OPTIMIZATION OF GROWTH MEDIA USING FLOWERS OF MADHUKA INDICA EXTRACT FOR *ASPERGILLUS SPP*.

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Abstract: Optimization of growth media for *Aspergillus* species was done in this study. For this purpose using semi synthetic preparation containing extract of flowers of Madhuka indica, media was optimized having pH of 5.5. At these conditions, maximum fungal growth observed when ratio of flowers extract and water maintained at 1:4. This study shows that the semi synthetic media prepared from flowers of Madhuka indica was superior to synthetic media for fungi, for instance Sabourauds agar used in research laboratories. It may provide cost savings for a possible increase in production scale if used industrially as alternative to molasses, starch based media and other agro based residues employed for growth of *Aspergillus.*

Index Terms - Madhuka indica, Semi synthetic media, *Aspergillus* species, Sabourauds agar, Optimization

INTRODUCTION

Flowers of Madhuka indica J.F.Gmel plant, belonging to family Sapotaceae is abundant in the forests of Andhra pradesh, Chhattisgarh, Chandrapur and Gadchiroli districts of Maharashtra, India [1]. Madhuka indica flowers season generally starts in month of March and April. Yellowish green colored corollas fall on the ground. The moist dry flowers are rich in sugars like glucose, sucrose, fructose, maltose, starch (60 - 67%) [2] and crude proteins (4-6 %), ferrous (21-48%), fat (0.9-1.3%), ash (5-5.2%) as well as 150 mg of calcium with vitamins. [3]

Moisture content of dry flowers is 11% while carotenoids are present up to 33.09 µg per gram of flower. Besides these tannins, phenols, lipids, starch also present in it. They have good keeping qualities. [4]

Due to the above advantages present study is based on the usage of Madhuka indica flowers as key ingredients of media for fungi. It will become a great economic advantage in the Indian context as species fungi Aspergillus are widely used for production of citric acid, gluconic acid, and as biopesticide.[5] [6]

MATERIALS AND METHODS

1. Sterile Sabourauds agar plates.
2. Sterile Sabourauds broth containing tubes.
3. Whatman filter paper no-1
4. Cultures of *Aspergillus niger, Aspergillus carbonarius* and *Aspergillus flavus*

Collection of flowers- Madhuka indica flowers were collected in morning hours, in clean brown paper bags and brought to laboratory under hygienic conditions. Poorly developed, unhealthy and broken flowers were sorted out. [7]

Biochemical test of extract- 1 kg of flowers were washed twice with distilled water. Flowers were grounded into paste and then diluted with water in 1:5 (% w/v) ratios and then pressed to collect juice. Collected juice was then subjected to further analysis.
Total carbohydrates present in samples were estimated by phenol sulphuric acid method and for estimation of reducing sugars by di-nitrosalicylic acid method as well. Total proteins present in both samples were estimated by Folin Lowry method. [8][9]

Optimization of Media -
1 kg of flowers was grinded with water to make slurry. Slurry was diluted with water in 1:1, 1:2, 1:4, 1:8 ratios (w/v). Sets of diluted preparation containing flasks were labeled appropriately. In 100 ml slurry containing flask, following ingredient were added.
1) Urea 0.05 % and Ammonium sulfate, 0.06% as a source of Nitrogen.
2) 0.12 gm % Sodium potassium tartarate as a chelating agent.
pH was maintained to 5.5.
3) 2 gms of Agar powder.
Media was sterilized at pressure of 10 lb/ $inch^2$
Sterile media containing plates were prepared from each set.
Simultaneously for comparison purpose Sabourauds media containing plates were prepared.
Inoculations of test cultures of *Aspergillus spp*. were made on all test media and Sabourauds plates and incubated at 35^0 C for 48 to 72 hours.

Biomass measurement- 3 sets of tubes, each containing 20 ml of Sabourauds broth and 3 sets of tubes containing broth of media under testing were prepared, and both sets were inoculated with 10^4 conidial dosages of *Aspergillus niger*, *A. carbonarius* and *A. flavus* separately. Liquid shaken cultures were allowed to grow for 3 days at 35^0C. After growth, it was filtered using whatman filter paper number 1, (which was weighed initially, before filtration).The process was applied for all the 6 tubes of 3 sets. The biomass then dried in the oven at 50^0C, and weighed. Results were recorded of all the 3 sets. Initial weights of whatman filter were subtracted from weight of paper with biomass (final weight).

RESULTS AND DISCUSSION

Total carbohydrates in the flowers of Madhuka extract were estimated by phenol sulphuric acid method and were found 56%. While reducing sugars were 30% when estimated by DNSA method. 5% total proteins were estimated using Folin lowery method.

When *Aspergillus* species cultured on test media having ratios of Flowers: water of 1:1, 1:2, 1:4, 1:8, it was observed that maximum growth was obtained on plates containing 1:4 dilution of slurry of flowers. Similarly luxuriant growth of *Aspergillus* observed *on* test media when compared with growth on Sabourauds media. (Fig-1, 2, 3)

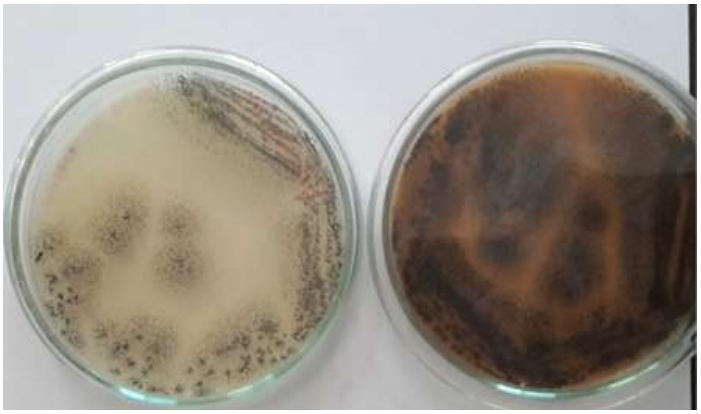

fig-1: *Aspergillus carbonarius* on sabouraud's agar and on test media respectively

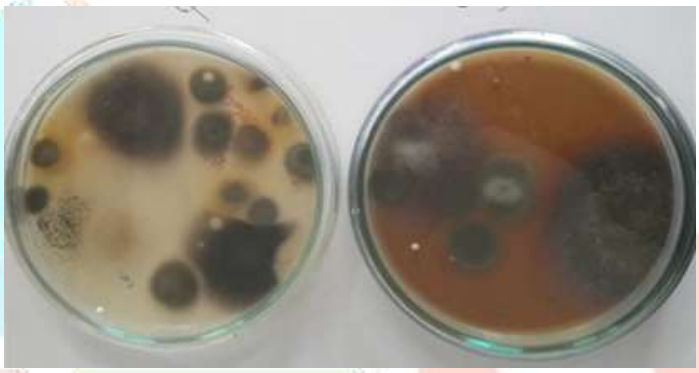

fig-2: *Aspergillus niger* on sabouraud's agar and on test media respectively

fig-3: *Aspergillus flavus* on test media

Results of measurement of biomass weight are as per table number 1.

Table: 1 weight of biomass on sabourauds media and on test media

Fungi	Weight of biomass on Sabourauds media	Weight of biomass on Test media
A. niger	1.390 gm	2.099 gm
A. carbonarius	1.260 gm	2.100 gm

A. flavus	0.900 gm	1.700 gm

CONCLUSION

In this work, the semi synthetic culture medium was optimized for fungi *Aspergillus*, which is extensively used for production of citric acid as well as for biopesticide preparation. The composition of synthetic media like Sabourauds media was based on mycological peptone, dextrose, which can be expansive for laboratories working on these fungi. For industrial applications, usually carbohydrate sources for these fungi are molasses, starch based media and other agro based residues. The current study shows that, media prepared from Madhuka indica flowers have shown enhanced growth in terms of biomass when compared with growth on Sabourauds media. Madhuka indica will be alternative source for the operation at high volume fermenters. For this reason use of slurry of flowers of Madhuka indica with added nitrogen source will be cost-effective method.

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