ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Design of Medium Components for the Enhanced Production of Mycoprotein by *Fusarium venenatum* Using Plackett Burman Model.

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**ABSTRACT**

Mycoprotein is the ingredient that is completely meat-free form of high quality protein and is also a good source of dietary fibre, hence developed as a food source to combat food shortage. The present study aims to evaluate the influence of different media components on the growth of *Fusarium venenatum* which is considered to have high protein content. A Plackett Burman design (8 run) was adopted with 7 variables namely Jaggery water, date extract, $KH_2PO_4$, $K_2HPO_4$, $MgSO_4$, incubation time and inoculum size to identify the most significant parameter influencing the growth of the fungi. The experimental results showed that date extract, Jaggery water and $KH_2PO_4$ were found to substantially influence the growth followed by inoculum size, incubation time and $MgSO_4$. Under the mentioned conditions, the amount of biomass obtained was 4.9g/l and the protein content was found to be 33 (w/w).

**Keywords**: *Fusarium venenatum*, Mycoprotein, Jaggery water, Date extract, Plackettt Burman model

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ISSN: 0975-8585

## INTRODUCTION

The most critical step in the optimization of any fermentation process is the identification of different nutrients in the appropriate quantities as this determines the yield and overall economic feasibility of the process [1]. Conventional method involved screening for each category of source at an arbitrary level, one factor at a time, keeping the other parameters constant. The results that were obtained were used to select a few compounds in each category based on the highest product promotion followed by finding the optimum level of that compound. The scientists face an arduous task of screening a large number of components in the precise quantities involving large number of experiments over a long period of time consuming significant infrastructural facilities [2-4]. This situation can be well handled by Plakett Burman designs in which the number of sources/ parameters used in the experiment is one less than the number of experiments, thereby shortlisting few reliable sources for further optimization. The present study uses Plakett Burman design for optimizing the media components for the enhanced production of *Fusarium venenatum* which is considered to be a significant contributor for the production of mycoproteins.

Free from animal fat, trans fat and cholesterol, mycoprotein is an effective source of protein as well as fiber, which aids digestion. It provides vitamins, specially B vitamins and minerals, including zinc, along with essential amino acids. Mycoprotein is also low in sodium and hence considered as an alternative protein source at a time when the world was about to face a severe protein shortage.

In this context, the present study aims to increase the production of *Fusarium venenatum* in terms of its biomass by using different carbon and nitrogen sources using the Plackett Burman design. This would reveal the significant nutrient sources required for the maximum production of the biomass along with the ideal concentration and finally determine the mycoprotein obtained at the optimal conditions.

## MATERIALS AND METHODS

### Fungal strain

*Fusarium venenatum* was obtained from Fungal Biodiversity Centre, Netherland in lyophilized form. Oat Meal was used as the medium for the activation of fungi. The active fungus was maintained on the oat meal agar slants as monosporic cultures at 4°C.

### Inoculum Preparation

The fungus was cultivated in 100 ml of Vogel's minerals medium [5] (10 g glucose, 2.6 g $Na_3C_6H_5O_7 \cdot 2H_2O$, 2.52 g $KNO_3$, 2.88 g $(NH_4)H_2PO_4$, 1.6 g $KH_2PO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g, $CaCl_2 \cdot 2H_2O$, 2.5 mL of biotin solution and 5 mL of trace elements per liter). The trace elements solution consisted of 0.1 g citric acid, 0.1 g $ZnSO_4 \cdot 7H_2O$, 0.02 g $FeSO_4$, $(NH_4)_2SO_4 \cdot 6H_2O$, 5 mg $CuSO_4 \cdot 5H_2O$, 1 mg $MnSO_4 \cdot H_2O$, 1 mg $H_3BO_3$, 1 mg $Na_2MoO_4 \cdot 2H_2O$ per 100 mL. The pH of the medium was adjusted to 5.8. 1ml of spore suspension (95 spores/ml) was inoculated into the media and the fungal spore was collected after 15 days by scrapping off with a sterilized glass rod. A homogenous spore suspension was prepared in sterile distilled water by adding a few drops of the wetting agent Tween80 (0.01%). The spore concentration of the suspension was determined using an improved Neubauer haemocytometer (Germany).

### Placket Burman Design

Plackett-Burman design is statistically based experimental design to optimize fermentation media and is considered as an efficient approach to study the effects of several factors and to improve product yields. In the present study, 7 variables namely Jaggery water (50 g in 1000 ml of water), Date extract (100 g in 1000 ml of water), $KH_2PO_4$, $K_2HPO_4$, $MgSO_4$, inoculum size and incubation time were considered in an 8 run Plackett-Burman design [6]. Table-1 shows the variables employed and the concentration used.

ISSN: 0975-8585

**Table 1: Variables used for Plackett Burman model**

| Variables | | High | Low |
|---|---|---|---|
| A | Jaggery water | 50ml | 25 ml |
| B | Dates extract | 100 ml | 50 ml |
| C | $K_2HPO_4$ (g/L) | 1g/L | 500 mg/L |
| D | $KH_2PO_4$ (g/L) | 1g/L | 500 mg/L |
| E | $MgSO_4$ | 100 mg/L | 50 mg/L |
| F | Inoculum size | 5% | 2.5% |
| G | Incubation time | 92 h | 72 h |

**Biomass Estimation**

Biomass was estimated by wet weight method. Prior to biomass estimation, the broth was kept at 64 -65°C for 20-30 min to remove RNA content [7]. After the heat treatment, media was filtered through Whatman No.1 filter paper, the filtrate was discarded. Biomass transferred to pre dried Whatman No.1 filter paper, kept in sterile Petri plate, dried using an oven at 60°C to a constant weight.

## RESULTS AND DISCUSSION

In the present study, seven components were examined using Plackett-Burman Statistical experimental design. The selected components were Jaggery water (carbon source), Dates extract (nitrogen source), dipotassium hydrogen phosphate, dihydrogen potassium phosphate, $MgSO_4$, Inoculum size and Incubation time. Basic design was used for these experimental studies (8 run), three replicates were used and experiments were randomized.

The main effect was determined by the difference between the average of the H (high) and L (low) responses for each independent and dummy variable and given in equation 1. The effect of an independent variable on the response is the difference between the average response for the four experiments at the high level and the average value for four experiments at the low level and given in Eq 2.

$$\boldsymbol{\Sigma} A\,(H) - \boldsymbol{\Sigma} A\,(L) \quad \text{..Eq 1}$$

$$\boldsymbol{A} = \Sigma A\,(H)/4 - \Sigma A\,(L)/4 \quad \text{..Eq 2}$$

$$2(\Sigma A\,(H) - \Sigma A\,(L))/8 \quad \text{..Eq 3}$$

The experimental error can be calculated by averaging the mean squares of the dummy effects of E and G and provided in equation 3. The final stage is to identify the factors which are showing large effects. In the above experiment, this was done using an F-test which is calculated by using the formula

Factor mean square/error mean square.

The regression coefficient, F value and P value of the factors were calculated for biomass production using statistical design. [8]

**Table 2: Experimental Plackett - Burman Design Matrix and biomass obtained**

| Trials | A | B | C | D | E | F | G | Response Biomass in g/L |
|---|---|---|---|---|---|---|---|---|
| 1 | H | H | H | L | H | L | L | 3.5 |
| 2 | L | H | H | H | L | H | L | **5** |
| 3 | L | L | H | H | H | L | H | 4 |
| 4 | H | L | L | H | H | H | L | 3.4 |
| 5 | L | H | L | L | H | H | H | 4.3 |
| 6 | H | L | H | L | L | H | H | 3.7 |
| 7 | H | H | L | H | L | L | H | 4.2 |
| 8 | L | L | L | L | L | L | L | 3.4 |

ISSN: 0975-8585

Table 2 gives the details of the trails with varying concentrations along with the response in terms of biomass produced. It was observed that trial 2 was favorable for the maximum production of biomass which was found to be 4.9 g/L. Statistical analysis of Plackett Burman design was provided in Table 3.

**Table 3: Statistical analysis of Plackett-Burman design of each variable at different levels for Biomass production by *Fusarium venenatum***

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| | **Jaggery water** | **Date Extract** | **Di potassium Hydrogen Phosphate** | **Di Hydrogen potassium Phosphate** | **$MgSO_4$** | **Inoculum Size** | **Incubation time** |
| ΣH | 14.8 | 16.9 | 16.1 | 16.5 | 15.2 | 16.3 | 16.2 |
| ΣL | 16.5 | 14.4 | 15.2 | 14.8 | 16.1 | 15 | 15.1 |
| DIF EFF | -1.80 | 2.40 | 0.80 | 1.60 | -10 | 1.20 | 10 |
| MS | 0.405 | 0.72 | 0.08 | 0.32 | 0.125 | 0.18 | 0.125 |
| MSE | 0.20 | | | | | | |
| F TEST | 3.24 | 5.76 | 0.64 | 2.56 | 1 | 1.44 | 1 |
| P Value | 0.0001 | 0.0001 | 1.0000 | 0.0064 | 0.9830 | 0.6766 | 0.9830 |

It can be seen that the most significant parameters were date extract, Jaggery water, $KH_2PO_4$ as their *p*-value was less than 0.05, followed by inoculum size, $MgSO_4$ and incubation time.

It is well justified in earlier report provided by Wiebe [7] where in *Fusarium venenatum* was used as food and feed, Prakash et al [9] also reported the potential antioxidant and anticancer activity of *Fusarium venenatum*. The extraction of the mycoprotein was successfully demonstrated by Prakash et al [10] using the simple process of grinding.

In the present investigation, the first significant component identified for the growth of *Fusarium venenatum* was found to be the date extract. Dates being rich in sugars and proteins can be used both as carbon as well as nitrogen source. Hosseini et al [11] reported that *Fusarium venenatum* was identified as a noteworthy producer of mycoprotein with date sugar (14g/l) being the significant medium component for maximum production of biomass and mycoprotein (47.34 %). Similarly Chauhan et al [12] also reported that date sugars were also used in enhancing the lactic acid production from *Lactobacillus* sp. KCP01.

The second significant component identified in the present study is Jaggery water for the growth and production of mycoprotein. It contains natural goodness of minerals and vitamins especially iron [13] inherently present in sugarcane juice and hence crowned as one of the most wholesome and healthy sugars in the world.

The third important compound necessary for the growth of the fungi is dihydrogen potassium phosphate (p value: 0.0064). This result is supported by the data obtained by Hosseini et al [11] who also reported Dihydrogen potassium phosphate to be an important component in the production of mycoprotein.

A study by Hosseini et al [11] reported the ideal combination of media components to be date syrup 14 g/l, (NH4) H2PO4 3.5g/l, KH2PO4 1.6 g/l, temperature 30 °C, time 72 h, seed age 48 h, and seed size 10% v/v for the maximum production of 5.46 g/l *Fusarium venenatum* biomass. The present study suggests the inclusion of jaggery water as one of the media components as this also greatly supported the growth of the biomass (5 g/L) which is almost on par with the biomass produced by Hosseini [11]. Jaggery water apart from being a source of nutrition for the growth of the fungi, is also beneficial in terms of economy.

## CONCLUSION

The use of Plackett Burman model for designing the media components for the growth of *Fusarium venenatum* was demonstrated in the present study. This study reported the maximum production of biomass

(5 g/L) with the media composition being date extract, Jaggery water, $KH_2PO_4$, $MgSO_4$, inoculum size and incubation time. The most significant parameters that influenced the growth of the fungi was found to be date extract, Jaggery water, $KH_2PO_4$ as their p value was less than 0.05 followed by inoculum size, $MgSO_4$ and incubation time. Therefore, this study successfully demonstrated the use of Plackett Burman model for the effective growth of *Fusarium venenatum* in the designed media to enhance the mycoprotein production.

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