# Enhanced production of Lovastatin by filamentous fungi through solid state fermentation

**Naveed Munir[1,4], Muhammad Asghar[2], Mian Anjum Murtaza[3], Naheed Akhter[4], Ghulam Rasool[5], Syed Muhammad Ali Shah[6*], Imtiaz Mahmood Tahir[4], Fahad Said Khan[7], Muhammad Riaz[5], Sabira Sultana[6], Abid Rashid[8], Muhammad Akhlaq[9] and Muhammad Akram[6]**

[1]Department of Biochemistry, Government College University, Faisalabad, Pakistan
[2]Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan
[3]Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan
[4]College of Allied Health Professional, Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan
[5]Department of Allied Health Sciences, Sargodha Medical College, University of Sargodha, Sargodha, Pakistan
[6]Department of Eastern Medicine, Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan
[7]Department of Eastern Medicine, Faculty of Medical and Health Sciences, The University of Poonch, Rawalakot, AJK
[8]Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan
[9]Faculty of Pharmacy, Gomal University, Dera Ismail Khan, KPK, Pakistan

**Abstract**: Lovastatin is a natural competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme-A (HMG-CoA) reductase and inhibits specifically rate limiting step in cholesterol biosynthesis. Further, lovastatin in comparison with synthetic drugs has no well-reported side effects. Four pure isolated filamentous fungal strains including *Aspergillus niger* IBL, *Aspergillus terreus* FFCBP-1053, *Aspergillus flavus* PML and *Aspergillus nidulans* FFCBP-014 have been cultured by solid state fermentation (SSF) using rice straw as substrate for the synthesis of lovastatin. After selecting *Aspergillus terreus* FFCBP-1053 as the best producer of lovastatin, various selected physical parameters including pH, temperature, inoculums size and moisture content were optimized through response surface methodology (RSM) under center composite design (CCD) for lovastatin hyper production. Maximum lovastatin production of 2070±91.5 was predicted by the quadratic model in the medium having moisture content 70% and pH 4.5 at 35°C which was verified experimentally to be 2140±93.25µg/g DW of FM (microgram/gram dry weight of fermentation medium), significantly ($P<0.05$) high as compared to un-optimized conditions while it was noted that lovastatin production is independent on inoculum size ($P>0.05$) measured by spectrophotometer at 245 nm against standard. It was determined that optimized conditions for the hyper-production of lovastatin from fungal sources have a significant effect.

**Keywords**: *Aspergillus,* 3-hydroxy-3-methyl glutaryl coenzyme A reductase, lovastatin, fermentation.

## INTRODUCTION

Hypercholesterolemia; linked with inadequate diet plans, sedentary life style, lack of proper physical activities or exercise and many other socioeconomic glitches; is fetching underdeveloped countries to an alarming threat. World Health organization (WHO) estimated till 2015, Non communicable diseases (NCDs) have been caused about 40 million deaths. It was reported that globally CVDs are the major cause of these deaths than any other cause due to NCDs deaths. Among four major death causes of NCDs, CVDs accounts 45% (about 17.7 million deaths), 22% deaths due to different type of cancers (accounting about 8.8 million deaths); 3.9 million people die due to chronic respiratory disease (accounting about 10%) and 4% deaths occurred due to diabetes (1.6 million deaths). Further, it was reported that low and middle income countries are affected more than ¾ of CVD deaths than others. People with CVDs or at high CVD risk required appropriate early diagnosis and adequate management using counseling and medication (WHO, 2017). It has also been reported that high cholesterol level increases the risk of several nervous system diseases like dementia/Alzheimer's disease (Kivipelto, 2002, Notkola, Sulkava *et al*., 1998) and Ischemic heart stroke (IHS) (Lewington *et al*., 2007). Further, hypercholesterolemia stimulates the chances of diabetes development, obesity and certain types of cancers. That is why; high blood cholesterol level and supplementary risk factors put the advanced world at one of the chief worldwide socio-economic and medical confront (Hui and Howles, 2005).

Like many enzymes inhibiting drugs, anti-cholesterol agents including lovastatin and mevastatin also competitively inhibit cholesterol biosynthesis rate-limiting step catalyzed by HMG-CoA reductase, i.e. conversion of HMG-CoA to mevalonate (Alberts *et al*., 1980, Hajjaj *et al*., 2001, Tahir *et al*., 2016). So lovastatin inhibits the production of non-sterol isoprenoid compounds to prevent stroke (Hebert, 1997) and to

**Corresponding author:* e-mail: smalishah@hotmail.com

suppress the tumor development (Rollini, 2002 and Tobert, 2003). Various strategies and medical remedies like fibrates, dietary plans, bile acid sequesters including cholestyramine and colestipol, nicotinic acid and probucol were used till 1987 when lovastatin was unavailable to control the hyperlipidemia; though, such precautionary managements to regulate blood cholesterol have limited admissibility or value, or both (Tang *et al*., 1998). Introduction of lovastatin in market as being used to reduce low density lipoprotein (LDL) cholesterol significantly was highly acknowledged with no well reported side effects, easy to take and due to very short dosage size as anti-cholesterol agent. Fermentation biotechnology as an emerging field used agro-industrial wastes as supporting substrates has significant and cost effective role for lovastatin production as secondary metabolite. Different types of filamentous fungi including *Penicillium* species *Aspergillus* species, and *Monascus* species have higher capability for lovastatin production through this technique. It was also reported that species of *Scopolariopsis, Paecilomyces, Doratomyces, Pleurotus, Trichoderma, Phythium, Phoma, Gymnoascus* and *Hypomyces* have also potential to produce lovastatin during the course of fermentation (Gunde-Cimerman *et al*., 1995 and Shindia, 1997). So, the major rationale of this research work was to explore the potential of local *Aspergillus* species for lovastatin hyper production under optimized fermentation conditions and interaction of these parameters using RSM statistical model.

## MATERIALS AND METHODS

### *Fermentative organisms*

*Aspergillus terreus* FFCBP-1053 and *Aspergillus nidulans* FFCBP-014 were purchased from First Fungal Culture Bank of Pakistan (FFCBP), Department of Mycology & Plant Pathology, University of the Punjab, Lahore, Pakistan. *Aspergillus flavus* PML *Aspergillus niger* IBL were available in the Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan. 10 mL of sterilized 0.1% Tween-80 solution was added to make spore suspension to a 7-day old pure culture slants of the fungi and the number of spores in the inoculum were counted using hemocytometer method and adjusted at 5× $10^7$to 5× $10^8$spores per/ml (Kolmer *et al*., 1951).

### *Experiment design*

During the first phase of this work, the efficacy of lovastatin production with selected strains using rice straw as substrate was examined for 10 days by using pre-optimized conditions as 30°C temperature, pH 5.0, 5.0g substrate conc., 60% v/w moisture content, and 2.0mL inoculum size. Samples were harvested after every 24 hours for successive 10 days to select the best lovastatin producing fungal strain (fig. 1). In the next phase, different levels of fermentation conditions were used to enhance lovastatin production, viz. pH (4.5, 5.0, 5.5, 6.0 and 6.5), temperature (°C) (25, 30, 35, 40 and 45) inoculums size (mL) (1.0, 1.5, 2.0, 2.5 and 3.0) and moisture content (%) (50, 60, 70, 80 and 90). Experimental design was formulated according to CCD of RSM using DESIGN EXPERT software (table 1).

## STATISTICAL ANALYSIS

### *Lovastatin extraction and determination*

Solid culture media was dried for two days at 60°C and 15mL methanol was used to extract lovastatin from 0.5g of the dry culture media with continue shaking in a shaker for 60 min at 220 rpm (Ajdari *et al*., 2011). Whatman filter paper 1 (0.2*µ*m) was used to filter extracted lovastatin and lovastatin concentration in the filtrate was measured using efficient and reliable method of Rajput and Raj (2009) by spectrophotometer at 245 nm. This method was successfully applied. A slightly modified linearity (r > 0.999) was found while the estimation of lovastatin concentration ranging from 0 to 2500 µg/mL range set by standards (Rajput and Raj 2009; Jaivel and Marimuthu, 2010). The data collected was subjected to Analysis of Variance (ANOVA). The Response Surface Methodology plots (RSM plots) and contour graph were drawn by using state software Ver. 7.0 (Trial version, USA). To determine the optimum condition point, prediction tools were used. Further, calculations to determine the means and standard errors of means (Mean ± S.E) for each treatment were performed (Steel *et al*., 1997).

## RESULTS

For the production of lovastatin from selected *Aspergillus* species, many studies have already been done to optimize the cultural conditions. So, in the first phase of study pre-optimized conditions were used (fig. 1) and the results of current study explored that *Aspergillus terreus* FFCBP-1053 is the best producer of lovastatin (1562±19.2 µg/gDW of FM), on seventh ($7^{th}$) day of incubation (Venkateswaran and Vijayalakshmi, 2010) among selected strains i.e. *Aspergillus nidulans* FFCBP-014 (391±23.1 µg/g DW of FM), *Aspergillus niger* IBL (77±8.0 µg/g DW of FM), *Aspergillus flavus* PML (504±22.µg/g DW of FM) (fig. 1) (Kamath, Dwarakanath *et al*., 2015, Osman, Khattab *et al*., 2011). Statistical analysis of the second phase data revealed that *Aspergillus terreus* FFCBP-1053 in medium having 70% moisture content on incubating at 35°C with pH 4.5 during SSF showed significant (*P*<0.05) response in the form of lovastatin production (figs. 2-4) while, independent on inoculum size (*P*>0.05) (fig. 2).

## DISCUSSION

### *RSM for optimization of Lovastatin production*

Experiment design was mapped up using modern statistical design RSM to check the response (lovastatin

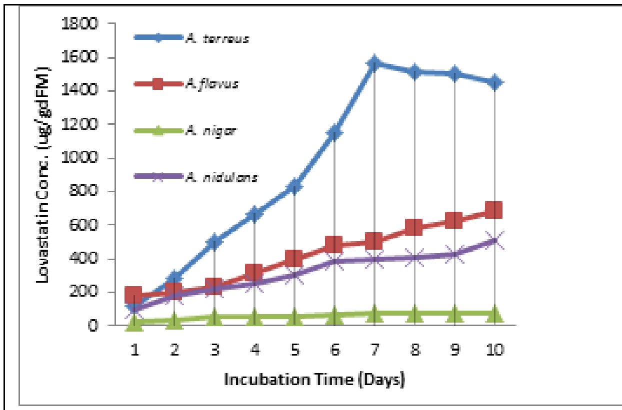


**Fig. 1**: Concentration of lovastatin produced by selected fungal strains on rise straw used as substrate during 10 days (Temperature 30°C, pH 5.0, Substrate concentration 5.0g, Moisture content 60% v/w, Inoculum size 02mL)

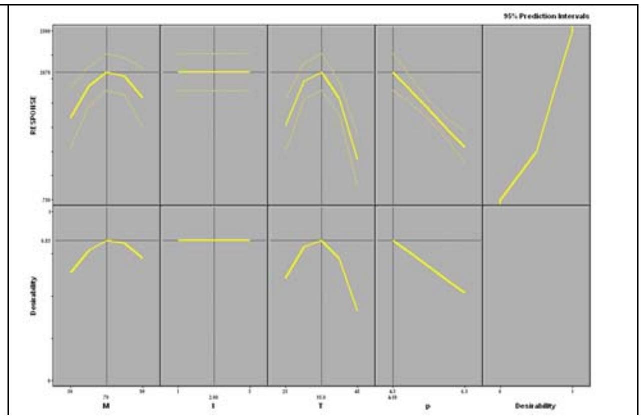


**Fig. 2**: Prediction profile plot for the production of lovastatin (response) with various levels of factors presenting optimized points
M= Moisture content (%v/w), T= Temperature (°C), P= pH, I= Inoculum Size, Response = Lovastatin production (µg/g DW of FM)

production) and to manage time as well as cost of study at minimum. Thirty runs were proceeded using four different factors with five levels of each (table 1) and the evaluation of model fitting was checked on the basis of lowest residual sum of squares (error) (SSE) (Allen and Yu, 2002).
The results of analysis of variance showed that the model is highly significant (F-value >4.0) derived by MS regression and MS residual (table 2). Further, the significance of model was cleared from *P* value (Prob> F) of the model ($P<0.05$) (table 2) and the $R^2$ value is 0.798 closer to 1 which indicated that mathematical model is better fit and very reliable. Coefficient estimates and *P* values of used variables revealed that pH, temperature and moisture content are significant ($P<0.05$) model parameters while non-significant ($P>0.05$) effect of inoculum size was observed on Lovastatin production.

The quadratic model equation given below was obtained after analysis of the experimental data:
Lovastatin Yield (µg/g DW of FM) = -9364.14 + 133.5866*M + 494.0609*T - 389.107*P - 0.917369*M*M - 7.316564*T*T

Three dimensional (3D) graphs of significant factors were constructed to represent the data in concise manner e.g. in pairwise combination and constructed contour graphs using RSM software were analyzed further to explore optimum points of the variables at a time by point prediction statistical software (figs. 3, 4 & 5).

***Screening for lovastatin production***
Lovastatin produced as a secondary metabolite by various filamentous fungi so in the first phase of study pre optimized conditions were used to find out the fermentation duration for maximum production of this secondary metabolite by selected fungal species. It was explored that *Aspergillus terreus* FFCBP-1053 is the best producer of lovastatin among selected fungal strains on 7th day of incubation at selected pre optimized conditions on rise straw as substrate for 10 days (fig. 1) (Venkateswaran and Vijayalakshmi, 2010); (Kamath, Dwarakanath *et al*., 2015, Osman, Khattab *et al*., 2011).

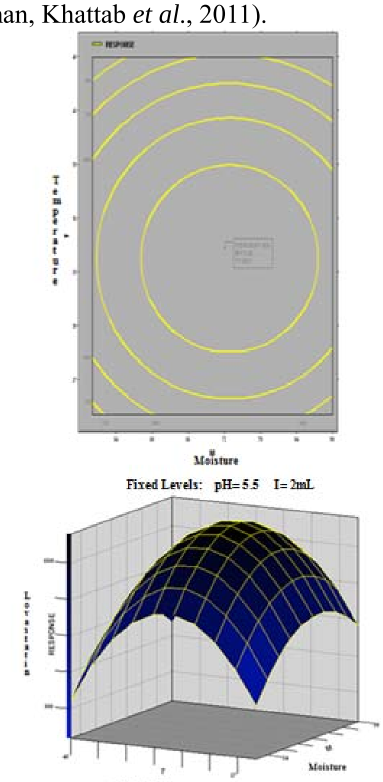


**Fig. 3**: The contour plot and the corresponding response surface plot showing the effects of Moisture content (%v/w) and temperature (°C) on lovastatin production by *Aspergillus terreus* FFCBP-1053 with fixed levels of Inoculum (2mL) and pH (5.5)

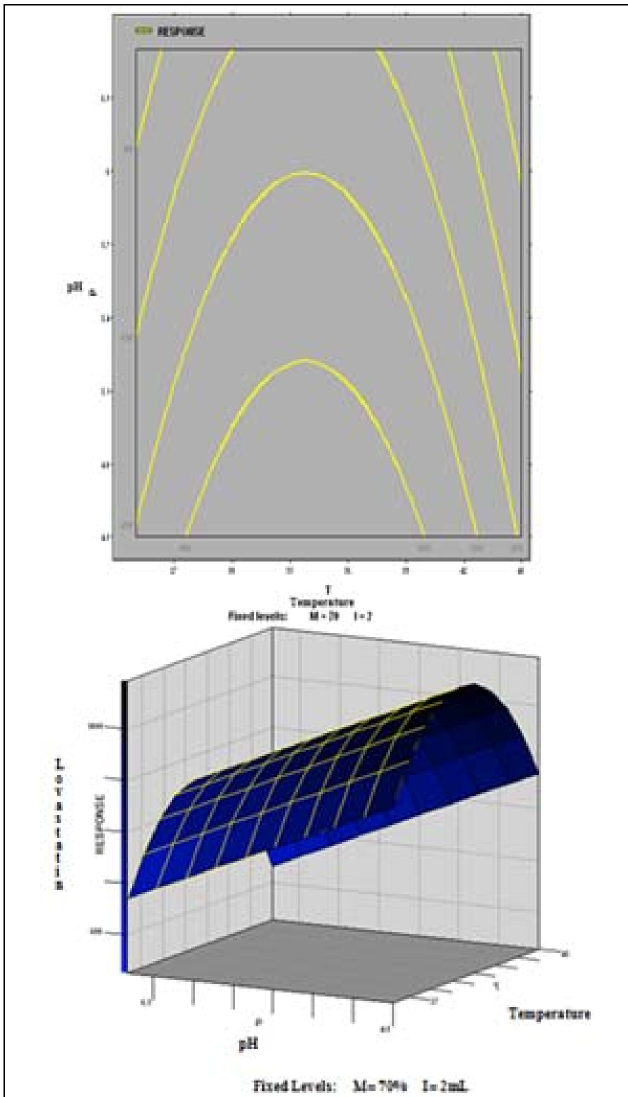


**Fig. 4**: The contour plot and the corresponding response surface plot showing the effects of pH and temperature (°C) on lovastatin production by *Aspergillus terreus* FFCBP-1053 with fixed levels of Inoculum (2mL) and moisture (70%)

Where P= pH, T=Temperature (°C), Response= Lovastatin production (µg/g DW of FM)

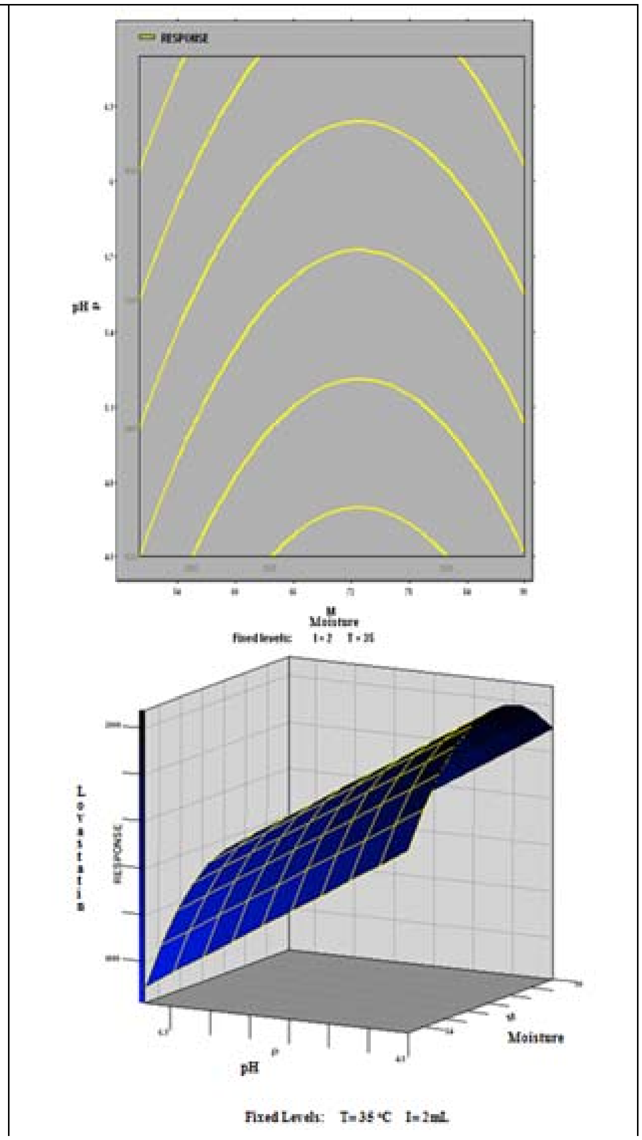


**Fig. 5**: The contour plot and the corresponding response surface plot showing the effects of Moisture content (%v/w) and pH on lovastatin production by *Aspergillus terreus* FFCBP-1053 with fixed levels of Inoculum (2mL) and temperature (35°C)

Where M= Moisture content (%v/w), P= pH, Response= Lovastatin production (µg/g DW of FM)

Where M= Moisture content (%v/w), T=Temperature (°C), Response= Lovastatin production (µg/g DW of FM)

### *Moisture Content and lovastatin Production*

Several studies revealed that increasing or decreasing %age moisture content significantly affects the lovastatin biosynthesis. Moisture contents basically influence the lovastatin production by affecting voidage volume and surface area of medium. Alteration in air supply directly change the oxygen availability required for cellular metabolism, so, when the moisture content is low it enhance the oxygen supply but at low water concentration production of heat might influence metabolic activity of fungi (Jaivel and Marimuthu, 2010). Further, when the moisture content is above 70% it decreased the passage of oxygen due to decreased aeration ultimately decreased lovastatin production (Faseleh Jahromi, Liang *et al*., 2012). It was found that at moisture content of 70% *Aspergillus terreus* UV 1718 gave highest production of lovastatin (3004±25µg $g^{-1}$ DFM) (Pansuriya and Singhal, 2010, Prabhakar, Lingappa *et al*., 2012).

### *Incubation temperature and lovastatin production*

Fermentation temperature results showed that increasing temperature more than 35°C had negative response in the form of lovastatin production. Change in temperature

**Table 1**: Optimal design for optimization of lovastatin production

| Run | Moisture Content (%v/w) | Inoculum Size (mL) | Temperature (°C) | pH | Response (Lovastatin Yield) (µg/g DW of FM) |
|---|---|---|---|---|---|
| 1 | 60 | 1.5 | 30 | 5.0 | 1583 ± 57.5 |
| 2 | 80 | 1.5 | 30 | 5.0 | 1675 ± 59.0 |
| 3 | 60 | 2.5 | 30 | 5.0 | 1500 ± 43.59 |
| 4 | 80 | 2.5 | 30 | 5.0 | 2140 ± 93.25 |
| 5 | 60 | 1.5 | 40 | 5.0 | 1540 ± 51.0 |
| 6 | 80 | 1.5 | 40 | 5.0 | 1710 ± 63.0 |
| 7 | 60 | 2.5 | 40 | 5.0 | 1380 ± 37.85 |
| 8 | 80 | 2.5 | 40 | 5.0 | 1270 ± 39.0 |
| 9 | 60 | 1.5 | 30 | 6.0 | 1230 ± 42.50 |
| 10 | 80 | 1.5 | 30 | 6.0 | 1590 ± 52.00 |
| 11 | 60 | 2.5 | 30 | 6.0 | 1451 ± 46.00 |
| 12 | 80 | 2.5 | 30 | 6.0 | 1130 ± 48.55 |
| 13 | 60 | 1.5 | 40 | 6.0 | 1125 ± 34.50 |
| 14 | 80 | 1.5 | 40 | 6.0 | 1253 ± 38.00 |
| 15 | 60 | 2.5 | 40 | 6.0 | 1045 ± 29.50 |
| 16 | 80 | 2.5 | 40 | 6.0 | 1360 ± 53.85 |
| 17 | 50 | 2.0 | 35 | 5.5 | 990 ± 27.25 |
| 18 | 90 | 2.0 | 35 | 5.5 | 1245 ± 52.15 |
| 19 | 70 | 1.0 | 35 | 5.5 | 1660 ± 72.50 |
| 20 | 70 | 3.0 | 35 | 5.5 | 1680 ± 81.50 |
| 21 | 70 | 2.0 | 25 | 5.5 | 1635 ± 77.45 |
| 22 | 70 | 2.0 | 45 | 5.5 | 1035 ± 41.25 |
| 23 | 70 | 2.0 | 35 | 4.5 | 1150 ± 53.00 |
| 24 | 70 | 2.0 | 35 | 6.5 | 1005 ± 43.69 |
| 25 | 70 | 2.0 | 35 | 5.5 | 1690 ± 79.50 |
| 26 | 70 | 2.0 | 35 | 5.5 | 1710 ± 84.50 |
| 27 | 70 | 2.0 | 35 | 5.5 | 1635 ± 69.90 |
| 28 | 70 | 2.0 | 35 | 5.5 | 1730 ± 65.99 |
| 29 | 70 | 2.0 | 35 | 5.5 | 1670 ± 71.65 |
| 30 | 70 | 2.0 | 35 | 5.5 | 1780 ± 81.50 |

Incubation time 7 days, Substrate conc. 5g

**Table 2**: The results of hypothesis tests for linear, quadratic, and cross product terms

| Regression | DF | Type I sum of Squares | R-Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Linear | 4 | 677440 | 0.2816 | 5.04 | 0.0089 |
| Quadratic | 4 | 1127625 | 0.4687 | 8.38 | 0.0009 |
| Cross product | 6 | 96429 | 0.0401 | 0.48 | 0.8144 |
| Total Model | 14 | 1901493 | 0.7904 | 4.04 | 0.0055 |

Significant (p<0.05), Non-Significant (p>0.05)

significantly influenced the production of metabolites from microorganisms as well as enzymes activity, protein functioning and cell viability (Chanakya, 2011), so, lower concentration of lovastatin production with increase or decrease in temperature might be due to the effect of temperature on enzymes activity involved in metabolic process for lovastatin synthesis (Pyo, 2007). *Monascus pilosus* 'KFRI 1140' in soybean medium as substrate at 35°C gave supreme response in the form of mevinolin (2.9g /g of dry wt) and *Monascus pilosus* 'M12-69' with inoculum size 5 mL gave highest production of monacolin K (lovastatin) at 30°C (2.5mg monocolin K/g of substrate) by SSF (Chen and Hu, 2005, Wang, Lee *et al*., 2003).

***pH of culture media and lovastatin production***

A change in pH significantly affects the growth as well as productivity of microbes. Further, a small variation in pH also influences the activity of biocatalysts and fluctuation in pH retardates the fungal growth (Munir, Asgher *et al*., 2015). The pH of fermentation medium could have a significant role in the behaviors of lovastatin production as secondary metabolite and on the growth of fungi (Buckland, Gbewonyo *et al*., 1989) (Lai, Tsai *et al*., 2005). It was explored, by using several substrates including fermented raw rice, broken wheat, maize, germinated finger millet and parboiled rice for lovastatin synthesis, that medium having pH 4.4 showed the maximum level of statins (5.2g/kg dry wt.) (Venkateswaran and Vijayalakshmi, 2010). Valera *et al*. (2005) determined that lovastatin synthesis enhanced in a medium having pH 4.8 with a continue slightly stirring two-liter bioreactor.

***Inoculum size and lovastatin Production***

Varying size of inoculum (1-3 mL with fungal spores 5 x $10^7$/mL) for enhanced production of lovastatin showed non-significant effect (fig. 2) by *Aspergillus* species SSF process (Faseleh Jahromi, Liang *et al*., 2012). Small inoculum size less $5\times10^7$ spores/mL showed a depressive behavior of microbes due to higher level of nutrients or prolonged lag phase (Pansuriya and Singhal, 2010) and on the other hand fungal spores more than $5\times10^7$ might exhaust the available nutrient before stipulated time period and impose a toxic effect on fungal growth and fungal products (Chanakya, 2011).

## CONCLUSION

The study showed that the optimization of nutritional and physical parameters by RSM under CCD is a time saving as well cost effective statistical design for the hyper-production of lovastatin by *Aspergillus terreus* FFCBP-1053 using rice straw through SSF process. It was also reported that optimized conditions significantly improved the lovastatin production from indigenous fungal source. The promising high concentration of lovastatin also suggests the application of fermentation biotechnology for the large scale production of this drug which would be a cheap source of lovastatin as a natural drug could be used to treat hypercholesterolemia. In future, this study can be expanded through running animal trials to investigate the beneficial as well as detrimental effects of fungal origin drugs.

## ACKNOWLEDGMENTS

The authors highly appreciate and acknowledge the contribution of Industrial Biotechnology Lab (IBL), Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan.

## REFERENCES

Ajdari Z, Ebrahimpour A, Abdul Manan M, Hamid M, Mohamad R and Ariff AB (2011). Assessment of Monacolin in the fermented products using *Monascus purpureus* FTC5391. *J. Biomed. Biotechnol*., pp.1-9.

Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J and Springer J (1980). Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proceed. National Acad. Sci.,* **77**(7): 3957-3961.

Allen TT and Yu L (2002). Low-cost response surface methods from simulation optimization. *Qual. Reliab. Engineer. Inter.,* **18**(1): 5-17.

Buckland B, Gbewonyo K, Hallada T, Kaplan L and Masurekar P (1989). Production of lovastatin, an inhibitor of cholesterol accumulation in humans. *Novel Microb. Products Med. Agri.,* pp.161-169.

Chanakya P (2011). Solid state fermentation for the production of lovastatin by *Aspergillus fischeri*. *Res. J. Pharm. Sci. Biotechnol*., **1**(1): 45-52.

Chen F and Hu X (2005). Study on red fermented rice with high concentration of monacolin K and low concentration of citrinin. *Inter. J. Food Microbiol.,* **103**(3): 331-337.

Faseleh M, Liang J, Mohamad R, Goh Y and Shokryazdan P (2012). Lovastatin production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation. *J. Biomed Biotech*., **196264**: 1-11.

Gunde-Cimerman N, Plemenita AA and Cimerman A (1995). A hydroxymethylglutaryl-CoA reductase inhibitor synthesized by yeasts. *FEMS Microbiol. Lett.,* **132**(2):39-43.

Hajjaj H, Niederberger P and Duboc P (2001). Lovastatin biosynthesis by *Aspergillus terreus* in a chemically defined medium. *Applied Environ. Microbiol.,* **67**(6): 2596-2602.

Hebert PR (1997). Cholesterol lowering with statin drugs, risk of stroke, and total mortality. *J. Amer. Med. Assoc.,* **278**(4): 313-315.

Hui DY and Howles PN (2005). Molecular mechanisms of cholesterol absorption and transport in the intestine. *Semin. Cell Develop. Biol.,* **16**(2): 183-192.

Jaivel N and Marimuthu P (2010). Optimization of lovastatin production in solid state fermentation by *Aspergillus terreus*. *Int. J. Eng. Sci. Technol.,* **2**(7): 2730-2733.

Kamath PV, Dwarakanath BS, Chaudhary A and Janakiraman S (2015). Optimization of culture conditions for maximal lovastatin production by *Aspergillus terreus* (KM017963) under solid state fermentation. *HAYATI J. Biosci.,* **22**(4): 174-180.

Kivipelto M (2002). Apolipoprotein E ε4 Allele, Elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. *Ann. Inter. Med.,* **137**(3): 149-152.

Kolmer JA, Spaulding EH and Robinson HW (1951). Approved laboratory techniques. Appleton Century Crafts, New York 1090.

Lai LST, Tsai TH and Cheng TY (2005). The influence of culturing environments on lovastatin production by

*Aspergillus terreus* in submerged cultures. *Enzyme Microb. Technol.,* **36**(5): 737-748.

Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R and Collins R (2007). Blood cholesterol and vascular mortality by age, sex, and blood pressure: A meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet.* **370**(9602): 1829-1839.

Munir N, Asgher M, Tahir IM, Riaz M, Bilal M and Shah SA (2015). Utilization of agro-wastes for production of ligninolytic enzymes in liquid state fermentation by *Phanerochaete chrysosporium*-IBL-03. *Inter. J. Clin. Biol. Sci.,* **7**: 9-14.

Notkola I-L, Sulkava R, Pekkanen J, Erkinjuntti T, Ehnholm C, Kivinen P, Tuomilehto J and Nissinen A (1998). Serum total cholesterol, apolipoprotein E {FC12}e4 allele and Alzheimer's disease. *Neuroepidemiol.,* **17**(1): 14-20.

Osman M, Khattab O, Zaghlol G. Abd El-Hameed R (2011). Screening for the production of cholesterol lowering drugs (lovastatin) by some fungi. *Aus. J. Basic Appl. Sci.,* **5**(698): 703-706.

Pansuriya RC and Singhal RS (2010). Response surface methodology for optimization of production of lovastatin by solid state fermentation. *Braz. J. Microbiol.,* **41**(1): 164-172.

Prabhakar M, Lingappa K, BABU V, Amena S and Vishalakshi N (2012). Characterization of physical factors for optimum lovastatin production by *Aspergillus terreus* KLVB28mu21 under solid state fermentation. *J. Recent Advances Applied Sci.,* **27**: 45-50.

Pyo YH (2007). Production of a high value-added soybean containing bioactive mevinolins and isoflavones. *J. Food Sci. Nutr.,* **12**(1): 29-34.

Rajput SJ and HA Raj (2009). Simultaneous estimation of ezetimibe and lovastatin by derivative spectroscopy. *Int. J. pharm. Tech. Res*., **1**(3): 894-899.

Rollini MMM (2002). Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. *Applied Microbiol. Biotechnol.,* **58**(5): 555-564.

Shindia AA (1997). Mevinolin production by some fungi. *Folia Microbiol*., **42**(5): 477-480.

Steel R, Torrie J and Dickey D (1997). Principles and procedures of statistics: A biometrical approach, McGraw-Hill, New York, NY, USA.

Tahir IM, Iqbal T, Saleem S, Mehboob H, Akhter N. Riaz M (2016). Effect of acetaminophen on sulfamethazine acetylation in male volunteers. *Int. J. Immunopathol. Pharmacol.,* **29**(1): 17-22.

Tang JL, Armitage JM, Lancaster T, Silagy CA, Fowler GH, Neil HAW, Smith GD and Ebrahim S (1998). Systematic review of dietary intervention trials to lower blood total cholesterol in free-living subjects Commentary: Dietary change, cholesterol reduction, and the public health what does meta-analysis. *Brit. Med. J.,* **316**(7139): 1213-1220.

Tobert JA (2003). Case history: Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nat. Rev. Drug Discover.*, **2**(7): 517-526.

Valera HR, Gomes J, Lakshmi S, Gururaja R, Suryanarayan S and Kumar D (2005). Lovastatin production by solid state fermentation using Aspergillus flavipes. *Enzyme Microbial*. *Technol*., **37**(5): 521-526.

Venkateswaran V and Vijayalakshmi G (2010). Finger millet (*Eleusine coracana*) an economically viable source for antihypercholesterolemic metabolites production by *Monascus purpureus*. *J. Food Sci. Technol.,* **47**(4): 426-431.

Wang JJ, Lee CL and Pan TM (2003). Improvement of monacolin K, ?-aminobutyric acid and citrinin production ratio as a function of environmental conditions of *Monascus purpureus* NTU 601. *J. Indus. Microbiol. Biotechnol.,* **30**(11): 669-676.

World Health organization, Cardiovascular diseases (CVDs), 2017. http://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)