ORIGINAL RESEARCH PAPER

Botany

STUDY ON EFFECT OF PHYSICOCHEMICAL PARAMETERS ON GROWTH OF *FUSARIUM EQUISETI* (MTCC9658)

KEY WORDS: Biomass, Dextrose, *Fusarium equiseti*, Microorganisms, Nutrients

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ABSTRACT

The microorganisms like fungi proliferate in different environmental and ecological conditions depending upon the availability of nutrients and the form of nutrient that they absorb. In addition to this the physical conditions like temperature, pH, salinity and light etc. also effect the growth and biomass production of fungal species. The present study was carried out to determine the effect of variation in physical and chemical parameters on growth and biomass production of the fungal strain *Fusarium equiseti* (MTCC9658). A maximum biomass yield of 12.24 g/L at pH 5 and temperature of 30°C was recorded; and minimum of 5.21 g/L at pH 9 and temperature of 25°C. The observations clearly indicate that pH 9 and above did not support higher biomass production at any of the temperatures, while pH 5 to pH 6 supports higher dry biomass production in all temperature ranges applied. The different carbon and nitrogen sources used in the medium corresponded to variation in growth of the strain. Dextrose in combination with Yeast extract as nitrogen source was best for biomass production.

INTRODUCTION

The microorganisms thrive in different environmental and ecological condition differently. Their growth depends on the nutritional elements present in the surrounding they proliferate in, synthetic medium or, development on host and also the absorbable forms of nutritional elements. The different carbon and nitrogen sources comprising a synthetic culture medium have great effect on biomass production of the microorganisms. The culture medium composition has immense effect on the production of the extracellular proteins, carbohydrates, secondary metabolites and other valuable compounds. *Fusarium* spp. are producers of many mycotoxins and are responsible for causing frequent contaminations of cereal and fruit rots too. Since they produce type A trichothecenes, a type of sesquiterpenoid differing from other trichothecenes because they do not have a carbonyl group in position C8. Chromic exposure to trichothecenes causes weight loss, bloody diarrhea and pathological changes in liver and stomach as well as stunted growth and reproductive defects (Gil- Serna *et al*, 2014). On the other hand few species have been investigated for useful natural products too. The marine fungi, fungal endosymbionts and lichens are being investigated for natural colorant sources. In one of the studies by Dawoud *et al* in 2020, a yellow colored pigment was produced from endolichenic *Bacillus* sp. that was isolated from a lichen known as *Dirinaria aegialita.* The bacterial strain characterized as *Bacillus gibsonii* was able to produce yellow pigments in Luria- Bertani medium. It was isolated and partially purified using thin layer chromatography and FTIR and GC-MS analysis showed its possibility of carotenoid formation. Additionally the pigment exhibited antifungal activity against 3 fungal pathogens: *R. solani*, *F. oxysporum* and *S. rolfsi*, and antioxidant properties too.

Fusarium equiseti is synonymously also known as *Gibberella intricans* Wollenw. 1930 (Schoch *et al*, 2020). *F. equiseti* has not been much investigated for its brighter prospects in terms of industrial and biotechnological research. The undertaken study has been carried out to investigate the effect of variation in physical and chemical parameters on the growth and biomass production of *Fusarium equiseti.*

The availability of nutrients and essential matter for growth provided by the host to the pathogenic fungi are important tools to study the host pathogen interaction and their control strategy. The *in vitro* studies of cultivating the fungal pathogens in a number of manipulated synthetic medium helps to know the effect of the physical and chemical parameters on their growth and sporulation. A number of physical and chemical factors are there that affect the growth and sporulation of different fungi. Physical factors like- temperature, pH, light and aeration etc. (Dhingra and Sinclair, 1995) and chemical factors that are basically the chemical composition of the culture media like- carbon sources, nitrogen sources, mineral salts/ions and other undefined supplements. In one of the studies by Ramteke & Kamble (2011) sucrose was also considered the best carbon source for the strain *Fusarium solani.* In another study by Sharmin *et al.* (2012) it was found that Peptone and Sucrose were most suitable nitrogen and carbon source for *Fusarium oxysporum*. Mailto *and* coworkers in 2017, found that temperature and pH affected the spore formation and spore size in pathogenic fungi *Fusarium oxysporum* isolated from Chickpea. They found that the temperature of 30°C and pH from 6 to 7 supported higher sporulation and dry mycelia weight yield.

MATERIALS AND METHODS

Revival of fungal cultures

Fungal strain *Fusarium equiseti* (MTCC9658) acquired from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh, India; was revived and maintained on Potato Dextrose Agar medium (PDA- Potato infusion 200g/L, Dextrose 20g/L and Agar 20 g/L).

Effect of pH and temperature

To study the effect of pH and temperature on growth and biomass production of *Fusarium* (MTCC9658), the fungal spores in concentration of 10^6 spores/ml was inoculated in Potato Dextrose Broth (PDB) medium. The pH was set ranging from 3 to 9, and each set was incubated at three different temperatures i.e. at 25°C, 30°C and 35°C. The cultures were incubated for 4 weeks for growth. Each set of experiment was performed in triplicates and observations recorded as average of three values.

Effect of different carbon, nitrogen sources and N/C ratio

The fungal strain *Fusarium equiseti* was cultured in varying *nitrogen to carbon ratio* (N: C) as nutrient in the ratio- 1:6, 1:4 and 1:2. The 3 carbon sources namely dextrose, sucrose and

lactose, each in combination with four different nitrogen sources -Yeast Extract, Peptone, Potassium nitrate [KNO_3] and Ammonium Sulphate [$(NH4)_2SO_4$] supplemented in basic PDA medium, in above mentioned three different ratios were used to optimize the culture media for growth and biomass production in submerged culture condition. Each set of experiment was performed in triplicates and observations recorded as average of three values.

RESULTS AND DISCUSSION

Effect of pH and temperature

Fusarium equiseti (MTCC9658) cultivated at 25°C temperature, the maximum biomass production of 8.98g/L was recorded at pH 6 while minimum of 5.21g/L at pH 9 (Table 1). At 30°C the highest dry biomass produced was 12.24g/L at pH 5 and lowest of 6.01g/L at pH 9. A maximum biomass yield of 9.21g/L and minimum of 5.39g/L at pH 6 and pH 9 respectively was produced at 35°C. The observations clearly indicate that pH 9 did not support higher biomass production at any of the temperatures, while pH 5 to 6 supports higher dry biomass production in all temperature ranges applied (Fig 1).

Effect of different carbon, nitrogen sources and N/C ratio:

DEXTROSE: As carbon source Dextrose was used in combination with 4 different nitrogen sources supplemented to basic PDB medium in three different N:C ratios, resulted in highest biomass production of 10.76g/L at N:C =1:6 with Yeast extract as nitrogen source and lowest of 4.22g/L with Ammonium Sulphate as nitrogen source in the ratio N:C=1:2.

SUCROSE: When Sucrose was supplemented to basic PDB medium in place of Dextrose with 4 different nitrogen sources in 3 ratios, it showed maximum dry biomass yield of 8.64g/L with Peptone as nitrogen source in the ratio N:C=1:6 whereas a minimum of 3.20g/L with yeast extract in the ratio N:C= 1:2. There was no growth seen at all in Ammonium Sulphate in any ratio with Sucrose.

LACTOSE: When Lactose was supplemented to basic PDB medium in place of Dextrose with 4 different nitrogen sources in 3 ratios, highest yield of 6.22g/L dry mycelia obtained at N:C= 1:6 with Yeast extract as nitrogen while minimum biomass of 2.14g/L with Potassium Nitrate as nitrogen source at ratio N:C=1:2 (Fig 2).

pH	Mycelial weight in g/L at 25° celsius	Mycelial weight in g/L at 30° celsius	Mycelial weight in g/L at 35° celsius
pH3	6.12	8.64	8.51
pH4	6.11	11.42	7.23
pH5	8.62	**12.24**	8.65
pH6	**8.98**	6.33	**9.21**
pH7	7.36	7.35	8.27
pH8	7.25	7.65	6.66
pH9	**5.21**	**6.01**	**5.39**

Table.1. Dry mycelial weight at the end of 4 weeks recorded at 3 different temperatures with pH ranging from pH3 to pH9

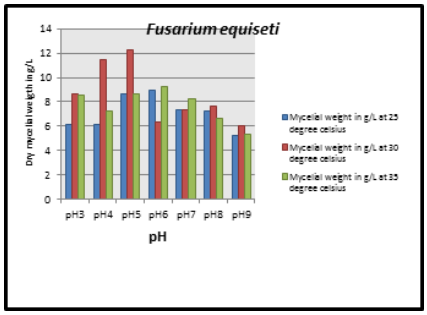

Fig. 1 Graph showing dry mycelial weight at the end of 4 weeks recorded at 3 different temperatures with pH ranging from pH3 to pH9

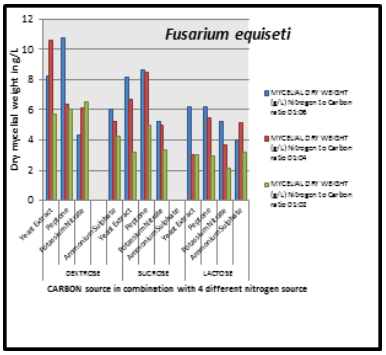

Fig. 2 Dry mycelial weight at the end of 4 weeks recorded in with 3 different carbon sources in combination with 4 different nitrogen sources used in 3 different N: C ratio (ie. 1:6, 1:4 and 1:2)

CONCLUSIONS

In the present study two physical parameters that are pH and temperature were studied. The investigation shows that the strain *Fusarium equiseti* (MTCC9658) gave high dry biomass yield at pH range of 5 to 6, and decrease in biomass was observed as the pH decreased to pH 3 and also upon increasing towards neutral and then to pH 9. Temperature of 30°C was best for the strain while at 25°C dry biomass production was moderate. The best pH and temperature combination to produce high biomass was pH 5 at 30°C.

In the undertaken study the chemical parameters examined for the growth and biomass production of *Fusarium equiseti* (MTCC9658) using basic PotatoDextrose Agar medium modified by adding other carbon and nitrogen sources revealed that Dextrose was the best carbon source producing high yield of dry biomass, although the strain was able to utilise sucrose and lactose as well but gave lesser growth as compared to dextrose. The combined effects of carbon and nitrogen sources were also analysed and it indicated that Yeast extract and Peptone were best nitrogen sources, Yeast extract being on top. Ammonium sulphate and Potassium nitrate gave lesser dry biomass yield with all carbon sources in all N: C ratios. Sucrose in combination with Ammonium sulphate produced no spores at all in submerged culture. Lactose with Yeast among different nitrogen sources gave high dry biomass yield. The results obtained in this study are in agreement with one of the studies by Maitlo *et al*. (2017) that depicts dextrose as best carbon source for the growth of pathogenic fungi *Fusarium oxysporum* isolated from Chickpea.

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