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Pigment Production From *Monascus purpureus* MTCC 369 Using Rice Malt

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Abstract

Monascus purpureus pigment fermentations have been experimented largely in hard cultures, though the production yields have been too low to allow be commercial range production. Numerous agents that can manipulate the color production by *Monascus* gaseous environments, agitation and aeration, source of carbon and nitrogen have been reported as parameters that robustly control growth and the secondary metabolite assembly. The current study describes the environment for pigments production by *M. purpureus* in flooded cultures using an kitchen waste rice malt as substrate. The circumstances recognized for pigment production by *M. purpureus* were using 0.1-1.0% of Sodium nitrate, 6-12 days of incubation, 4.0-6.0 pH and 25-35°C of temperature of incubation. The inoculum level of fungal spores was kept similar in all the flasks of incubation. RSM exposed the following circumstances to be most favorable 0.1% of Sodium nitrate at 30°C of temperature of incubation at 5.0 pH and 12 days of incubation resulted in maximum fungal biomass and pigment production.

Keywords: *Monascus purpureus*, RSM, Rice Malt

Introduction

Food colorants are pigments or dyes supplementary to food to attain some objectives which embrace to retain the original color of the food stuff intensify the original color, add color to a colorless food and preserve the food stuff (Barrows et al., 2003). The foremost objectives of toting up food colorants are to advance the superiority and make the food more eye-catching to regulars. With a steady boost in human population and development, there is an increased demand for food with extended shelf life and other striking qualities. In the early on epoch, the sources of food colorants were primarily result of *Monascus* culture, due of monascidin A, deep-rooted by technical investigations, was proved against some bacterial and fungal strains. The brute pigment obtained by growing the *Monascus* strain in exterior culture had an antifungal action against some species of *Aspergillus*, *Mucor*, *Penicillium* and *Fusarium* genus. The yellow pigment isolated from red yeast rice also inhibits bacteria of the genera of *Bacillus*, *Pseudomonas* and *Escherichia*. These bacteriostatic and antifungal effects have lead to the deliberation of the additive value of the pigments of *Monascus purpureus* further the pictorial properties. *M. purpureus*, cultured in a shrimp and crab shell powder medium, displayed protease activities and the ability of enhancing the expansion of rape (Wang *et al.*, 2004). Countless of the studies relating *Monascus purpureus* have dealt

with the universal culture conditions to improve pigment production. *Monascus* is probably a xerophilic fungus, which grows in a broad diversity of natural substrates. A few usual substrates that have already been tested, besides rice and other cereals, are cassava starch , wheat bran, wheat meal, bread meal, corn meal , as banana peel, chikoo peel, papaya peel, rice malt and dairy milk. Malt is generated as waste while cooking rice hence it can serve as the cheapest source for biopigment production. It can bring down the production cost when used for large scale production. Previous research and studies has shown that growth of *Monascus* require sucrose or dextrose as carbon source. Rice malt has rich source of sucrose and easily availability makes it a better substrate. Therefore, the key intention of this study was to examine the potential of rice malt as a substrate for *Monascus* stain production by submerged fermentation process. Though, with the increase in demand for food colorants, these usual sources of colorants became insufficient and unreal food colorants were introduced into the market. Fake food colorants were extensively used but people have become more cognizant of the health implications of unreal colorants in food and pharmaceuticals (Arnold et al., 2012; Stevens et al., 2013, 2014). People's concerns in natural sources of food colorants have been recharged and explore in ways of mounting sources of natural food colorants have become significantly revolutionized. The sky-scraping demand for natural food colorants is reflected in the global market size which has been escalating gradually. It was anticipated at 1.15 billion US dollars in 2007 (Mappari et al., 2008) and projected to reach 1.7 billion US dollars in 2020 (Singh and Tyagi, 2015, Rohan 2012) has estimated the value to reach 2.3 billion US dollars in 2019. The mounting demand for and steady boost in the market size of natural food colorants has led to investigations into microbial pigments as natural sources of food colorants. even if, numerous groups of microorganisms such as fungi, algae, lichen and bacteria have been explored for pigment production,. Pigments are another group of fungal secondary metabolites with various useful applications. Fungal pigments are natural colorants and have lots of advantages over their unreal counterparts. Natural colorants are environmentally friendly. The majority of them are safe, and can be produced using cheap underdone materials. Diverse color shades are produced by unstable the culture setting (Shi et al., 2015) while the majority of them are stable over a ample range of light intensity, temperature and pH (Mappari et al., 2005). conversely, for any pigment to be used as food colorant, it should be safe for human eating. The employ of fungal pigments as food colorant has been in exercise early in the past even before 1884 when the French Botanist Tieghem characterized the fungus *Monascus purpureus* (Tieghem, 1884). Among the fungi, members of the Class, Ascomycetes are the broadly studied group for pigment production. Some of the fungal pigments that have been permitted and presently used take in account Arpink red from *Penicillium oxalicum*, riboflavin from *Ashbya gossipii*, lycopene and Beta-carotene from *Blakeslea trispora* and *Monascus pigments* (Dufosse et al., 2013). Aside from adding preferred colors to foods, fungal pigments have other eye-catching qualities such as antimutagenic and antimicrobial (Visalakchi and Muthumary, 2010; Geweely, 2011; Teixeira, 2012); antioxidants (Shcherba et al., 2000; Cassia et al., 2005; Li et al., 2009;Gessler 2013); anti-cancerous and anti-obesity activities (Visalakchi and Muthumary, 2010; Feng et al.,2012). Manufacture of fungal pigments is exaggerated by both nutritional and ecological factors. Some review articles on production and uses of fungal pigments (Dufosse et al., 2014; Chen et al., 2015; Abdel Ghany, 2015; Vendruscolo et al., 2015) have shown that fungi are major sources of renewable and reliable natural food colorants. Though, commercial assembly of these pigments requires good indulgent of the factors that affect their production. Malt is generated as waste while cooking rice hence it can serve as the cheapest source for biopigment production. It can bring down the production cost when used for large scale production. Previous research and studies has shown that growth of *Monascus* require sucrose or dextrose as carbon source. Rice malt has rich source of sucrose (Ayernor *et al.,* 2007) and easy accessibility makes it a better substrate. Thus, the main intent of this study was to examine the potential of rice malt as a substrate for *Monascus* pigment production by submerged fermentation process.

Materials and Methods

Culture: A culture of *Monascus purpureus* (MTCC 369) was obtained from the Microbial Type Culture Collection (MTCC, Chandigarh, India) and used for the experiment. It was maintained on potato dextrose agar medium (Hi-Media, Mumbai, India); preserved at 4°C.

Inoculum preparation: Inoculum was prepared in potato dextrose broth (Hi-Media, Mumbai, India). It was incubated for 6 to 8 days, at 30°C. The sterilized substrate is directly inoculated from the broth culture.

Variables: Temperature (25, 30, 35ºC) , pH (4, 5, 6), Incubation time 6, 9 and 10 days respectively, nitrogen source 0.55%, 0.1% and 1% respectively.

Substrate: Rice malt was chosen as a substrate. It was collected from (Hostel Mess MM University Mulana). Experiments were conducted in 150 ml Erlenmeyer flasks containing 25ml of substrate. To get red pigment various pH4, 5 and 6 respectively of substrate was maintained using pH meter. Substrate was autoclaved at 121°C for 15min and cooled to room temperature. It was inoculated with *M. purpureus* from the broth culture and incubated at 25°C, 30°C and 35°C respectively. These conditions were maintained throughout the experiment.

Pigment extraction: From the fermented liquid substrate, the cells were removed using Watmann's filter paper #1. Cells were dried at 55°C in a hot air oven till it gets fully dried and then powdered. Pigment was extracted using 99% acetone as a solvent and separated using separating funnel. The solvent fraction was collected and concentrated by keeping in rotary shaker at 30 rpm for one hour.

RSM Experimental blueprint and Statistical examination

A Box-Behnken (Box and Behnken, 1960) factorial plan was used in the optimization of culture settings for the pigment production. Four-factors and five-level face-centered cube design requiring a total 29 experiments flask were adopted in this study. The self-determining variables studied were pH (X1), incubation temperature (X2,°C), incubation time (X3, days) and (X4, nitrogen source). The response (dependent variable) was pigment production (AU/g dry substrate). Each self-determining variable was studied at three coded levels (-1,0,+1). The minimum and maximum levels of each independent variable and the experimental design with respect to their coded and un coded levels are presented in Table.1

Table1 Levels of the four independent variables (factor) used in RSM.

Variables	Range of Levels		
	-1	0	+1
pH (X1)	6.0	7.0	8.0
Incubation temperature (X2)	30	35	4 0
Incubation time (X3)	24	48	72
Nitrogen source(X4)	$NaNO_3$ (0.1%)	$NaNO_3$ (0.5%)	$NaNO_3$(1%)

Results and Discussion

Cultural characteristics of *Monascus purpureus* MTCC 369

The growth of *Monascus purpureus* MTCC 369 is shown in Fig.1.A.The fungal mycelium started from 1mm diameter to 80mm after 6 days of incubation. In the initial stages, the mycelium was white in colour, followed by yellowish colour after 3 days of incubation and red colour after 6 days of incubation. Different stages of pigments can also been seen in Fig.1.B in a PDA slants and Fig.1.C to Fig.1.D. in rice malt as well.

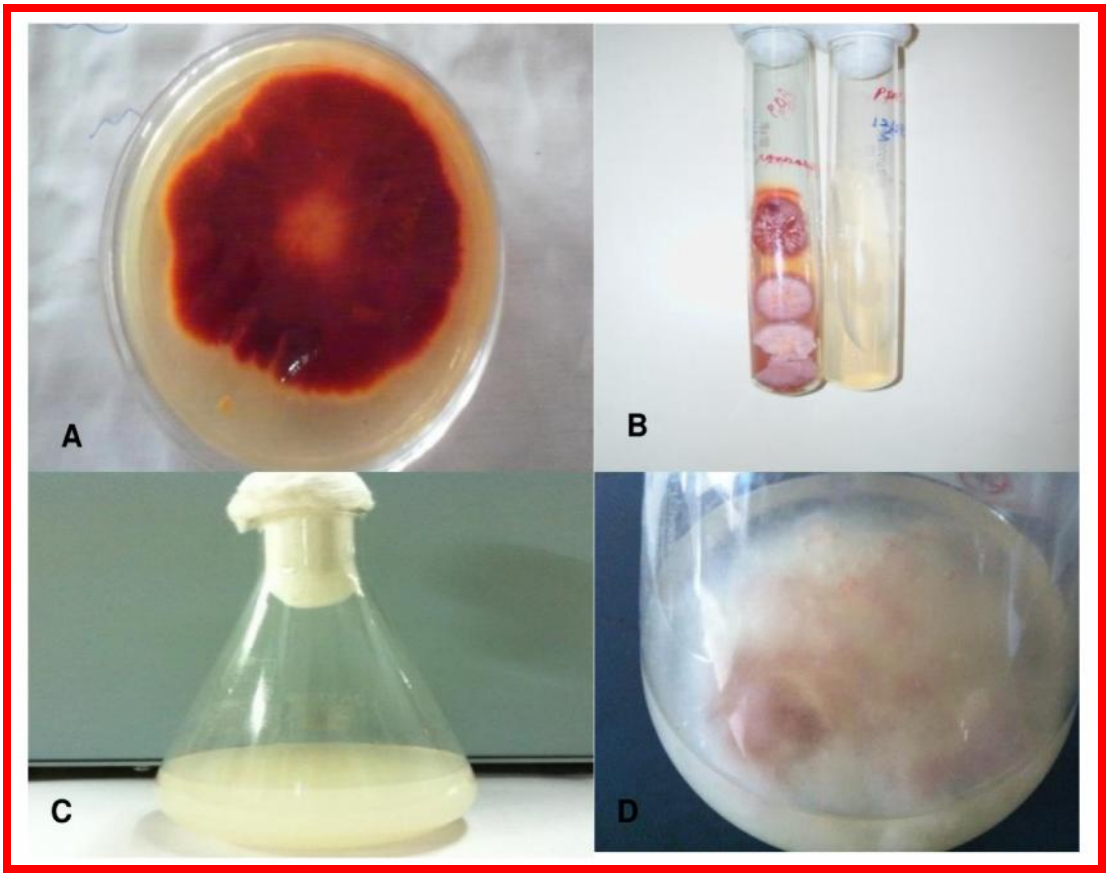


Fig.1.(A) Culture of *Monascus purpureus* MTCC 369 On PDA .(B) The fungal mycelium started from 1mm diameter to 80mm after 6 days of incubation. In the initial stages, the mycelium was white in colour, followed by yellowish colour after 3 days of incubation. (C) In the initial stages, the mycelium was white in colour, followed by yellowish colour after 3 days of incubation and (D) red colour after 6 days of incubation.

Optimization of Culture Conditions Using RSM

The statistical system is extensively used as means for checking the competence of several processes. In the current study it has been used with the purpose of obtaining information about the culture conditions (pH, incubation temperature, incubation time and nitrogen source) for the pigment production. To examine the combined effect of four different conditions (self-regulating variables) on pigment production, a Box-Behnken factorial design having five centre points leading to a total 29 experiments were performed. Equation (3) represents the mathematical model relating the production of pigment with the independent process variables, Xi and the second order polynomial coefficient for each term of the equation determined through the multiple regression analysis using the design expert. The experimental and predict value of yields of pigment production are given in table 2 and table 3. It was observed that the predicted values for pigment production were in high-quality accord with RSM plots. The p values are used as a tool to check the connotation of each coefficient, which also indicate the interaction strength between each independent variable; the smaller the p values, the bigger the significance of the corresponding coefficient table 4. The coded values of self-determining variables are also given.

Table 2.Experimental design used in RSM studies by using four variables at each three levels

Run	Temp	Time	pH	N	Actual	Predicted
1	35	216	4	0.55	0.49	Value
2	30	216	4	0.1	0.765	0.725625
3	30	216	6	1	0.564	0.589792
4	30	144	5	1	0.741	0.709625
5	30	216	5	0.55	0.646	0.6708
6	30	144	5	0.1	0.814	0.841292
7	25	216	5	1	0.548	0.55075
8	30	288	6	0.55	0.771	0.79325
9	30	288	5	0.1	0.986	1.007792
10	30	216	5	0.55	0.686	0.6708
11	25	216	6	0.55	0.645	0.666125
12	30	144	6	0.55	0.701	0.69075
13	30	144	4	0.55	0.63	0.630917
14	35	144	5	0.55	0.548	0.569458
15	30	216	5	0.55	0.684	0.6708
16	35	216	6	0.55	0.471	0.443625
17	35	216	5	0.1	0.578	0.598417
18	30	288	5	1	0.786	0.749125
19	30	216	5	0.55	0.664	0.6708
20	25	288	5	0.55	0.855	0.819958
21	30	288	4	0.55	0.701	0.734417
22	25	216	5	0.1	0.875	0.876417
23	30	216	4	1	0.589	0.606958
24	35	288	5	0.55	0.601	0.595458
25	30	216	6	0.1	0.893	0.861458
26	35	216	5	1	0.512	0.53375
27	30	216	5	0.55	0.674	0.6708
28	25	216	4	0.55	0.514	0.531792
29	25	144	5	0.55	0.648	0.639958

Table 3. Experimental design for pigment production by *Monascus purpureus* MTCC 369

Run	Temp	Time	pH	N	Actual	Predicted
1	35	216	4	0.55	Value	Value
2	30	216	4	0.1	0.398	0.382542
3	30	216	6	1	0.401	0.376542
4	30	144	5	1	0.542	0.566375
5	30	216	5	0.55	0.424	0.439375
6	30	144	5	0.1	0.548	0.562875
7	25	216	5	1	0.634	0.635542
8	30	288	6	0.55	0.381	0.379375
9	30	288	5	0.1	0.391	0.376042
10	30	216	5	0.55	0.669	0.658583
11	25	216	6	0.55	0.492	0.496083
12	30	144	6	0.55	0.359	0.341083
13	30	144	4	0.55	0.374	0.370583
14	35	144	5	0.55	0.355	0.335583
15	30	216	5	0.55	0.575	0.555917
16	35	216	6	0.55	0.462	0.46725
17	35	216	5	0.1	0.488	0.493583
18	30	288	5	1	0.44	0.455375
19	30	216	5	0.55	0.372	0.381875
20	25	288	5	0.55	0.479	0.483042
21	30	288	4	0.55	0.425	0.423542
22	25	216	5	0.1	0.49	0.505042
23	30	216	4	1	0.729	0.703875
24	35	288	5	0.55	0.32	0.359042
25	30	216	6	0.1	0.408	0.406875
26	35	216	5	1	0.589	0.606985
27	30	216	5	0.55	0.541	0.4994
28	25	216	4	0.55	0.893	0.861458
29	25	144	5	0.55	0.512	0.53375

Statistical analysis and modeling

The data of dry weight obtained was subjected to analysis of variance (ANOVA), appropriate to the design of experiments. The mathematical relationship of the independent variables and the responses were calculated by the second equation.

The second order model used to fit the response to the independent variables is shown in the equation 3 given below-

Eq.3 $Y = \beta_0 + \sum \beta i x i + \sum \beta i i x^2 i + + \sum \beta i j x i x j$

Where, Y is the response (dry weight of fungal biomass) ; β_o , βi, $\beta i i$, $\beta i j$ are regression coefficients for intercepts, linear, quadratic and interaction term, respectively and xi and xj are independent variable.

Final equation in term of actual factors

Fungal biomass (R1)= -4.63211+0.27196*A+(-0.00271)*B+0.7495*C+(-0.82984)*D-0.00011*A*B-0.0075*A*C-0.029*A*D+(-3.5E-06)*B*C+(-0.00098)*B*D-0.085*C*D-0.00403*A^2-1.66E-05*B^2-0.04473*C^2-0.345144*D^2

Final equation in term of coded factors

Fungal biomass (R1) = 0.6708-0.07375*A+0.0515*B+0.029667*C-0.09758*D-0.0385*A*B-0.0375*A*C-0.06525 *A*D + (-0.00025) *B*C+(-0.03175)*B*D-0.03825*C*D-0.10086*A^2-0.086267*B^2-0.04473*C^2-0.69892*D^2

Where R1 is the Fungal biomass of the fungal biomass, as a function of the coded levels of Temp (A), Time (B), pH (C) and Nitrogen Source (D).

Determination of Significant Variables

Model Validation The adequacy of the model and fitness were evaluated by ANOVA (Analysis of variance) and regression coefficients for the experimental design used (table numb). The ANOVA for the quadratic model was highly significant with an F value of 30.35922 as shown by fisher's F- test, along with very low probability value (P model> F=0.0001), which has significant at 95% confident interval.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The " lack of Fit F- Value" of 5.183104 implies lack of Fit is non significant. The insignificant lack of fit test also indicated that the model was suitable to navigate the design space.

Analysis of variance

The coefficient of determination (R^2) was calculated as 0.968414 for dry biomass which is given in table 4. The highest R^2 value also showed the good agreement between the experimental results and the theoretical values predicted by the model 9 Weisberg, 1985) and it showed that the model was suitable to represent the real relationship among the selected factors. This clearly shows that this model is an adequate predictor of the experimental conditions and confirms that selected process parameters significantly influence fungal biomass production.

Table 4. Model validation used for production of pigment by *M. purpureus* MTCC 369 (O.D/ mg dry biomass)

ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	Df	Square	Value	Prob > F	
Model	0.255275	14	0.018234	30.65951	< 0.0001	significant
A-A	0.013267	1	0.013267	22.30746	0.0003	
B-B	0.045633	1	0.045633	76.73045	< 0.0001	
C-C	0.003605	1	0.003605	6.06221	0.0274	
D-D	0.147187	1	0.147187	247.4881	< 0.0001	
AB	0.00366	1	0.00366	6.15455	0.0264	
AC	4.9E-05	1	4.9E-05	0.082391	0.7783	
AD	0.009216	1	0.009216	15.4963	0.0015	
BC	0.009409	1	0.009409	15.82082	0.0014	
BD	0.0057	1	0.0057	9.58472	0.0079	
CD	0.001444	1	0.001444	2.428023	0.1415	
A^2	0.011804	1	0.011804	19.84736	0.0005	
B^2	0.001565	1	0.001565	2.631627	0.1271	
C^2	0.002802	1	0.002802	4.711134	0.0477	
D^2	0.000628	1	0.000628	1.05641	0.3215	
Residual	0.008326	14	0.000595			
Lack of Fit	0.006111	10	0.000611	1.103452	0.5037	not significant
Pure Error	0.002215	4	0.000554			
Cor Total	0.263601	28				

Table 5.Analysis of variance for fungal biomass (mg) production

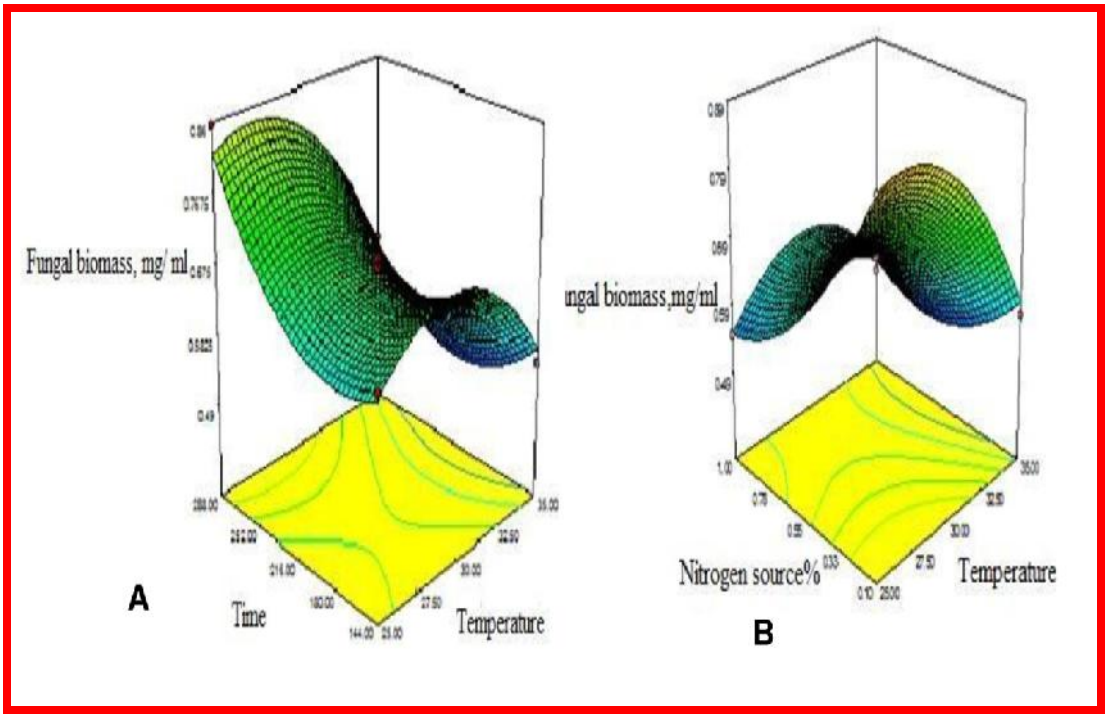
Std. Dev.	0.032765
Mean	0.675172
C.V. %	4.852848
PRESS	0.082051
R-Squared	0.968111
Adj R-Squared	0.936223
Pred R-Squared	0.825912
Adeq Precision	23.94139

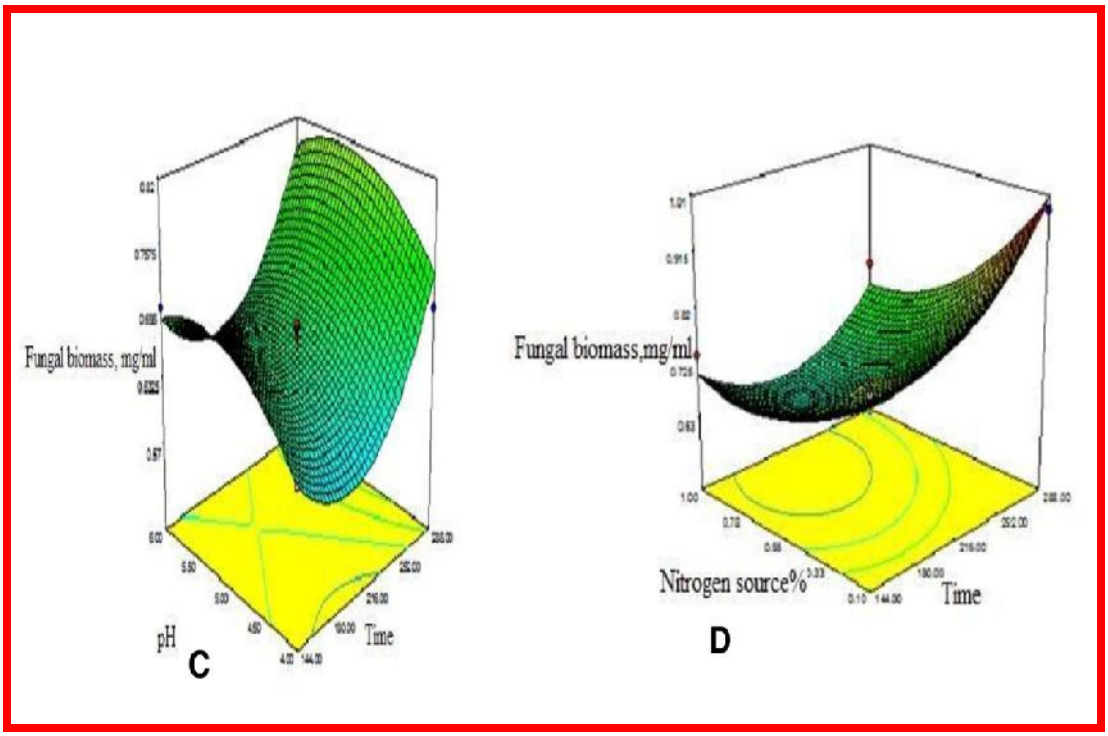
" Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 20.1 indicates an adequate signal. This model can be used to navigate the design space. At the same time, relatively lower value of coefficient of variation (CV=5.179969%) indicated a better precision and reliability of the experience carried out.

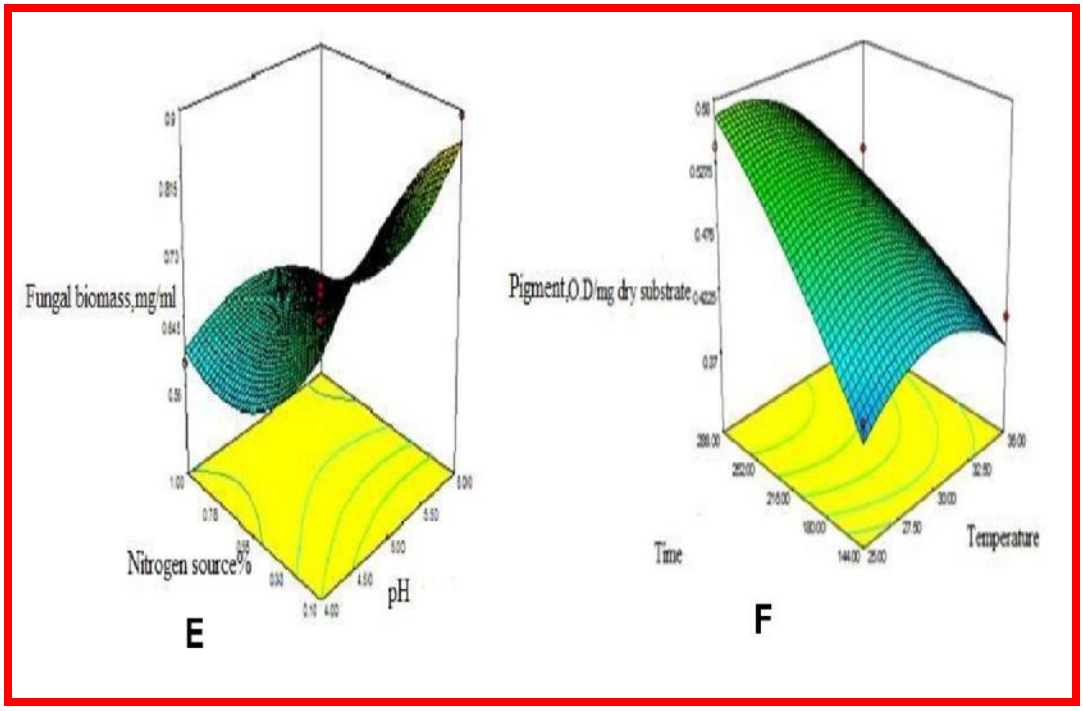
Box-Behnken (Box and behnken 1960), factorial design was used in optimization of cultural conditions for pigment production by *M. purpureus* MTCC 369. A set of 29 experiments were adopted in the study. The variables studied were pH, incubation time, concentration of N-source, temperature of incubation. Each variable was studied at three coded levels (-1,0,+1)

The three-dimensional (3-D) response surfaces (Fig.3.A-Fig.3.F) were plotted on the basis of the model equation to investigate the interaction among variables and to determine the optimum concentration of each factor for maximum pigment production. The three-dimensional plots (Fig.3.A) show that the increase in time and temperature resulted an increase in the fungal biomasss production to optimum values of 288 h and 30°C, respectively, where further decrease with time and temperature. The three-dimensional plots (Fig.3.B) show that the increase in temperature and pH caused an increase in the fungal biomass production to optimum values of 30°C and pH 6.0 respectively, whereas further increase in temperature and pH decreased the pigment production. The three-dimensional plots (Fig.3.C) show that the increase in nitrogen level and temperature resulted an increase in the ethanol production to optimum values of 0.01% and 30◦C respectively, whereas further increase in nitrogen level decreased the pigment production. The three-dimensional plots (Fig.3.D) show that the increase in pH and time increased in the fungal biomass production to optimum values of 6.0 and 288hr respectively.

The three-dimensional plots (Fig.3.E) show that the decrease in nitrogen level and increase time increased the fungal biomass production at an optimum value of 0.01% and 288 hr respectively. The three-dimensional plots (Fig.3.F) show that the decrease in nitrogen level increased in the fungal biomass production to optimum values of 0.01%whereas further increase resulted in less fungal biomass production.




Fungal biomass, mg/ml
pH
Time
C
Fungal biomass,mg/ml
Nitrogen source%
Time
D


Fungal biomass,mg/ml
Nitrogen source%
pH
E
Pigment,O.D/mg dry substrate
Time
Temperature
F

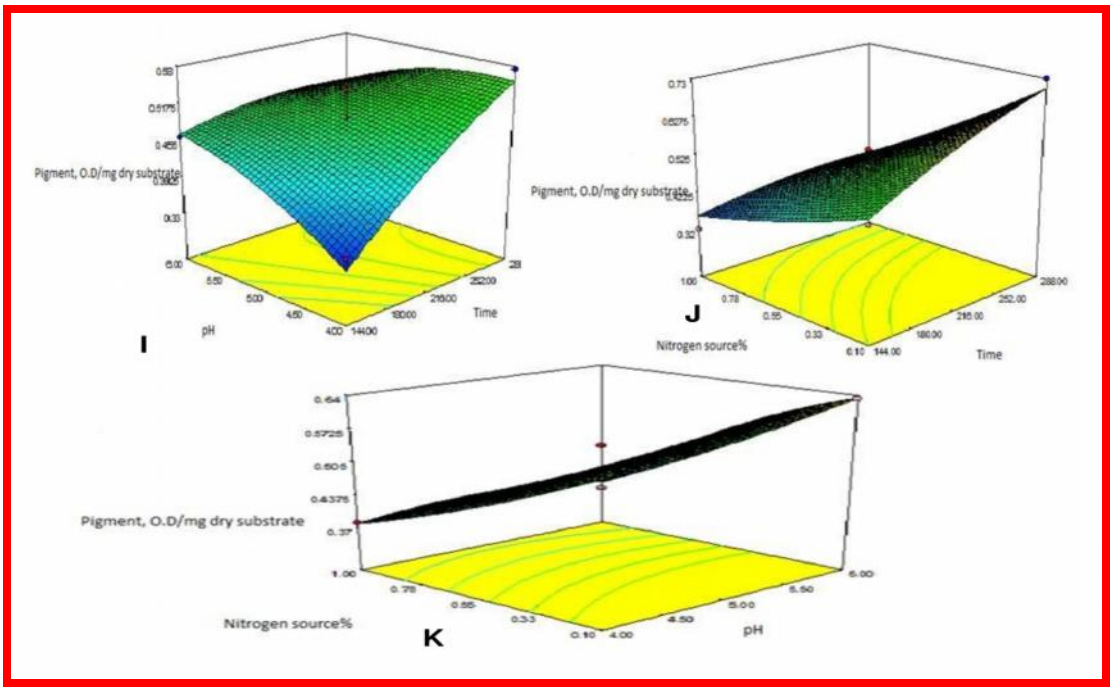


Fig.3 (A) Effect Of Time And Temperature On Fungal Biomass Production (B) Effect Of pH And Temperature On Fungal Biomass Production (C) Effect Of Nitrogen Source And Temperature On Pigment Production (D) Effect Of pH And Time On Fungal Biomass Production (E) Effect Of Nitrogen Source And Time On Fungal Biomass Production (F) Effect Of Nitrogen Source And pH On Fungal Biomass Production (G) Effect Of Time And Temperature On Pigment Production (I) Effect Of Nitrogen Source And Temperature On Pigment Production (J) Effect Of pH And Time On Pigment Production (K) Effect Of Nitrogen Source And pH On Pigment Production

Effect of incubation time on pigment production:

Maximum pigment production was obtained after 10 days of incubation. From the results it was evident that up to 6 days of incubation, the maximum growth of the fungus was observed. After 4 days the pigment production took place which was increased till 10 day of observation. However, Mukherjee and Singh, 2011 reported the pigment production was observed from 3 days of incubation. As shown in Fig.2A-Fig.2D.

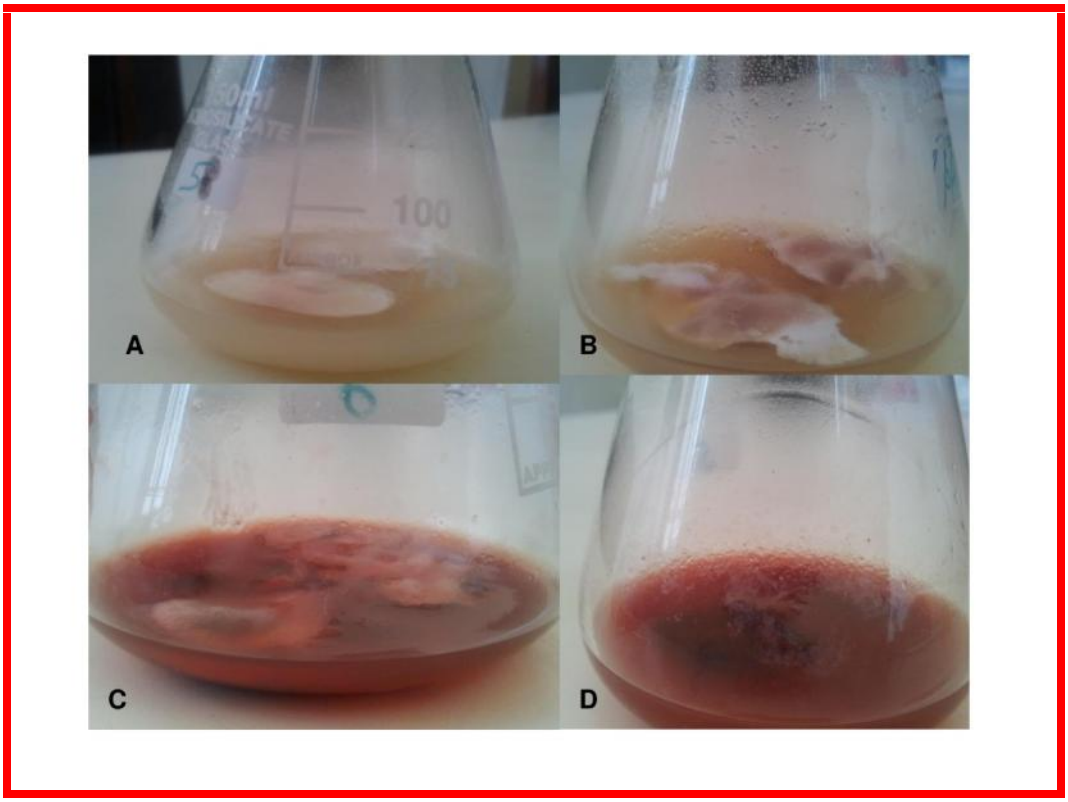


FIG.2.Different stages of growth and pigment level of *Monascus purpureus* MTCC 369 in rice malt
(A) *Monascus purpureus* MTCC 369 In Rice Malt After 3th Day Of Incubation (B) 6th Day Of Incubation Start Forimg Red Colour Pigment. (C) 9th Day Of Incubation Concentration Of Pigment Increasing (D)12th Day Of Incubation Complete Suspension Of Pigmention In Rice Malt

Effect of temperature on pigment production: Temperature is an important factor which has greater impact in pigment production as well as in growth of the fungus. It was observed that maximum production of red pigment was obtained at 30°C (Domsch *et al*., 1980). As temperature was increased the concentration of red pigment decreases (Carvalho *et al.,* 2005).

Effect of initial pH of the medium: The pH of the medium is a major factor in pigment production. It was observed that at lower pH, there was no red pigment produced. The higher pH, between 6 to 8 has shown the appearance of red pigments (Yongsmith *et al*., 2013). Maximum red pigments were observed at pH 6. Red pigment was more evident at pH 6.0 (500nm) but acidic pH supported yellow pigment production. At low initial pH (2.0–4.0), yellow pigment was subjugated (at 400nm) but declined stridently beyond pH 5.0. Though, the orange portion maintained a secure state irrespective of pH condition (at 480 nm). At higher pH (6.0–8.0), the red pigment was found to be dominating.

Effect of N-Source: Two nitrogen sources were tried in preliminary trial for pigment production (Sodium nitrate and Ammonium Sulphate). The addition of ammonium sulphate resulted in less pigment production, therefore Sodium nitrate (at 0.1-1.0%) was used in the experimental design. Mukherjee and Singh, 2011 reported monosodium glutamate at 0.3% as nitrogen source suitable for pigment production. However, peptone-supplemented medium was not found suitable as it declined the pigment production, drastically.

Summary and Conclusion

Monascus purpureus pigment fermentations have been performed chiefly in solid cultures, however production yields have been too low to allow commercial scale production. a number of factors can manipulate the pigment production by *Monascus* gaseous environments, agitation and aeration, source of carbon and nitrogen have been reported as parameters that sturdily manipulate growth and secondary metabolite production. RSM uses experimental plan, algorithm inference, and system analysis to reveal the effect of individual factors on the outcome of the multi-factor experiment, and to seek

the optimum condition for each variable, which can be expressed in terms of a mathematic function. The figures generated illustrate the effect of the variables on the products and the interaction between variables. RSM has several advantages, including requiring fewer experiments, suitability for multiple factor experiments, demonstration of relations between factors, determination of the most suitable conditions, and the ability to forecast responses. At present, because of the high-cost processes in *Monascus pigment* production, various agro industrial materials such as wheat bran, corn meal, grape waste, dioscorea and jackfruit seed powder have been performed as substrates in solid-state fermentation. However, the low pigment productivity compared with rice fermentation seems cannot satisfy the request of industrialization. In fact, the sorts of rice selected have a great influence to the metabolites of *Monascus*. Indica rice, sticky rice and non-glutinous rice were acted as basic substrate respectively. The rice malt added with other carbon sources (dextrose) had been shown for better yield of pigment. Since, malt is generated as waste while cooking rice hence it can serve as the cheapest source for biopigment production. It can bring down the production cost when used for large scale production. Previous research and studies has shown that growth of *Monascus* require sucrose or dextrose as carbon source. Rice malt has rich source of sucrose and easily availability makes it a better substrate.

The present work describes the conditions for pigments production by *M. purpureus* in submerged cultures using an kitchen waste rice malt as substrate. The conditions established for pigment production by *M. purpureus* were using 0.1-1.0% of Sodium nitrate, 6-12 days of incubation, 4.0-6.0 pH and 25-35°C of temperature of incubation. The inoculum level of fungal spores was kept similar in all the flasks of incubation. RSM exposed the following conditions to be optimal: 0.1% of Sodium nitrate at 30°C of temperature of incubation at 5.0 pH and 12 days of incubation resulted in maximum fungal biomass and pigment production.

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Conflict of Interests

The authors have not declared any conflict of interests.

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