSO_4.7H_2O$in the fermentation medium. The yield of ergot alkaloids from the extracellular extracts of *Penicillium commune* ranged from 5.42 mg/ 100 ml to 14.64 mg/100 ml as given in Table 7. These observed values were also compared with predicted values using the polynomial equations. A highest yield of ergot alkaloids was obtained from extracellular extract of run No. 6 (14.64 mg/ 100 ml) of *Penicillium commune.* The yield of run No. 6was compared with the predicted yield (14.99 mg/100 ml) which was calculated by the STATISTICA version 7 (Stat-Ease Inc., Minneapolis, USA) software. The lowest value of ergot alkaloids yield was observed from extracellular extract of run No. 11 (5.42 mg/100 ml) and this was also compared with the predicted value (5.15 mg/100 ml).

Residual regression analysis: The residual regression study was also performed by calculating the variation between observed (Y) and predicted (Y') response of ergot alkaloids (dependent variable). The regression was calculated using the following formula:

$$e = Y - Y'$$

The residual plot for the yield by *Penicillium commune* strain is shown in Fig. 2. This chart shows a linear relationship (straight line) among the predicted and observed values of the response of ergot alkaloids from extracellular extracts of *Penicillium commune*.

Figure 2clearly indicated that BBD model was proved as a best model to explain the influence of optimized factors (sucrose, yeast extract and $FeSO_4$) as important individual factors on the yield of ergot alkaloids.

ANOVA – BBD model: The adequacy of BBD model was checked using ANOVA. In the step, Fisher's statistical analysis was done as shown in Table 8. The individual and combined interaction effects of sucrose, yeast extract and $FeSO_4$ were analyzed using ANOVA. It was found that sucrose substantially enhanced the yield of ergot alkaloids in extracellular extracts (15.34 mg/100 ml). The lowest amount (1.72 mg/100 ml) of ergot alkaloids was obtained from the extracellular extracts of *Penicillium commune* after the addition of yeast extract in the fermentation medium. Among the combined interaction effects of sucrose, yeast extract and $FeSO_4.7H_2O$, the combination of yeast extract-$FeSO_4$enhanced the yield of ergot alkaloids in extracellular extract (13.74 mg/100 ml). The lowest yield of ergot alkaloids (0.40 mg/100 ml) was obtained from the extracellular extract of sucrose-$FeSO_4$.

Table 8 indicated that the experimental design of BBD model was highly reliable to optimize the significant selected variables. The *p*-values described the significance of the co-efficient and also it helped in the understanding of the pattern of combined interaction effects among selected the factors. The results obtained from the BBD model were analyzed by a second order polynomial formula to explain the reliance of total yield of ergot alkaloids on the fermentation medium components. The final equation achieved after putting all the values of ergot alkaloids yield was as follows:

$$Y=22.6674 -0.3443\ n^1 - 0.0425\ x^2 -78.5246\ n^3 + 0.008\ n1^2 +0.003\ n2^2 +427.75\ n3^2 - 0.0046\ n^1n^2 -0.3542\ n^1n^3 + 2.1806\ n^2n^3$$

Table 6. ANOVA chart of PBD using *Penicillium commune* for the production of ergot alkaloids.

Source	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	F-value	*p*-value
Intercept	0.46	1	0.46	8.26	0.21
Sucrose	147.78	1	147.78	2670.95	0.012
Yeast extract	22.54	1	22.54	4.7.44	0.032
Succinic acid	1.30	1	1.30	23.64	0.13
$MgSO_4.7H_2O$	1.16	1	1.16	20.96	0.14
KH_2PO_4	0.16	1	0.16	2.86	0.34
$FeSO_4.7H_2O$	12.87	1	12.87	232.57	0.042
$ZnSO_4.7H_2O$	0.50	1	0.50	9.06	0.20
Asparagine	0.96	1	0.96	17.41	0.15
Tryptophan	0.36	1	0.36	6.47	0.24
pH	3.06	1	3.06	55.33	0.085
Error	0.06	1	0.06	-	-

Table 7. Yield of ergot alkaloids using BBD (observed and predicted yields).

Runs	Sucrose (g/100ml)	Yeast extract (g/100ml)	$FeSO_4$ (g/100ml)	Alkaloids yield observed	Alkaloids yield predicted
1.	41	5	0.06	7.86	7.52
2.	41	39	0.06	9.46	8.62
3.	41	22	0.01	7.38	7.96
4.	41	22	0.11	7.98	8.59
5.	5	5	0.06	7.42	8.27
6.	5	39	0.06	14.64*	14.99*
7.	5	22	0.01	11.48	10.88
8.	5	22	0.11	13.36	12.78
9.	23	5	0.01	7.83	7.61
10.	23	39	0.01	7.53	7.82
11.	23	5	0.11	5.42	5.15
12.	23	39	0.11	12.53	12.76
13.	23	22	0.06	6.39	6.39

Table 8. ANOVA of ergot alkaloids production by *Penicillium commune* using BBD model.

Variable	Sum of square	Degree of freedom	Means square	F-value	*p*-value	t-value
Intercept	33.21	1	33.21	30.25	0.01	5.50
Sucrose	10.21	1	10.21	9.30	0.06	-3.05
$Sucrose^2$	15.34*	1	15.34*	13.97*	0.03*	3.74*
Yeast extract	0.14	1	0.14	0.12	0.75	-0.35
Yeast $extract^2$	1.72	1	1.72	1.57	0.31	1.25
$FeSO_4$	4.44	1	4.44	4.04	0.14	-2.01
$FeSO_4{}^2$	2.61	1	2.61	2.38	0.22	1.54
Sucrose, yeast extract	7.91	1	7.91	7.19	0.075	-2.68
Sucrose, $FeSO_4$	0.40	1	0.40	0.37	0.59	-0.60
Yeast extract, $FeSO_4$	13.74*	1	13.74*	12.51*	0.04*	3.54*
Error	3.29	3	1.09761			

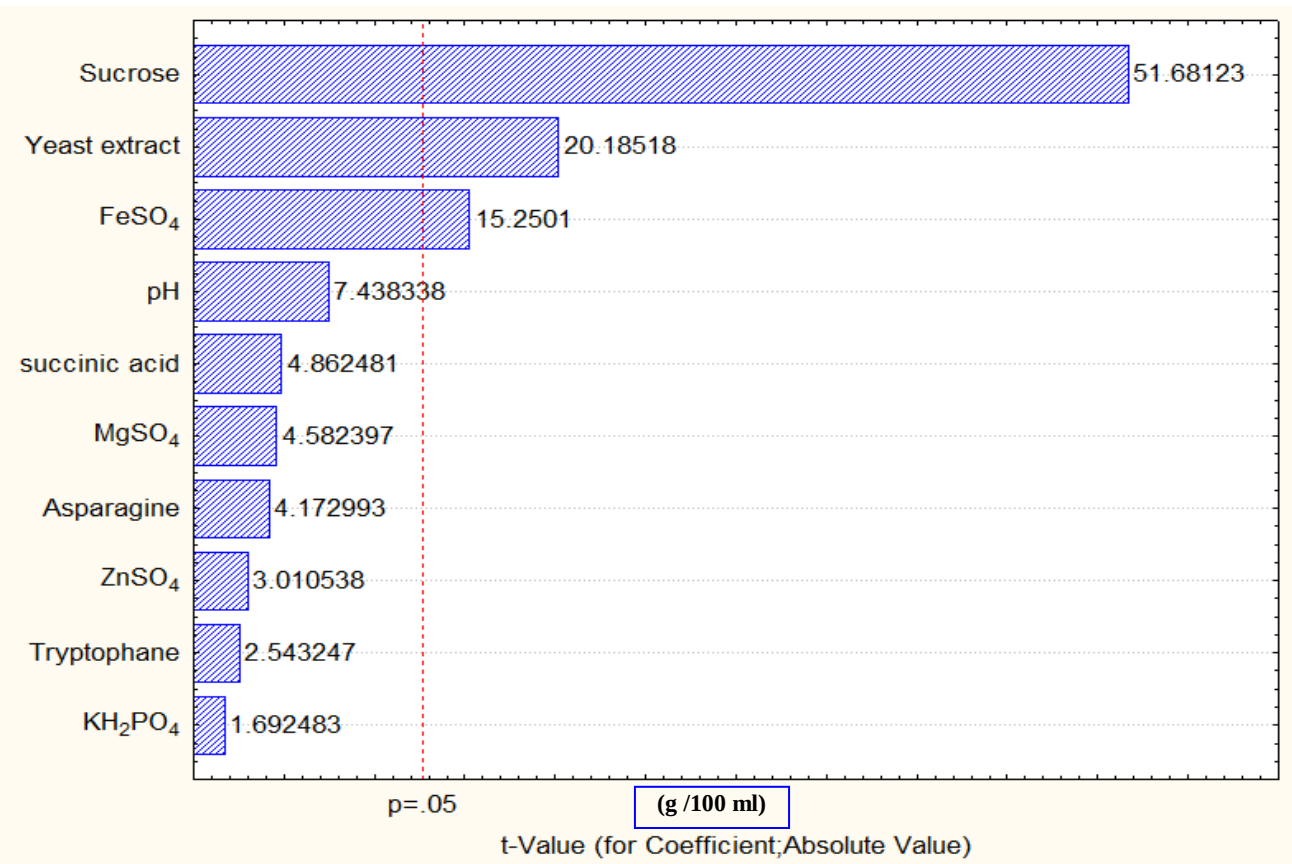

Fig. 1. Pareto chart indicating the key factors for the production of ergot alkaloid by *Penicillium commune*.

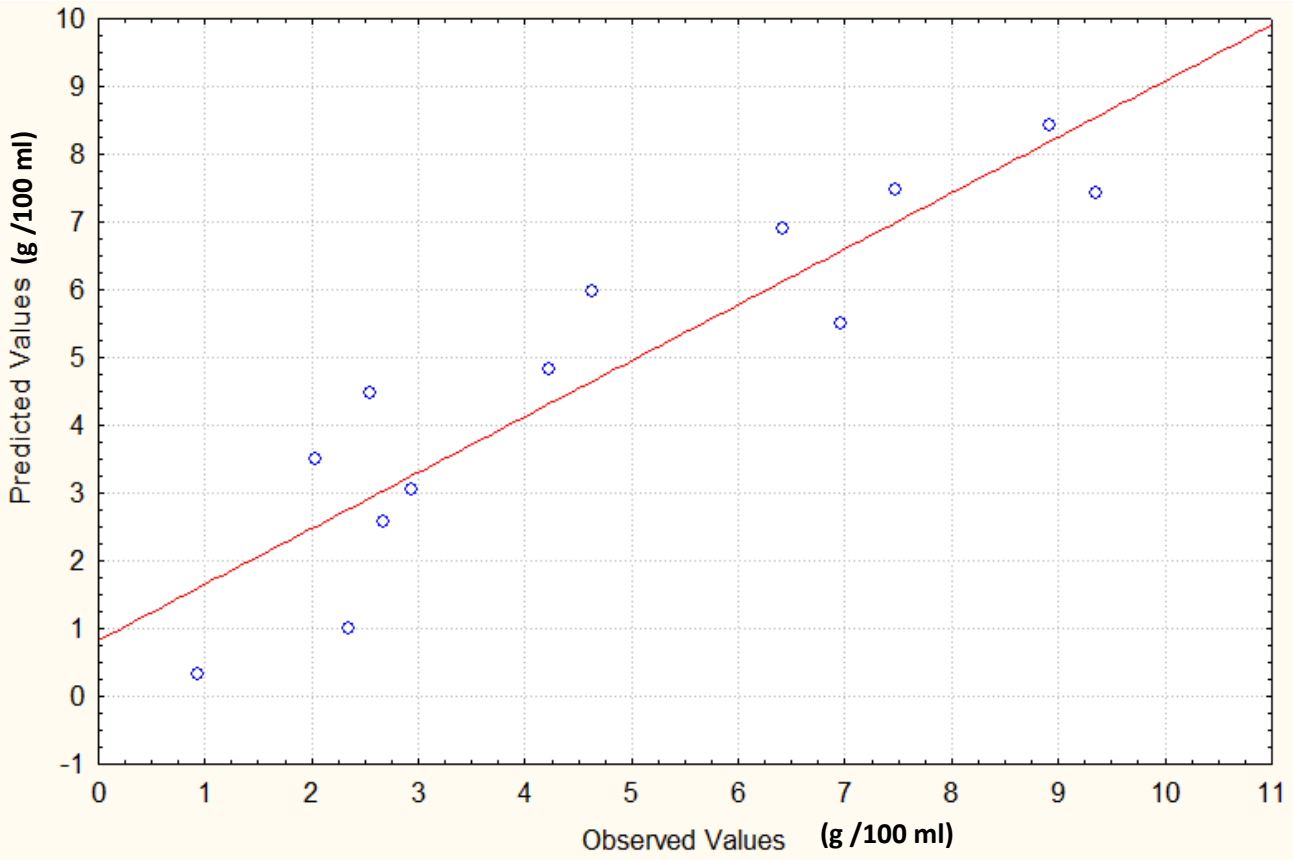

Fig. 2. BBD observed and predicted values of ergot alkaloid yield using *Penicillium commune.*

In the above equation, 'Y' is the expected/predicted yield of ergot alkaloids (total response),n^1, n^2 and n^3 are the values of sucrose, yeast extract and $FeSO_4.7\ H_2O$, respectively.

The above results of the regression analysis determined the productivity of ergot alkaloids using *Penicillium commune* by employing BBD experimental model. The combined interaction effects of sucrose-yeast extract, sucrose-$FeSO_4.7H_2O$and yeast extract-$FeSO_4.7H_2O$are given in Figs. 3, 4 and 5. Three-dimensional graphs were prepared for each combination of selected factors for cumulative ergot alkaloids production. These graphs showed the curves that reflect the maximum and enhanced yield of ergot alkaloids in extracellular extracts.

Figure 3 showing an upward curve which indicated the insignificant impact of sucrose and yeast extract on the yield of ergot alkaloids. Thus, this combination was not useful for the production of ergot alkaloids.

Figure 4 also indicated the less significant interaction of sucrose-$FeSO_4.7H_2O$ on the production of ergot alkaloids. During the fermentation process with this combination, a slight increase in the ergot alkaloids yield was observed. Fig. 5 represented a dome-shaped curve which described that combined interaction of yeast extract-$FeSO_4$ was found to be the best for the maximum production of ergot alkaloids. The presence of both of these factors at a time in fermentation medium triggered the synthesis of ergot alkaloids and a maximum yield was recorded (15.64 mg/100 ml) in the fermentation medium.

Discussion

Ergot alkaloids are organic molecules, known as secondary metabolites, containing at least one nitrogen atom in their ring structures. Ergot alkaloids were initially recognized in *Claviceps purpurea* as the agent causing ergot of rye which is one of the important members of Ascomycetes. The composition and toxicity of ergot alkaloids make them practically very significant so that the secan be used in formulating various drugs/medicines. Approximately, more than 50 formulations of ergot alkaloids are present in international market of pharmaceutics. These formulations can cure migraine, control postpartum bleedings, and induce labour pains as well as can also be used as therapeutically important medicines (Shahid *et al*., 2016). A vast range of optimization and statistical techniques, such as OFAT (one factor at a time) are in use to enhance the yield of ergot alkaloids in fermentation medium. Hence, a statistical design, called response surface methodology (RSM), is a practical statistical procedure that can be used for various regression analyses of quantitative data received from multi-factorial experimental runs (Rao *et al.*, 2000; Venil & Lakshmanaperumalsamy, 2009b). This technique can be used preferably for different experiments of screening and optimization of fermentation factors (Prapulla *et al.*, 1992; Mayers & Montogomery, 2002; Mao *et al.*, 2005). It is an intelligent technique for analyzing various fermentation

parameters in a single run. In this technique, a lesser number of experiments are required as compared to one factor at a time (OFAT) technique. The traditional method of optimization studies by adding various concentrations of one factor at one time in a single flask is very time-consuming, unbearable and inaccurate. Therefore, statistical designs, such as response surface methods are efficient and frequently in-use now-a-days for statistical optimization of fermentation parameters by designing a fewer experiments (Nelofar *et al.,* 2011). These procedures are also significant for measuring the effects of combined interactions of fermentation factors that can affect the response/yield.

In the present study, two statistical designs of RSM were used. In the first step, Plackett-Burman design (PBD) was employed for the screening of fermentation factors and in the second step, Box-Behnken design (BBD) was applied for the optimization of selected fermentation factors to obtain the maximum ergot alkaloids yield using *Penicillium commune*. The PBD model was run for identifying and screening of fermentation medium factors (Table 5). The difference obtained in yield of ergot alkaloids reflected the significance of medium factors (variables) consumed in the fermentation broth to obtain highest yield. Sucrose, yeast extract and $FeSO_4.7H_2O$ were screened as notable factors by PBD model and it was also observed that these factors greatly influenced ergot alkaloids production (Fig. 1). Wu *et al.* (2011) used the same PBD model and screened significant factors of fermentation medium, which were utilized for the synthesis of fumigaclavine C and helvolic acid produced by endophytic *Aspergillus fumigatus* strain CY018. They also investigated that pH, phosphate and inoculum sizes were the three important independent variables that enhanced the fumigaclavine C and helvolic acid yield in extracellular filtrates of *Aspergillus fumigatus* strain CY018. In the present study, it was found that ergot alkaloids yield was significantly influenced by high concentration level (+1) of sucrose and yeast extract among the employed levels (-1 and +1) as shown in the Tables 7 and 8. With the boost in the concentrations of sucrose and yeast extract, a notable increase in yield of alkaloids concentration was achieved from extracellular extract of *Penicillium commune*. Guo *et al.* (2010) obtained the similar results by PBD model. In their experiment, they screened carbon and nitrogen sources as essential factors to achieve the maximum nisin yield in the fermentation medium of *Lactococcuslactis* subsp. *lactis*. Desai *et al.* (2006) and Singh *et al.* (2008) have worked on the same PBD statistical model to screen significant factors of fermentation medium to obtain the highest yield of nisin. Our results are in harmony with Wu *et al.* (2011) who worked on the synthesis of secondary metabolites and screened three key cultivation factors of fermentation medium such as pH, phosphate concentration and inoculum sizes using PBD (Plackett-Burman design) and CCD (Central-Composite design) models for fumigaclavin and havolic acid alkaloids production. Venil & Lakshmanaperumalsamy (2009a) also used PBD model to synthesize prodigiosin using *Serratiamarcescens* SWML08. Kim *et al.* (2008) also used the same PBD model for the production of prodigiosin by *Hahellache juensis* KCTC 2396 and Guo *et al.* (2010) for nisin production. Therefore, RSM had been used in the past by many researchers, frequently for the screening of medium components, fermentation process constituents and manufacturing of processed foods (Vazquez & Martin, 1997; Ramirez *et al.,* 2001; Park *et al.,* 2005).

In the second step, another statistical model BBD was used and sucrose, yeast extract and $FeSO_4$ were selected for the further optimization studies. Effect of combinations of sucrose, yeast extract and $FeSO_4$ was also studied such as sucrose-yeast extract, sucrose-$FeSO_4$ and yeast extract- $FeSO_4$. It was observed that yeast extract-$FeSO_4$ combination was found to be more efficient to enhance ergot alkaloids productivity in extracellular extracts of *Penicillium commune* (Table 8). Same BBD model was used by Venil & Lakshmanaperumalsamy (2009a) in their experiment and observed interaction effects of 3factorsof fermentation medium such as incubation temperature, $(NH_4)_2PO_4$ and trace salts on prodigiosin yield. Wang & Liu (2009) also worked on BBD to investigate the impact of various combinations of glucose, peptones and KH_2PO_4 on cell biomass production during fermentation process. In the present investigation, this design was also proved to be an effective statistical approach for the optimization of fermentation parameters.

In the present investigation, desirability charts were also prepared using BBD statistical model. It was observed that highest concentration levels of sucrose, yeast extract and $FeSO_4.7H_2O$ predicted via this statistical model was similar to the observed values. Therefore, it was concluded that *Penicillium commune* was a potential candidate for the ergot alkaloid production. Similar BBD model was employed by Krishnaa *et al.* (2013) to optimize and evaluate the effect of pH on biomass production of *Borasus flabellifer*. They observed the influence of pH on the adsorption of chromium in extracellular filtrates of 15 runs of *Borasus flabellifer.* They also proved that BBD was the best model to investigate the maximum number of runs in a single batch. The results of BBD model in this study are in accordance of BBD model used by Amara (2013) who optimized the culture conditions for the production of polyhydroxybutyrate and protease enzymes in fermentation medium using various *Bacillus* strains and achieved a maximum yield of protease.

The combined PBD and BBD statistical models for identification, screening and optimization of significant variables were found to be the most effective tools to obtain the maximum production of ergot alkaloids by *Penicillium commune* strain. This study also validates the efficiency of PBD and BBD models for the rapid analysis of fermentation factors that can maximize the yield of a product. The present investigation also identified the short durational and cost-effective technique that can be effectively applied to any fermentation process.

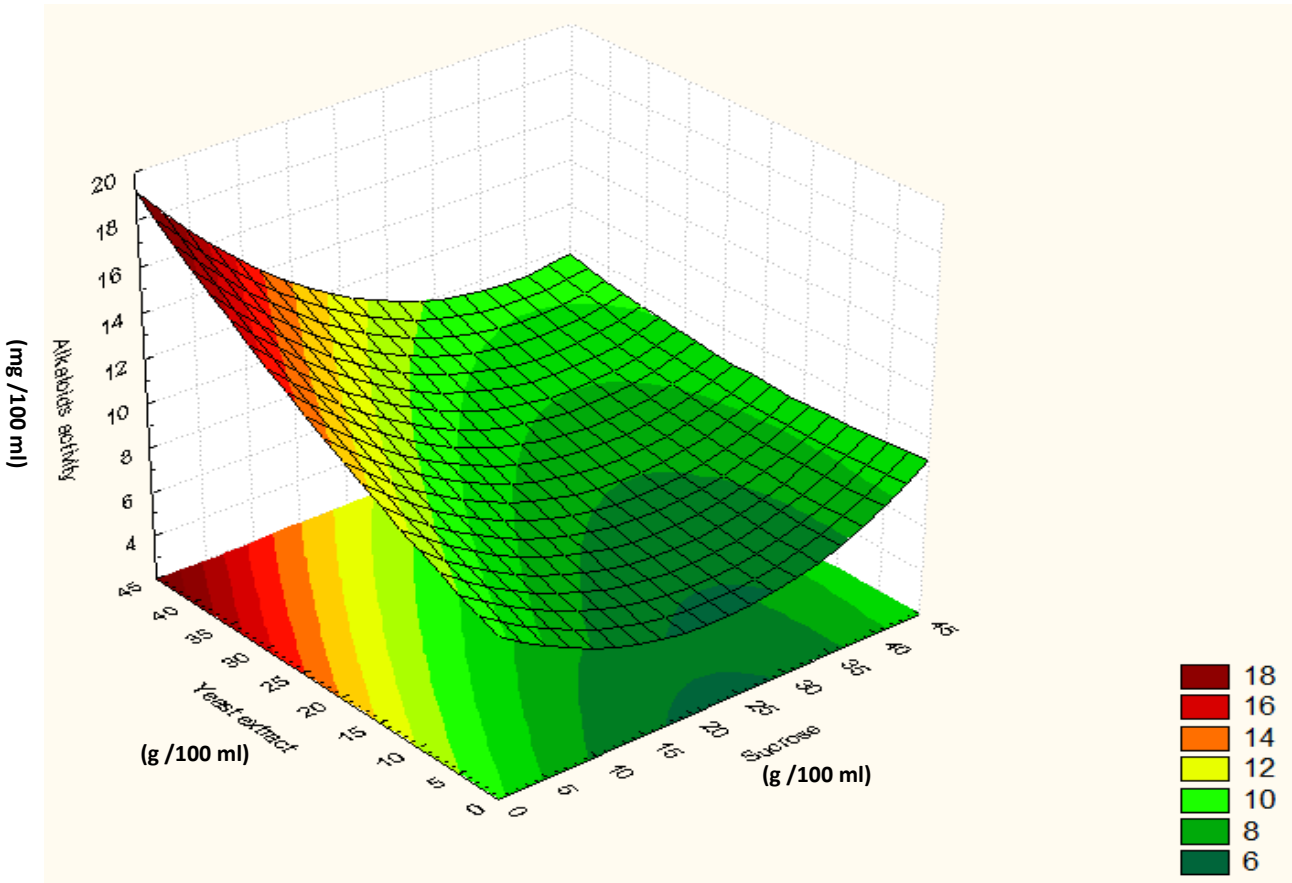

Fig. 3. Effect of combined interaction of sucrose-yeast extract on ergot alkaloid production by *Penicillium commune*.

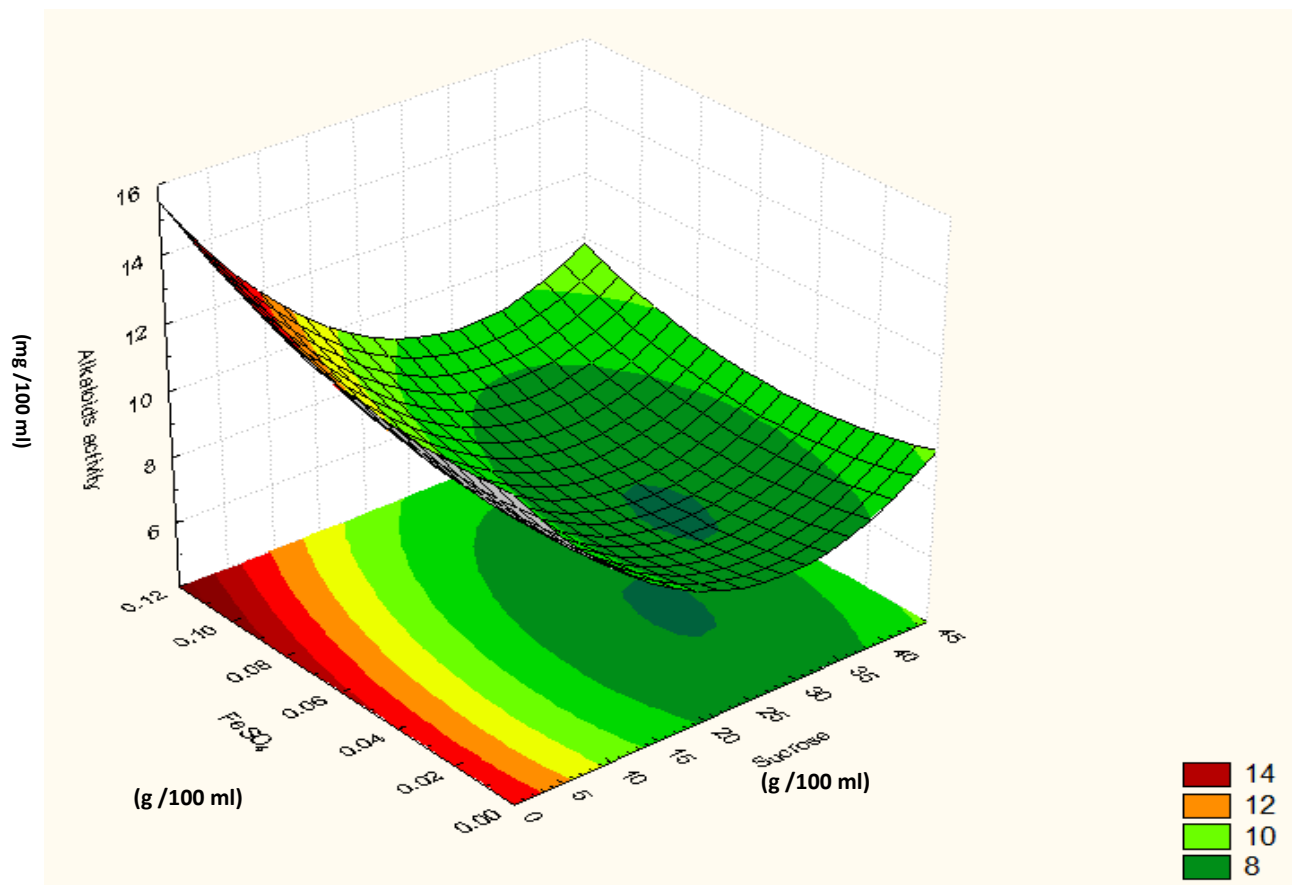

Fig. 4. Effect of combined interaction of sucrose-$FeSO_4.7H_2O$on ergot alkaloids production by *Penicillium commune*.

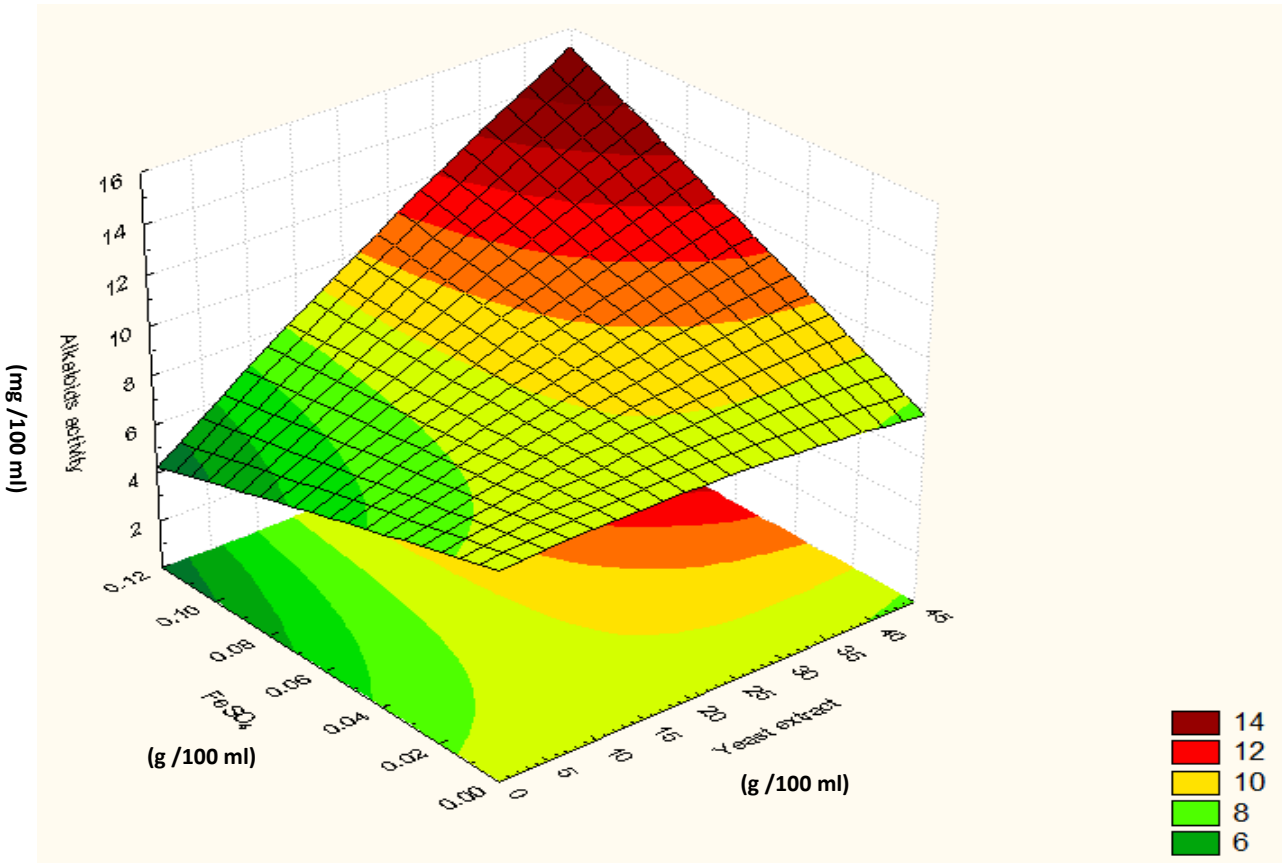

Fig. 5. Effect of combined interaction of yeast extract-$FeSO_4$ on ergot alkaloids production by *Penicillium commune*.

Conclusion

Ergot alkaloids are not only produced in plants but also in fungi. However, the amount of ergot alkaloids produced by fungal organism is comparatively lesser. But the enhanced yield of ergot alkaloids may be achieved by focusing on various fermentation factors. Ergot alkaloids have been in-use since many decades due to their pharmaceutical properties. In the present study, it was concluded that *Penicillium commune* was the potential candidate and static surface culture fermentation technique is vibrant technique for the production of ergot alkaloids. Optimization of fermentation conditions using RSM techniques revealed that statistical procedures, such as PBD and BBD are more reliable to enhance the yield of ergot alkaloids in a single step.

References

Amara, A.A.F. 2013. Optimizing PHB and protease production by Box-Behnken design. *J. IILUM Engin.*, 14(1): 15-28.

Box, G.E.P. and D.W. Behnken. 1960. Some new three level designs for the study of quantitative variables. *Technometrics*, 2: 455-475.

Burfening, P.J. 1973. Ergotism. *J. Am. Vet. Med. Assoc.*, 163: 1288-1290.

Deepak, V., K. Kalishwara lal, S. Ramkumarpandian, S.B. Venkatesh, S.R. Senthilkumar and G. Sangiliyandi. 2008. Optimization of media composition for nattokinase production by *Bacillus subtilis* using response surface methodology. *Biores. Techn.*, 99(17): 8170-8174.

Desai, K.M., S.K. Akolkar, Y.P. Badhe, S.S. Tambe and S.S. Lele. 2006. Optimization of fermentation media for exopolysaccharide production from *Lactobacillus plantarum* using artificial intelligence-based techniques. *Proc. Biotechnol.*, 41: 1842-1848.

Flieger, M., W. Wurst and R. Shelby. 1997. Ergot alkaloids sources, structures and analytical methods. *Fol. Microbiol.*, 42: 3-30.

Guo, W.L., Z. Yi-bo, J. Lu, J. Jiang, T. Li-rong, Y. Wang and Y. Liang. 2010. Optimization of fermentation medium for nisin production from *Lactococcus lactis* subsp. *lactis* using response surface methodology (RSM) combined with artificial neural network-genetic algorithm (ANN-GA). *Afric. J. Biotechnol.*, 9(38): 6264-6272.

Katzung, B.G. and D.F. Julius. 2001.*Histamine, serotonin, and the ergot alkaloids.* In: Katzung BG, editor.*Basic and Clinical Pharmacology*. (Edn. 8th). New York, NY: McGraw-Hill, pp: 265-88.

Katzung, B.G. 2009. *Histamine, Serotonin and the Ergot Alkaloids', in Basic and Clinical Pharmacology,* (Eds.): Katzung, B.G., S.B. Masters and A.J. Trevor, McGraw-Hill Medical, pp: 271-292.

Khurana, S., M. Kapoor, S. Gupta and R.C. Kuhad. 2007. Statistical optimization of alkaline xylanase production from *Streptomyces violaceoruber* under submerged fermentation using response surface methodology. *Ind. J. Microbiol.*, 47(2): 144-152.

Kim, S.J., K.L. Hong and H.Y. Joung. 2008. Statistical optimization of medium components for the production of prodigiosin by *Hahellache juensis* KCTC 2396. *J. Microbiol. Biotechnol.*, 18(12): 1903-1907.

King, D.S. and K.C. Herndon. 2005. *Headache disorders*. In: J.T. Dipiro, R.L. Talbert, G.C. Yee, G.R. Matzke, B.G. Wells, L.M. Posey, editors. *Pharmacotherapy*. (Edn. 6th). New York, NY: McGraw-Hill, pp: 1105-21.

Krishnaa, D., K.S. Krishnaa and R. Padma-Sree. 2013. Response surface modeling and optimization of chromium (Vi) removal from aqueous solution using *Borasus flabellifer* coir powder. *Int. J. Appl. Sci. Eng.,* 11(2): 213-226.

Lüllmann, H., K. Mohr, A. Ziegler and A. Bieger. 2000.Color *Atlas of Pharmacology*. (Edn. 2nd). Stuttgart, Germany: Thieme, pp: 114-265.

Mao, X.B., T. Eksriwong, S. Chauvatcharin and J.J. Zhong. 2005. Optimization of carbon source and carbon/nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. *Proc. Biochem.,* 40: 1667-1672.

Mavungu, D.D.J., D.A. Larionova, S.V. Malysheva, C.V. Peteghem and S.D. Saeger. 2011. Survey on ergot alkaloids in cereals intended for human consumption and animal feeding. *A Report submitted to EFSA*. pp: 1-112.

Myers, R.H. and D.C. Montgomery. 2002. *Response Surface Methodology*. Wiley, New York.

Nelofer, R., R.N. Ramanan, R.N. Zaliha, R.A. Rahman, M. Basri and A.B. Ariff. 2011. Sequential optimization of production of a thermostable and organic solvent tolerant lipase by recombinant *Escherichia coli*. *Ann. Microbiol.,* 61: 535-544.

Nielsen, C.A.F., F**.** Christope, A. Hatsch, A. Molt, H. Schroder, S.E. Connor and M. Naesby. 2014. The important ergot alkaloid intermediate chanoclavine-I produced in the yeast *Saccharomyces cerevisiae* by the combined action of EasC and EasE from *Aspergillus japonicus. Microb. Cell Fact.,* 13: 95-100.

Park, P.K., D.H. Cho, E.Y. Kim and K.H. Chu. 2005. Optimization of carotenoid production by *Rhodotorula glutinis*using statistical experimental design. *Wor. J. Microbiol. Biotechnol.,* 21: 429-434.

Plackett, R.L. and J.P. Burman. 1946. The design of optimum multifactorial experiments. *Biomet.,* 33: 305-325.

Polak, B. and A. Rompała. 2007. Effect of acidic mobile phase additives on the TLC behaviour of some alkaloids. *Acta Chromatographica,* pp: 24-35.

Prapulla, S.G., S. Jacob, N. Chand, D. Rajalakshmi and N.G. Karanth. 1992. Maximization of lipid production by *Rhodotroula gracilis* CFR-1 using response surface methodology. *Biotechnol. Bioeng.,* 40: 965-969.

Ramirez, J., H. Gutierrez and A. Gschaedler. 2001. Optimization of astaxanthin production by *Phaffia rhodozyma* through factorial design and response surface methodology. *J. Biotechnol.,* 88: 259-268.

Rao, J.M., C. Kim and S. Rhee. 2000. Statistical optimization of medium for the production of recombinant hirudin from *Sacchromyces cerevisiae* using response surface methodology. *Proc. Biochem.,* 35: 639-647.

Shahid, M.G., M. Nadeem, S. Baig, T.A. Cheema, S. Atta and G.Z. Ghafoor. 2016. Screening and optimization of some inorganic salts for the production of ergot alkaloids from *Penicillium* species using surface culture fermentation process. *Pak. J. Pharm. Sci.,* 29(2): 407-414.

Singh, A., A. Majumder and A. Goyal. 2008. Artificial intelligence based optimization of exocellular glucansucrase production from *Leuconostocdextranicum* NRRL B-1146. *Biores. Technol.,* 99: 8201-8206.

Sinz, A. 2008. Pharm. *UnsererZeit*, 37: 306-309.

Vazquez, M. and A.M. Martin. 1997. Optimization of *Phaffia rhodozyma* continuous culture through response surface methodology. *Biotechnol.,* 57: 314-320.

Venil, C.K. and P. Lakshmanaperumalsamy. 2009a. Application of statistical design to the optimization of culture medium for prodigiosin production by *Serratia marcescens* SWML08. *Mal. J. Microbiol.,* 5(1): 55-61.

Venil, C.K. and P. Lakshmanaperumalsamy. 2009b. Applications of response surface methodology in medium optimization for protease production by the new strain of *Serratiamarcescens* SWML08. *Pol. J. Microbiol.,* 58(2): 117-124.

Wang, X.L. and G.Q. Liu. 2009. Preliminary select and optimization of submerged fermentation media of *Ganoderma sinense*. *Food Sci. Technol.,* 34: 14-16.

Wenster-Botz, D. 2000. Experimental design for fermentation media development: Statistical design or global random search. *J. Biosci. Bioengin.,* 90: 473-483.

Wu, Q., S. Yong-Chun, H. Xu, Y. Guo, J. Li and T. Ren-Xiang. 2011. Medium optimization for enhanced co-production of two bioactive metabolites in the same fermentation by a statistical approach. *J. Asi. Nat. Prod. Res.,* 13(12): 1110-1121.

Zafar, A. M., S.A. Waseemuddin, I. Azhar, M. Sualeh, M.T. Baig and S.M.S. Zoha. 2010. Bioactive alkaloids produced by fungi: updates on alkaloids from the species of the genera *Boletus, Fusarium* and *Psilocybe* (Review).*Pak. J. Pharm. Sci.,* 23(3): 349-357.

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