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OPTIMIZING THE PRODUCTION OF PECTINASE OF ORANGE PEEL WASTE BY *PENICILLIUM CHRYSOGENUM* MF318506 USING RESPONSE SURFACE METHODOLOGY IN SUBMERGED FERMENTATION

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ABSTRACT

Pectinases find wide application in different industrial applications, e.g., textile, food, tea, paper and pulp, vegetable oil extractions, saccharification of agricultural remains, and in fermented drinks. Three fungal strains were screened for pectinase production. Results indicated that all strains are capable of producing a pectinase enzyme. *Penicillium chrysogenum* MF318506 showed the highest pectinase activity (0.214 U/ml) on the recommended medium at 30 ◦C at 150 rpm, on 6 days. Different agricultural wastes (orange peel, banana peel, potato peel, pomegranate peel, wheat bran, and rice bran) were screened as a carbon source. The maximum pectinase activity of 0.48U/ml was obtained from Orange peel and was selected for further studies. Screening of important medium components for pectinase production by the Plackett-Burman design (PBD) showed that the maximum pectinase activity of 1.057 U/ml was achieved with 3 % orange peel, 2.0 g/l $(NH_4)_2SO_4$, 6.0 g/l peptone, 6.0 g/l KH_2PO_4, 6.0 g/l K_2HPO_4, 0.1 g/l $MgSO_4.7H_2O$, 0.5 g/l KCl and 0.1 g/l $FeSO_4$. Finally, the Box-Behken design (BBD) which uses the response surface methodology (RSM) was applied to further optimization of the selected factors using the PBD. The maximum pectinase activity of 1.292U/ml was observed at 2.5% orange peel and 4.5 g/l peptone. The Statistical optimization, enhanced the pectinase production by 6.04 folds using Orange peel waste as a substrate. The pectinase produced by *Penicillium chrysogenum* MF318506 has potential applications in different industrial fields.

Keywords: Statistical Design optimization, pectinase production, *Penicillium chrysogenum*, orange peel waste

INTRODUCTION

Pectinases are a cluster of enzymes that catalyze the breakdown of pectin-holding substrates. Pectinases are classified into polygalacturonase (EC 3.2.1.15), pectinesterase (EC 3.1.1.11), pectin lyase (EC 4.2.2.10), and pectate lyase (EC 4.2.2.2) constructed on their activity. Pectinase (EC 3.2.1.15) is an enzyme that breaks down pectin in plant materials and which hydrolyzes polygalacturonic acid hooked on monoglacturonic acid by opening the glycosidic bonds and contravention ester bonds amongst carboxyl and methyl groups **(Saranraj and Naidu 2014**). Since pectinase is one of the greatest vital industrialized enzymes, Pectinases find a widespread request in the arena of cloth trades, nutrition productions, tea trades, paper, and soft tissue commerce, plant oil, saccharification of agricultural remains, and in fermented drinks (Chen et **al. 2012); Praveen and Suneetha 2014).**

Plants and microorganisms are the main enzyme production sources, but the microbial sources are considered more promising from both methodological and commercial point of view **(Anisha and Girish 2014). Esmail et al. (2013)** mentioned that *Bacillus sp*., *Erwinia sp*., and *Pseudomonas sp*. are the main creators of pectinases. As well as, pectinase produced from fungi like *Aspergillus*, *Rhizopus*, and *Penicillium* **(Mrudula et al. 2011).** More recently, pectin lyase was obtained by *Penicillium (P) expansum* RSW_SEP1 using agricultural wastes **(Atalla et al. 2019).** A large number of bacterial strains, mainly *Bacillus sp.* **(Kuvvet et al. 2017)** and many filamentous fungi such as *A. niger*, *A. oryzae*, *A. awamori*, *A. sojae*, *T. viridiae*, *T. virens*, *P. griseoroseum*, and *Phanerochaete chrysosporium* are potential pectinase producers (**Ruiz et al. (2012); Tari et al. (2007); Teixeira et al. (2011); Benoit et al. (2012); Heerd et al. (2012)**. Owing to the extensive uses of this enzyme, it is essential to use low-cost and willingly obtainable raw material for its construction. Employment of inexpensive carbon sources such as fruit handling waste, several lignocellulosic resources, wheat bran, soybean, sugar cane molasses, and agro-industrialized wastelands, especially from citrus fruits brans and straws, etc. can help increase enzyme productivity at a low cost **(Ruiz et al. (2012); Martin et al. (2004); Martin et al. (2010); Demir and Tari (2014).**

The enzymes acquired from the microbes are usually extracellular and their yield as well as the cost is extremely exaggerated by the medium components and cultivation conditions (Awad et al. **2014; Hamed et al. 2015; Atalla, et al. 2020).** So, the improvement of an economically sustainable production medium needs the choice of procedure factors and their optimization approaches. The conventional method for optimal conditions of enzyme production is time waste and a daunting assignment of changing one factor at a time whereas keeping others at constant levels. An alternate and more actual method is the use of statistical analysis.

The scheming a real production medium for maximal enzyme yield is a vital procedure as the medium structure can significantly affect the enzyme activity **(Ahmed et al. (2015); Djekrif- Dakhmouche et al. (2006)**. Plackett-Burman (PB) design has been successfully used for its efficiency in screening the essential elements in limited investigations rounds. The Plackett-Burman Experimental Design (PBED) is constructed with the idea that each element requirements its individual improper level. Moreover, it needs 4^n experimentations to examine a maximal of 4^{n-1} elements at two levels **(Hibbert et al. 2012).** The Box-Behnken (BB) response surface design products second-order polynomial calculations to estimate responses in assuring areas. Together with designs usage statistical analysis which can develop pectinase creation rapidly, with a significant decrease in medium charges **(Tari et al. 2007).** Numerous investigations have been described on numerical optimization of pectinase construction by *A. niger* **(El Enshasy et al. (2018); Janveja and Soni (2016); Mahesh et al. (2014).**

The study aimed to use the statistical designs PBD and BBD of response surface methodology (RSM) to optimize medium components for enhancing pectinase production by *P. chrysogenum* MF318506 using orange peel wastes as substrate. First, we used PBD to screen the important factors that affect pectinase production. Second, the BBD was applied to further optimization of the selected important factors using the PBD.

MATERIALS AND METHODS

Microorganism

Three fungal strains *A. terreus* MN901491, *A. oryzae* MN894021 were isolated from red seawater at Sharm El-Shiesh province and identified by the 18S rRNA gene **(Nehad et al., 2020)** and *P. chrysogenum* MF318506 **(Abd El Aty et al. 2020**), were screened for pectinase production in this study. The fungal cultures were maintained on potato dextrose agar (PDA), incubated at 30°C for 7 days, and stored at 4°C.

Preparation of substrates

The different agricultural wastes as orange peel, banana peel, potato peel, pomegranate peel, wheat bran, and rice bran were used as a substrate for pectinase production. The proximate analysis of these substrates was mentioned by **Nehad et al. (2020).** These substrates were washed, dried at 70°C in an oven, and powdered using a blender before use. The pest substrate was further selected to give the maximum pectinase production.

Fermentation medium and pectinase production

The medium used for pectinase production was composed of (g/l): pectin 10; $(NH_4)_2SO_4$ 6; KH_2PO_4, 6; K_2HPO_4 6; $MgSO_4.7H_2O$ 1; at pH 7.0 (**Okafor et al.** (2010). Two disks (6mm) in diameter from the fungal strain were cultured in 50 ml of fermentation medium in 250 ml Erlenmeyer flasks and incubated at 30 ◦C in a rotary shaker at 150 rpm, after 6 days of fermentation, the medium was centrifuged at 4,000 rpm for 15 min, 4°C. The filtrate obtained was used in the enzyme assay.

Enzyme assay

Pectinase activity was carried out according to **Okafor et al. (2010)** who, using 1.0% (w/v) citrus pectin as the substrate. The reaction mixture containing 1 ml of enzyme and 1 ml pectin (1%), prepared in sodium acetate buffer (0.1 M, pH 5.5) was incubated in a water bath at 50°C for 30 min. After incubation, 1 ml of DNS reagent was added and kept for 5 min in a boiling water bath. The absorbance was recorded at 540 nm using a spectrophotometer. The galacturonic acid was used as a standard. One unit (U) of enzyme activity was defined as the "amount of enzyme that required to release one µmole of galacturonic acid per minute under the standard assay conditions" **(Minjares-carranco et al. 1997).** Enzyme activity was calculated as:

Enzyme activity (U/ml) =
Galacturonic acid released (µM) * Dilution factor / Incubation time (min)

Effect of different agricultural wastes on pectinase production

One gram of the different powdered agricultural wastes (orange peel, banana peel, potato peel, pomegranate peel, wheat bran, and rice bran) has been added as a carbon source in the fermentation medium, inoculated with *P. chrysogenum* MF318506, and incubated as the previous conditions (fermentation medium and production part). Then, pectinase activity was determined. One substrate was further selected to achieve the maximum pectinase activity.

Optimization of medium components for pectinase production by experimental designs

To determine variables that significantly affected on pectinase production by *P. chrysogenum* MF318506, the statistical software package MINITAB, Inc (version 18.1.0.0) was used, an analysis of variance (ANOVA) for the obtained results was calculated. The optimization of the important medium components was carried out in two stages.

Stage one: Plackett-Burman design (PBD)

The PBD is a two-level fractional factorial design. PBD was used to screen the important variables that significantly affect pectinase production. The total number of trials to be carried out according to Plackett-Burman is $k + 1$ where k is the number of variables (medium components). PBD based on the first-order model: $Y = \beta o + \Sigma \beta i Xi$, Where Y is the response (growth of microorganism), β0 is the model intercept, βi is the linear coefficient, and Xi is the level of the independent variable. Eight independent variables (Orange peel, $(NH4)_2SO_4$, Peptone, KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, KCl, $FeSO_4$) have been investigated using Plackett-Burman experimental design at two levels, low level (-1) and high level (+1), are shown in (Table 1). Eight independent variables were screened in 28 experimental runs. All experiments were carried out in duplicate and the average pectinase activity was recorded as the response. F value and P values and the proportion of variance R2 determined the model is significant at $P \leq 0.05$ levels.

Table 1 Variables and their levels of the Plackett-Burman experimental design

Code	Variable	Level	
		Low (-1)	High (+1)
A	**Orange peel (%)**	**1.0**	**3.0**
B	**$(NH_4)_2SO_4$ (g/l)**	**2.0**	**6.0**
C	**Peptone (g/l)**	**2.0**	**6.0**
D	**KH_2PO_4 (g/l)**	**3.0**	**6.0**
E	**K_2HPO_4 (g/l)**	**3.0**	**6.0**
F	**$MgSO_4.7H_2O$ (g/l)**	**0.1**	**0.5**
G	**KCl (g/l)**	**0.1**	**0.5**
H	**$FeSO_4$ (g/l)**	**0.1**	**0.2**

Stage two: Box-Behken design (BBD)

BBD, which uses the response surface methodology (RSM) was applied to further optimization of significant variables selected using the PBD. The variables affecting the pectinase production of the Plackett-Burman design were orange peel, peptone, and KH_2PO_4. These three factors were further studied for the optimal range in the Box-Behnken design using software package MINITAB, Inc (version 18.1.0.0). Each factor in this design was studied at three levels, the lower, center, and high levels (Table 2).

Table 2 Coded values of independent variables used in the Box-Behnken design

Code	Variables	Level		
		-1	0.0	1
A	Orange peel	2	2.5	3
B	Peptone	2	4.0	6
C	KH_2PO_4	3	4.5	6

A design of 15 experiments for 3 variables with 3 levels was performed. All experiments were carried out in duplicate and the average pectinase activity was recorded as the response.

RESULTS

Screening of microorganisms for pectinase production

Three fungal strains were screened for pectinase production. The results indicated that all strains are capable to produce pectinase. *P. chrysogenum* MF318506 showed the highest pectinase activity (0.214 U/ml) followed by *A. oryzae* MN894021 (0.179 U/ml) and *A. terreus* MN901491 (0.144 U/ml).

Production of pectinase using different agriculture wastes

Six different agricultural wastes (Orange peel, Banana peel, Potato peel, Pomegranate peel, Wheat bran, and Rice bran) were screened as a substrate for pectinase production from *P. chrysogenum* MF318506. The results in Fig. 1 revealed that orange peel gave the highest pectinase activity (0.48U/ml) followed by pomegranate peel (0.35 U/ml). While, the Rice bran, wheat bran, and potato peel gave (0.27, 0.25, 0.18 U/ml) respectively.

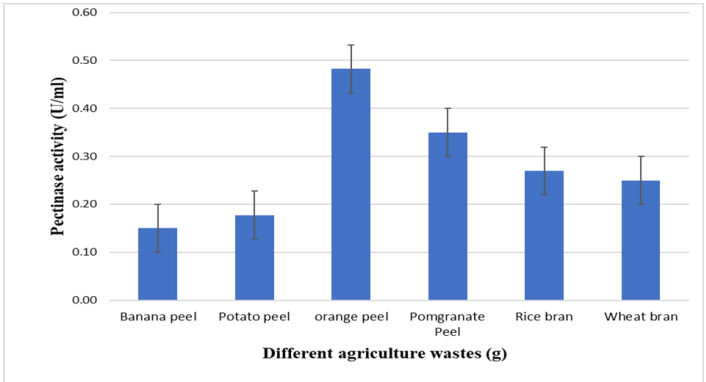

Figure 1 Production of pectinase from *Penicillium chrysogenum* using different agriculture wastes

Screening of essential medium constituents for pectinase production by the Plackett-Burman design

To screen the important medium components for enhancing pectinase production from *P. chrysogenum* MF318506 using orange peel as substrate Plackett-Burman

design was used. Eight media components (Orange peel, $(NH_4)_2SO_4$, Peptone, KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, KCl, $FeSO_4$) were investigated. Table 3 shows that the Plackett-Burman design with both the experimental and predicted response for the 28 experimental runs. The data indicated that there was a wide variation from 0.305 to 1.057 U/ml of pectinase activity in the 28 runs. This variation proposed that the optimization of the medium components had a noteworthy consequence of the pectinase activity.

Table 3 Plackett-Burman experimental design for the screening of important factors for pectinase production by *P. chrysogenum*

Run	A	B	C	D	E	F	G	H	Pectinase activity (U/ml)	
									Experimental	Predicted
1	3	6	2	3	6	0.5	0.5	0.2	0.521	0.492
2	3	6	6	3	3	0.1	0.5	0.2	0.697	0.706
3	1	6	2	3	3	0.5	0.1	0.1	0.373	0.309
4	3	6	6	3	3	0.1	0.1	0.2	0.675	0.714
5	3	6	6	3	3	0.1	0.5	0.1	0.719	0.792
6	3	6	2	6	6	0.1	0.1	0.2	0.641	0.615
7	1	2	2	6	3	0.5	0.5	0.2	0.316	0.382
8	1	2	6	6	3	0.1	0.5	0.1	0.807	0.679
9	1	2	6	3	3	0.5	0.1	0.2	0.486	0.485
10	3	2	2	3	3	0.5	0.5	0.1	0.613	0.652
11	1	6	2	6	3	0.1	0.1	0.2	0.429	0.339
12	3	2	2	3	6	0.1	0.1	0.2	0.576	0.592
13	1	6	2	3	6	0.1	0.5	0.1	0.305	0.321
14	3	2	6	6	3	0.5	0.5	0.2	0.835	0.850
15	1	2	2	3	6	0.5	0.5	0.2	0.450	0.285
16	3	6	2	6	6	0.5	0.1	0.1	0.662	0.679
17	3	2	2	6	3	0.1	0.1	0.1	0.753	0.775
18	1	2	6	3	6	0.1	0.1	0.1	0.529	0.590
19	1	6	6	3	6	0.5	0.5	0.1	0.474	0.490
20	3	2	6	6	6	0.5	0.1	0.1	0.884	0.941
21	1	6	6	6	6	0.1	0.5	0.2	0.584	0.519
22	3	2	6	6	6	0.1	0.5	0.1	1.057	0.955
23	1	2	2	3	3	0.1	0.1	0.1	0.460	0.402
24	1	6	6	6	3	0.5	0.1	0.2	0.455	0.508
25	1	2	2	6	6	0.1	0.5	0.2	0.369	0.400
26	3	2	6	3	6	0.5	0.1	0.2	0.816	0.761
27	1	6	6	6	6	0.5	0.1	0.1	0.566	0.592
28	3	6	2	6	3	0.5	0.5	0.1	0.752	0.675

The outcomes showed that the maximal pectinase yield of 1.057 U/ml was detected in run 22 and achieved under optimal experimental conditions with 3 % orange peel, 2.0 g/l $(NH_4)_2SO_4$, 6.0 g/l peptone, 6.0 g/l KH_2PO_4, 6.0 g/l K_2HPO_4, 0.1 g/l $MgSO_4.7H_2O$, 0.5 g/l KCl and 0.1 g/l $FeSO_4$, while the lowest activity of 0.305U/ml was achieved in run 13.

The main effects of the examined variables on the pectinase activity were calculated and presented in the Pareto graph. The Pareto graph is a significant tool for examining all the factors and emphasis on the most important variables. The Pareto chart results in Fig.2 indicated that orange peel, peptone, and KH_2PO_4 were recorded as a strong effect on pectinase production. The relationship between the medium components and the response obtained from the 28 experiments was predicted by the first-order model regression equation:

Y (Pectinase activity) =
0.206 + 0.1392 A - 0.01782 B + 0.04757 C+ 0.0313 D - 0.0009 E - 0.0532 F - 0.0189 G - 0.860 H

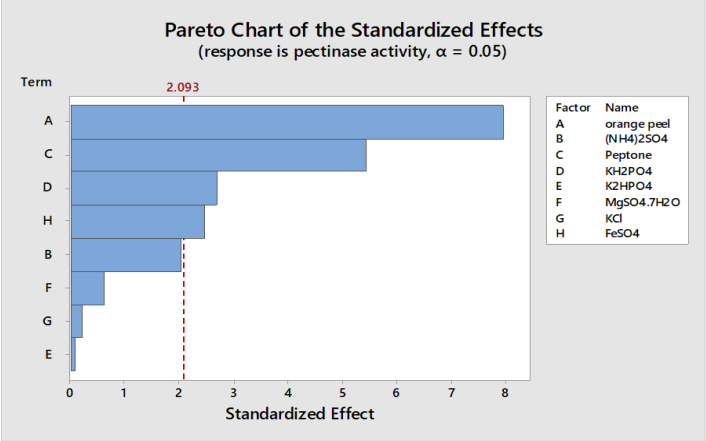

Figure 2 Pareto chart showing the effect of various variables on pectinase production

The results of pectinase activity were exposed to multiple linear regression analyses to estimate the t- and p values of each constituent. The analysis of the regression coefficients and the t values of 8 variables (Table 4) revealed that values for A, C, and D had positive effects on pectinase activity, whereas B, E, F, G, and H had negative effects. The significance of variables was identified as A, C, and D. The variables with confidence levels more than 95% was reflected as the important factors.

Table 4 Estimated effects and coefficients of the Plackett-Burman design

Term	Effect	Coeffi	SE Coeffi	T-Value	P-Value
Constant		0.5894	0.0175	33.71	0.000
A	0.2784	0.1392	0.0175	7.96	0.001
B	-0.0713	-0.0356	0.0175	-2.04	0.056
C	0.1903	0.0951	0.0175	5.44	0.024
D	0.0940	0.0470	0.0175	2.69	0.015
E	-0.0026	-0.0013	0.0175	-0.07	0.942
F	-0.0213	-0.0106	0.0175	-0.61	0.550
G	-0.0076	-0.0038	0.0175	-0.22	0.831
H	-0.0860	-0.0430	0.0175	-2.46	0.240

Analysis of Variance (ANOVA) of the PBD for the pectinase production was shown in Table 5. To test the fit of the model equation can be evaluated the 'determination coefficient R^2" Normally, a regression model having an R^2 value greater than 0.90 is reflected to have a very extraordinary relationship **(Haaland, 1989).** The R^2 value of the regression model was 0.8537. The model F value of 13.86 implies the Model is significant, the p values of variables A (Orange peel), C (Peptone), and D (KH_2PO_4) were 0.001, 0.024, and 0.015 respectively, which were considered as significantly influential for pectinase production. So, we selected the three variables: orange peel, peptone, and KH_2PO_4 as an important element for additional study to achieve the maximal enzyme activity. This showed that the PBD was a potent statistical method for selecting the significant parameters **(Plackett and Burman, 1946).**

Table 5 Analysis of Variance (ANOVA) of media components by Plackett-Burman design for pectinase activity +

Source	Sum of squares	df	Mean square	F-Value	P-Value	
Model	0.94893	8	0.118617	13.86	0.003	
orange peel	0.54266	1	0.542657	63.39	0.001	Significant
$(NH_4)_2SO_4$	0.03557	1	0.035572	4.16	0.056	
Peptone	0.25346	1	0.253461	29.61	0.024	Significant
KH_2PO_4	0.06185	1	0.061852	7.23	0.015	Significant
K_2HPO_4	0.00005	1	0.000046	0.01	0.942	
$MgSO_4.7H_2O$	0.00317	1	0.003172	0.37	0.550	
KCl	0.00040	1	0.000401	0.05	0.831	
$FeSO_4$	0.05177	1	0.051772	6.05	0.240	
Error	0.16265	19	0.008561			
Total	1.11159	27				

R^2 0.8537; R^2 (adj) 0.7921; R^2 (Pred) 0.6822

Optimization of medium Components for pectinase production by the Box-Behnken design

The box-Behnken design was implemented to further improve the concentrations of the most important constituents (orange peel, peptone, and KH_2PO_4) and the effect of their relations on pectinase activity. The BBD of variables in three levels with both the experimental and predicted response of the 15 experimental runs was shown in Table 6. The data indicated that the highest pectinase activity ranged from 1.286 to 1.292 U/ml was achieved by the central levels of all verified 3 constituents (Table 6: Runs 4, 12, and 15).

Table 6 Box-Behnken Design (BBD) of factors in coded levels with pectinase activity as a response

Run	A	B	C	Pectinase activity (U/ml)	
				Experimental	Predicted
1	-1	0	-1	0.824	0.829
2	0	1	-1	0.960	0.965
3	0	-1	-1	0.838	0.842
4	**0**	**0**	**0**	**1.286**	**1.287**
5	-1	1	0	0.915	0.904
6	-1	-1	0	0.786	0.776
7	1	1	0	0.995	1.005
8	0	-1	1	0.949	0.944
9	1	0	-1	0.936	0.921
10	-1	0	1	0.892	0.907
11	0	1	1	1.061	1.057
12	**0**	**0**	**0**	**1.283**	**1.287**
13	1	0	1	1.043	1.037
14	1	-1	0	0.885	0.896
15	0	0	0	1.292	1.287

Regression analysis, which was accomplished and fitted into the subsequent the 2[ed] order polynomial equation which shows the correlation between pectinase and the three variables

$$Y \text{ (pectinase activity)} = -6.525 + 4.271\,A + 0.4087\,B + 0.6161\,C - 0.8400\,A2 - 0.04544\,B2 - 0.06811\,C2 - 0.00475\,AB + 0.01300\,AC - 0.00083\,BC$$

Where Y is the pectinase activity (U/ml), A, B and C were the coded variables for orange peel concentration, Peptone and KH_2PO_4, respectively.

Analysis of Variance (ANOVA) of the Box-Behnken Design (BBD) for the pectinase production was shown in Table 7. From the data presented in Table 7, we found that the value of the "Predicted R^2" to be 0.9571 which is a reasonable agreement with R^2 of 0.9972 and Adjusted R^2 of 0.9922. This revealed that there is a good agreement and a high correlation between values predicted by the model, and the regression model offers an exceptional clarification of the correlation amongst the independent variables and the response (pectinase production). The data presented in Table 7 indicated that the model F value of 199.75 suggests the Model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise, The *P* values of the model were 0.002 ($p < 0.05$) which showed that the model terms were significant, the values of A, B, C, A2, B2, and C2 were significant whereas AB, AC, BC were non-significant.

Table 7 Analysis of Variance (ANOVA) for response surface quadratic model of Box-Behnken Design (BBD) for the production of pectinase

Source	df	Sum of squares	Mean square	F-Value	P-Value
Model	9	0.394871	0.043875	199.75	0.002
A- Orange peel	1	0.024420	0.024420	111.18	0.000
B-Peptone	1	0.027966	0.027966	127.32	0.000
C- KH_2PO_4	1	0.018721	0.018721	85.23	0.000
A2	1	0.162831	0.162831	741.32	0.000
B2	1	0.121968	0.121968	555.28	0.000
C2	1	0.086716	0.086716	394.79	0.000
AB	1	0.000090	0.000090	0.41	0.550
AC	1	0.000380	0.000380	1.73	0.245
BC	1	0.000025	0.000025	0.11	0.750
Error	5	0.001098	0.000220		
Lack-of-Fit	3	0.001056	0.000352	16.77	0.057
Pure Error	2	0.000042	0.000021		
Total	14	0.395969			

R^2 0.9972; R^2 (adj) 0.9922; R^2 (Pred) 0.9571

The optimal concentrations of medium components yielding maximum pectinase activity has been obtained from the response surface (3D) and contour plots (2D). Response surface and contour plots (Fig. 3(A-F)) represent relations among the experimental elements (orange peel, peptone, and KH_2PO_4) and the response of pectinase activity. Figure (3A-3B) displays the surface and contour plots of the effect of relations among orange peel (2-3 %) and peptone (2-6 g/l) while keeping KH_2PO_4 constant at 4.5 g/l. The maximum pectinase activity of 1. 292U/ml was observed at 2.5% orange peel and 4g/l peptone. Figure (3C-3D) shows the surface and contour plots of the effect of interactions between orange peel (2-3%) and KH_2PO_4 (3-6 g/l) while keeping peptone constant at 4 g/l. The outcomes presented that a higher pectinase activity was obtained at the orange peel (2.5%), while 4.5 g/l K_2HPO_4 was proper for constructing a maximum pectinase yield.

Alternatively, the response surface and contour plot for peptone and KH_2PO_4 as shown in Fig. (3E-3F) indicated that the orange peel concentration was fixed at 2.5%. As well as, the increase in both constituents progressively improved pectinase yield up to its maximum, and an additional increase caused in a reduced in pectinase yield. The last optimal peptone and KH_2PO_4 concentrations were 4 and 4.5 g/l, individually. Besides, from the model outcomes matched to our experimental runs, it can be noticed that the maximum experimental pectinase activity of 1.292 U/ml was in moral accordance with the model predicted activity of 1.287 U/ml.

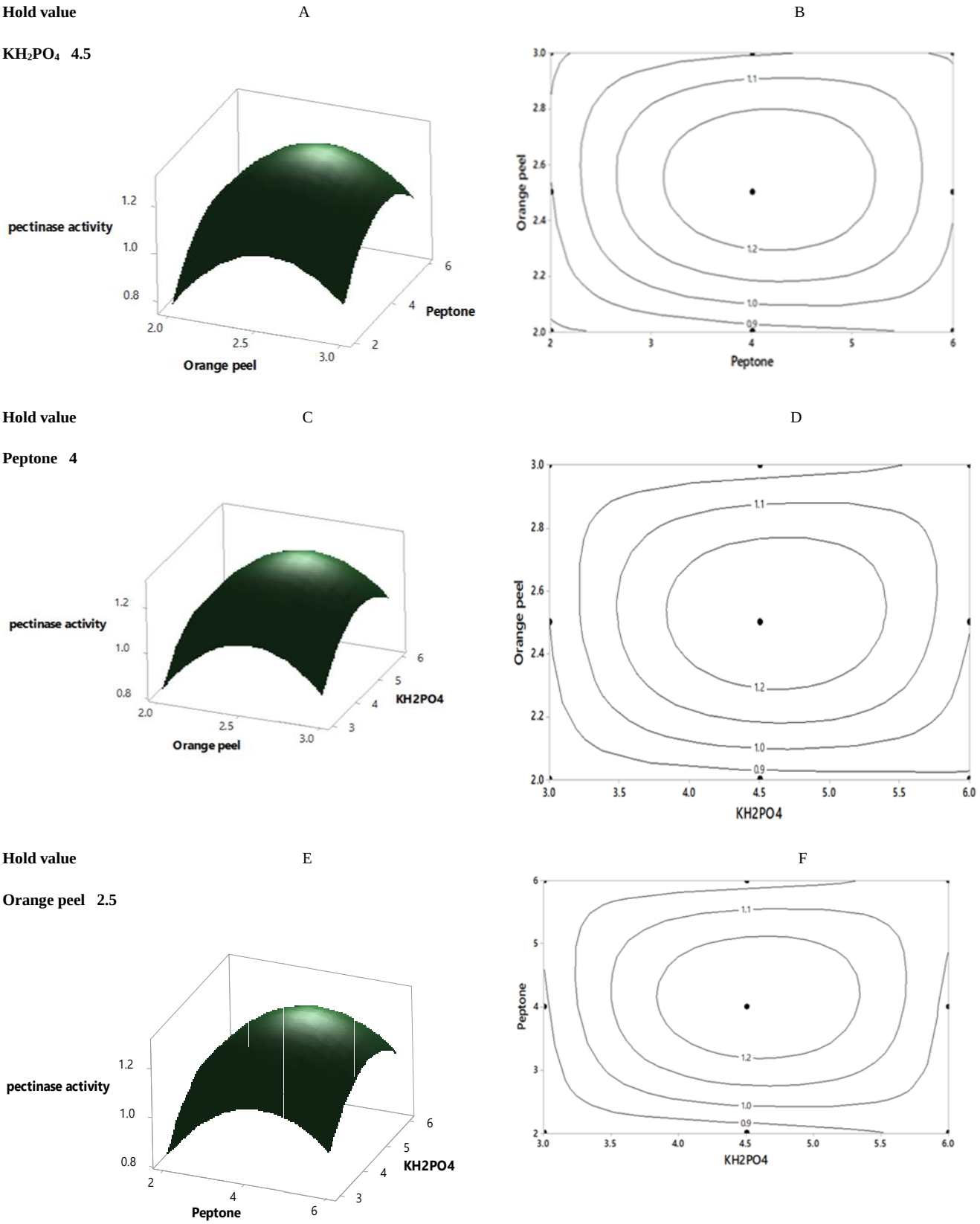

Figure 3 (A-F) Surface (A) and contour (B) plots showing the effect of interactions of orange peel concentration and peptone on pectinase activity, Surface (C) and contour (D) plots showing the effect of interactions of orange peel concentration and KH_2PO_4 on pectinase activity and Surface (E) and contour (F) plots showing the effect of interactions of peptone and KH_2PO_4 on pectinase activity

Discussion

Pectinase is one of the most significant industrialized enzymes, pectinases *find broad, delicate in the various fields*. Plants and microorganisms are the main sources of the enzyme production, *however*, microbial sources were considered the most promising.

A. oryzae MN894021 produced the pectinase yield of 0.214 U/ml from the first screening of different strains. The results agree with **Ibarra et al. (2017**) who

mentioned that the *Aspergillus*, *Penicillium*, and *Rhizopus* species are universally used fungi for pectinase production. Similar results have been reported by **Benzerra et al. (2012)**; **Thangaratham Manimegalai (2014) and Abdullah et al. (2018),** they indicated that *A. japonicus*, *Penicillium sp*., *A. flavus*, and *A. niger* IBT-7 showed pectinolytic activity. Based on the obtained results *P. chrysogenum* was *chosen* in this study for further experiments.

The orange peel gave the highest pectinase activity of 0.48 U/ml (Fig. 1). Comparatively less pectinase production has been observed with banana peel waste with a yield of 0.15 U/ml. These results showed that orange peel waste was the best substrate for pectinase production by *P. chrysogenum* MF318506. This is due to, the orange peel is a valuable source for pectinase production because it's rich in pectin, contains a considerable amount of valuable substances such as ash (7.39%), fat (1.85%), pectin (7.0%), lignin (6.4%), crude fiber (7.8%), total sugar (14.08%), reducing sugars (10.70%) and non-reducing sugar (3.70%), cellulose and hemicelluloses **(Ahmed et al. 2016)**.

Also, our results agree with *numerous* authors who indicated that the orange peel was the best substrate for the maximal pectinase production by *A. niger* **(Mrudulab and Anitaraj (2001);** from *Aspergillus* sp. **(Camargo et al., 2005);** by *P. oxalicum* **(Santos et al., 2008)**; from *A. niger* URM 4645 **by Maller et al. (2011); Miciel et al. (2013)**, **Miciel et al. (2014)**; from *Trichoderma viridi* **(Irshad et al., 2014);** by *A. niger* **(Ahmed et al., 2016);** from *A. flavus* **(Thangaratham and Manimegalai, 2014)**.

The Plackett-Burman design data in Table 3 assigned that there was an extensive difference from 0.305 to 1.057 U/ml of pectinase activity in the 28 runs. This variance fluctuation recommended that the optimization of medium components had *a significant* outcome on the pectinase yield. Our data was in good agreement with those previously expressed for statistical medium optimization of pectinase activity with either fungal or bacterial microorganism (**Reddy and Saritha (2016); Li et al. (2015); Yu et al. (2018)**. On the other hand, other parameters, e.g., NH_4Cl, pH 8.6, and temperature have a significant effect was noticed by **Li et al. (2014)** and **Yu et al. (2017)** who obtained pectinase by *P. oxalicum* PJ02 using 12 runs applying PBD and from *Bacillus subtilis* ZGL14, respectively. As well, the initial pH medium, inoculum size, and substrate concentration were indicated as a significant factor using PBD from *Geotrichum candidum* AA15 **(Asia et al. 2019).**

Analysis of Variance (ANOVA) of the PBD for the pectinase yield was revealed in Table 5. The data designated that the PBD was an influential statistical procedure for choice the essential elements **(Plackett and Burman, 1946).** In a previous study, **El Enshasy et al. (2018)** indicated that the K_2HPO_4, pectin, and $(NH4)^2SO_4$ were the most significant elements affecting pectinase yield through the fractional factorial design approach. On the other hand, **Zeni et al. (2014)** revealed that factors, e.g., pectin, yeast extract, and potassium phosphate had significant ($p<0.05$) positive effects on the pectin lyase production cultured by *P. brasilianum* employing the PBD.

The Box-Behnken design (BBD) of variables in three levels of data indicated that the maximal pectinase yield ranged from 1.286 to 1.292 U/ml was achieved using the middle levels of all tested three constituents (Table 6: Runs 4, 12, and 15). Comparable outcomes were accounted by **El Enshasy et al. (2018)** used the Box-Behnken design to further optimization of the concentrations of pectin, (NH4)2SO4, and K2HPO4 in the culture medium agreeing to the three levels and exhibited that the greatest pectinase yield was obtained by the middle levels of all verified three components.

As well, the Box-Behnken design was used for the optimum conditions of pectinase production from *Geotrichum candidum* AA15 and the results displayed that the strain formed 0.250 IU/ml pectinase under improved environments **(Asia et al. 2019)**. On the other hand, the highest yield of the pectinase by *Rhizopus* sp. C4 uses RSM to enhance the different environmental factors (temperature, moisture, and incubation days) for a total of 20 runs via central composite design was indicated by **Handa et al.** (2016).

Analysis of Variance (ANOVA) of the Box-Behnken Design (BBD) for the pectinase production *has appeared* in Table 7**.** The model of pectinase production was used to analyze the coefficient of determination (R2). The value of R2 comes closer to 1.0, this means that the model correlates fit **(Reddy and Saritha. 2016)**. This is indicating the better relationship between the predicted and actual values, suggesting the goodness of the model **(Li et al. 2008).** A small value of R2 indicated a poor relevance of the dependent variables in the model. The model can fit well with the actual data when R2 approaches unity **(Desai et al. 2017).**

From the data introduced in Table 7, the value of the "Predicted R^2" to be 0.9571 which is a reasonable agreement with R^2 of 0.9972, and Adjusted R^2 of 0.9922. These results agree with **El Enshasy et al. (2018)** who indicated that pectinase response has an R2 value of 93.69%, which designates a respectable fit of the model. The coefficient of determination (R2) value was calculated as 0.93 indicates 93.67% of the variance in response is demonstrated by this model (**Asia et al. 2019)**. Additionally, **Ajayi et al. (2018)** studied that the RSM used to optimize the parameters and the central composite design with a total of 30 experiments was successful in the pectinase production process by *A. niger* with a correlation coefficient (R2) of 0.901.

On the other hand**, Handa et al. (2016)** indicated that the variability in controlled response values by the experimental variables and their relations were estimated by R2, the predicted R2 value of 0.9545 by the model in a nearby contract with an actual R2 value 0.9883 disguised that the fitted linear, an interaction, and quadratic terms could elucidate 98.83% of the variation, viewing an acceptable demonstration of the process model.

The information introduced in Table 7 demonstrated that the model F value of 199.75, infers the Model is significant. Likewise, the achieved results are in agreement with those stated by **Tari et al. (2007)** where they indicated that the p-value reveals the correlation amongst the variables, and the response variable, p-value lower than 0.05, shows that the practical model is significant. The model F-value and Probability value (P model > F) of 170.45 and <0.0001 respectively, for pectinase activity, show that the applied model is highly significant (**Handa et al. (2016)**. *Likewise*, (**Asia et al. 2019)** reported that the significance of the model ($p<0.05$) was determined by Analysis of variance (ANOVA) that *indicated* an F-value of 8.22. On the other hand, **Kuvvet et al. (2019)** obtained a 2-fold increase in pectinase yield from apple pomace waste using the Box-Behnken response surface methodology by *Bacillus* sp.

The interactions amongst the experimental factors, e.g., orange peel, peptone, KH_2PO_4, and the pectinase activity as a response by Response surface and contour plots were shown in Figures (3a-f). **Ajayi et al. (2018)** explained that the relationship between different parameters of temperature, pH, and substrate concentration by the second-order polynomial. The two-dimensional contour plots were applied to establish the optimal values of the variables and found there was an important enhancement in the pectinase activity form *A. niger*.

CONCLUSIONS

Three fungal strains were screened for pectinase activity. Among of them, *P. chrysogenum* MF318506 gave the highest pectinase yield. Six different agricultural wastes were tested as substrates for pectinase activity and the orange peel gave the maximum pectinase activity (0.48U/ml). Employing the Plackett-Burman design denoted that the optimization of the medium components had a noteworthy effect on the pectinase activity. The maximal pectinase activity was achieved under optimum experimental conditions. By applying the Box-Behnken Design, the maximum pectinase activity of 1.292U/ml was obtained at 2.5% orange peel and 4g/l peptone. The obtained results indicated that the statistical optimization, enhanced the pectinase activity from the MF318506 strain and recommend using this strain as a pectinase producer in different industrial application fields.

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