ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

PRODUCTION AND OPTIMIZATION OF ECO-FRIENDLY NATURAL COLORANT BY A SOIL ISOLATE *JATHINOBACTERIUM SP*

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Abstract:

Now a days there is an impulse to search for eco-friendly or natural pigments particularly microbial pigments because there is an increasing awareness regarding the ill- effects of artificial pigments on food, environment and health. These microbial pigments are light absorbing compounds with unique physiochemical properties like stability to light, heat and pH. Microbial pigments are not only eco-friendly but also known for their numerous beneficial properties like antimicrobial, antiviral, anti-larval and anti-cancer.

In this study, pigment producer was screened from soil and the pigment production was done on a modified nutrient medium. The optimization of the pigment production under various fermentation conditions was carried out on laboratory scale and the pigment was extracted by solvent extraction method.

The organism was gram negative short bacillus of *Jathinobacterium*species. The higher yield of violacein pigment by *Jathinobacterium species* was under optimum nutrient concentrations which consist of 0.5% glucose, 1.5% peptone as carbon & nitrogen source respectively, and growth conditions of pH 8.0 at 37°C for 72 hr under steady state. However, this *Jathinobacterium*species can be exploited for large scale production of violacein pigment which can be further implemented in different industries for various applications.

Keywords: Violacein, *Jathinobacterium*species, Optimization, Production, Microbial pigments.

INTRODUCTION

The plants and microorganisms are the two potent naturalsources of bio-pigments. Among natural sources of pigments, microbial pigments are preferred over plants.Production of microbial pigmentsis not seasonal, and show a higher productivity in terms of availability, stability, cost efficiency, labour, yield and easy downstream processing **[1,2].** These pigments have been used widely in the field of pharmaceutical, dairy, fish, printing and textile industry. The various types of pigments produced by microorganisms are carotenoids, melanins, flavins, monascins, violacein and indigo. The microbial pigmentspossess important properties likestability to light, heat and pH **[3,4].** It has attracted increased interest owing to its important biological activitieslike bactericidal, antiviral, antioxidant, anti-protozoan, anticancer and pharmacological potential **[5].**

Violacein pigment (Violet) is produced as a result of secondary metabolite by microorganisms. This pigment is produced by a variety of different organisms including *Chromobacterium*, *Pseudoalteromonas*, *Janthinobacterium, Collimonas* and *Duganella* belonging to various

ISSN: 2278-4632
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genera.These microorganisms resides in a variety of ecosystems in tropical, subtropical regions and are a normal inhabitant of soil and water**[6,7].**The production of violacein pigment was affected by physical parameters, such as temperature, agitation and pH or nutritional factors. In *Chromobacterium violaceum*, production of violacein wasregulated via the quorum sensing molecule, N-hexanoyl-L-homoserine lactone (HHL)**[8].**

The use of synthetic colours has carcinogenic and other toxic effects on the human health and cause environmental pollution too. Thus use of synthetic colors is not appropriate. At the same time there is an increasing demand of pigments due to their varied applications in different industries.Therefore,to meet the demand it is necessary to explore eco-friendly source of natural coloranti.e.microbial pigments. Thus objective of the present study is to screen the pigment producing organismfrom various environments and to carry out optimization for its production in a controlled manner.

MATERIALS AND METHODS

Sample collection, Screening and identification

Soil samples were collected in a sterile container from various places in Ulhasnagar, Maharashtra.

The screening of pigment producer was done by sprinkling sterile polished rice grains over the surface of 5 gm. of moist soilin a petri plate. The plates were incubated at 37^0C for 5 days **[9].** The isolate was purified by streaking desirable growth aseptically on nutrient agar and rice agar plate. Then the culture was maintained on 0.1% (w/v) peptone water for each experiment**[10].** Identification of the isolates was done as per **Bergey's manual of determinative bacteriology, 1987 [11].**

Production and extraction of pigment

The production of crude violacein was done in100 ml of the modified nutrient broth (Glucose, Peptone, Yeast extract, Beef extract) at 37^0C for 48 hr. For the extraction of pigment, the cells were separated by centrifugation and lysed by SDS. The pigment was extracted using ethyl acetate and ethanol (1:1) v/v and centrifugation done at 16000xg for 10 minutes to get the upper organic phase containing violacein. The extract was concentrated in a rotaevaporator under low pressure at 40°C and dried in glass petridishfor 2days at 60^0C **[12,13].**

Optimization of fermentation conditions for production of Violacein pigment

Effect of different carbon sources

Sterile modified nutrient broth (100 ml in 250 ml flask) was used for media optimization. Different carbon sources were used at 1% concentration like pure sugars (glucose, mannitol and sucrose) and cereals (jowar, bajra, maize, rice and wheat). After inoculation, the flasks were incubated at 37^0C for 48 hr.

Effect of glucoseconcentration

To determine the optimum glucose concentration, sterile modified nutrient brothsupplemented with different concentrations of glucose (0.1%, 0.5%, 1.5%, and 2.0%) was used.The flask were inoculated and incubated at 37^0C for 48 hr **[5].** The optimum glucose concentration was estimated for the bestpigment production.

Effect of peptone concentration

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The modified nutrient broth with varying concentration of peptone (0.5%, 1.0% and 1.5%) was used. The flask were inoculated and incubated at 37^0C for 48 hr.The optimum peptone concentration as the nitrogen source for the pigment production was recorded**[5].**

Effect of pH

The optimum pH for the highest yield of pigment was evaluated by using modified nutrient broth with varying pH 5,6,7,8, and 9. After inoculationand incubation at 37^0C for 48 hr., the optimum pH for violacein pigment production was estimated **[5].**

Effect of temperature

To evaluate the optimum temperature for the violacein production, the modified nutrient broth was inoculated and incubated under different temperatures of 15^0C, R.T., 37^0C and 52^0C**[5].**

Effect of agitation:

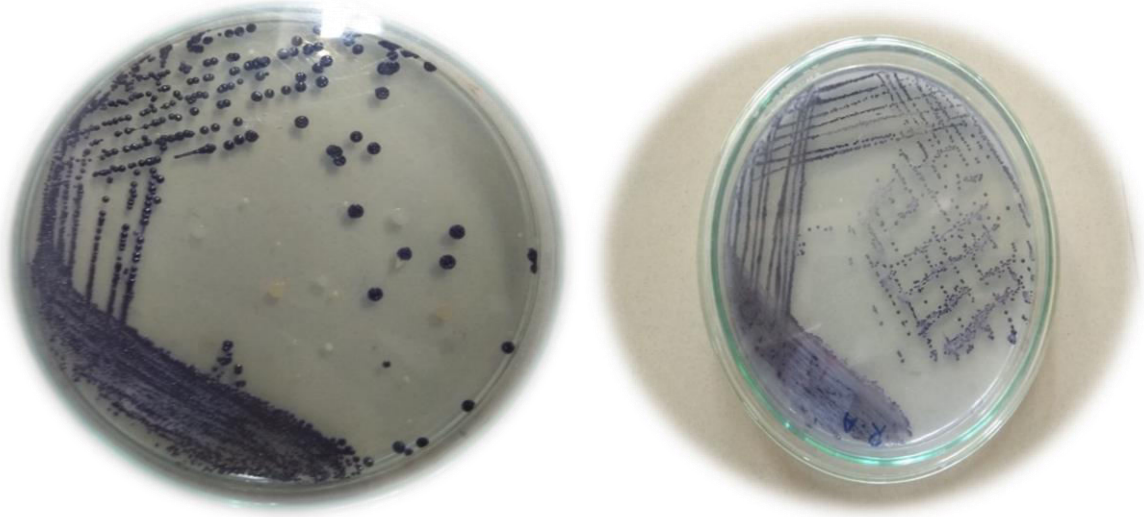
The effect of agitation on the production yield of violacein was determined by incubating fermentation flask of modified nutrient broth under static and shaker condition at 37^0C for 48 hr. **[5].**

Effect of incubation period

To determine optimum time duration for the best yield of pigment, the production flasks of modified nutrient broth were incubated for different time period ranging from 24 hr to 92 hr at the interval of 24 hr.

RESULTS AND DISCUSSION

The plates containing soil and covered with sterile rice grains were screened for violet coloured growth covering the rice grains. The growth of pure culture from rice grain was as shown in **Fig 1.** The morphological and biochemical characteristics of anisolate proved it to be *Jathinobacterium*species. Further identification is required by 16 S rRNA gene sequencing.



(A) Nutrient agar plate (B)Rice Agar plate

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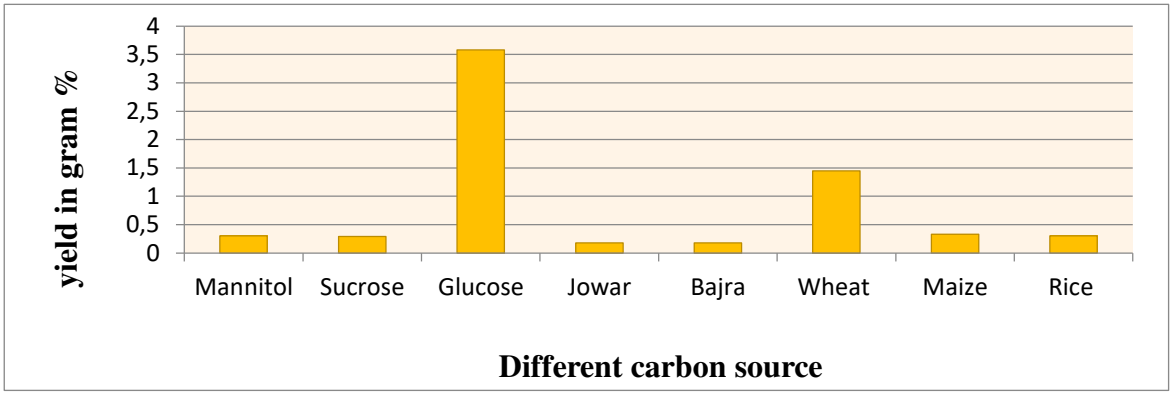
Fig 1: Violet colonies of pigment producers on agar plate.

Optimization of fermentation conditions for production of violacein pigment

Among the pure sugars used in present study, glucose was the best carbon source with yield of 3.58 gm% but among the cereal grains the highest yield was with wheat (1.45 gm%) followed by maize (0.33 gm%), rice (0.302 gm%), jowar and bajra (0.18 gm% each). The yield of violacein pigment by different carbon sources used during study was as depicted in **graph 1.**This may be because glucose is the easily and fast metabolizable sugar.The study of effect of cereal grains on the yieldof pigment revealed wheat as the best natural source for the production of pigment. Similar studies were reported where glucose was found to be the best carbon sources that gave pigment yield of nearly 2300 µg/L**[5]**. The highest yield of violacein pigment produced by *J. lividum* was reportedas approx. 90mg/100ml using nutrient broth supplemented with 1% glucose [**13].**



Graph 1: Effect of carbon source on the yield of violet pigment.

Glucose being the best carbon source among the sources tested in this study, its optimum concentration was determined and results were as shown in **graph 2**. The highest pigment yield was 1.39gm% at 0.5% glucose concentration.There was decrease in the yield with further increase in the glucose concentration. Somewhat similar results were reported by Cortés-Osorio Natalia *et al.*,in 2017. They reportednutrient broth supplemented with 1% glucose as the best carbon source with a yield of approx. 90mg/100ml **[13]**. Vishnu T.S and Palaniswamy M in 2017reported2% glucose as optimum concentration for production of violacein pigment **[5].** This discrepancy in optimum concentration of glucose may be due to variations in the microbial strain used for production.

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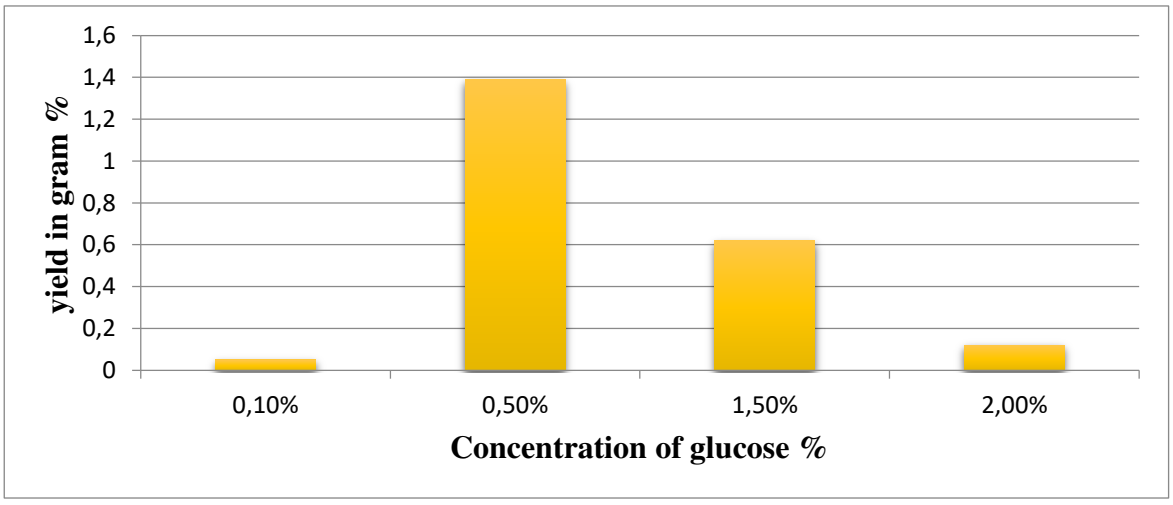
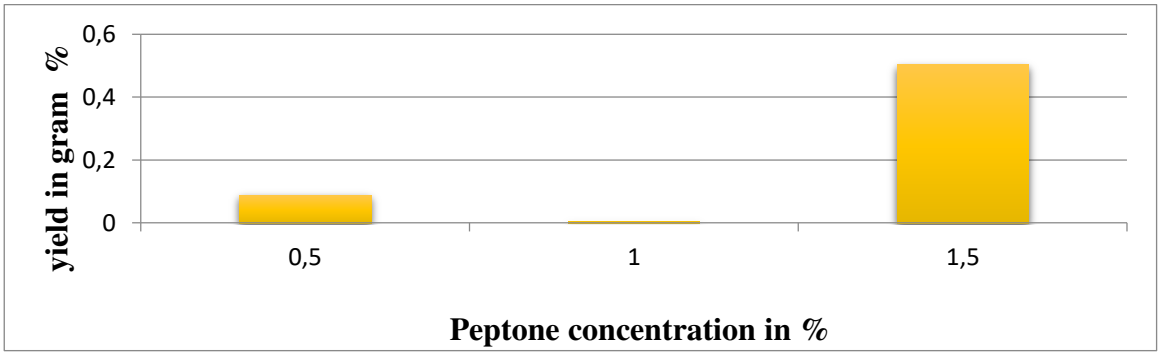


Graph 2: Effect of glucose concentration on the yield of violet pigment.

Jathinobacteium spp under study produced 0.5gm% of violacein pigment when supplemented with 1.5% peptone as the source of nitrogen.The yield of violacein pigment was negligible at concentration less than 1.5% as given in **Graph 3**.

Somewhat similar optimum concentration of peptone was reported by Palanichamy V. in2011wheresodium caseinate and peptone with 1% were the best nitrogen source by *Streptomyces violaceoruber* **[14]**.



Graph 3: Effect of concentration of peptone on the yield of violet pigment.

The pigment production was completely inhibited at pHbelow 7 and above 8.The pH 8 was optimumfor *Jathinobacterium* spp.in the present study (**Graph 4**).At acidic pH, production of violacein completely stopped. The fluctuation in the yield at different pH of the medium may be due to its effect on cell membrane, cell morphology and structure, solubility of salts, ionic state of substrates, uptake of various nutrients and product biosynthesis**[5].**Similar result was reported for violacein production by *Streptomyces violaceoruber*with a pH of 7.6 -8**[14].**

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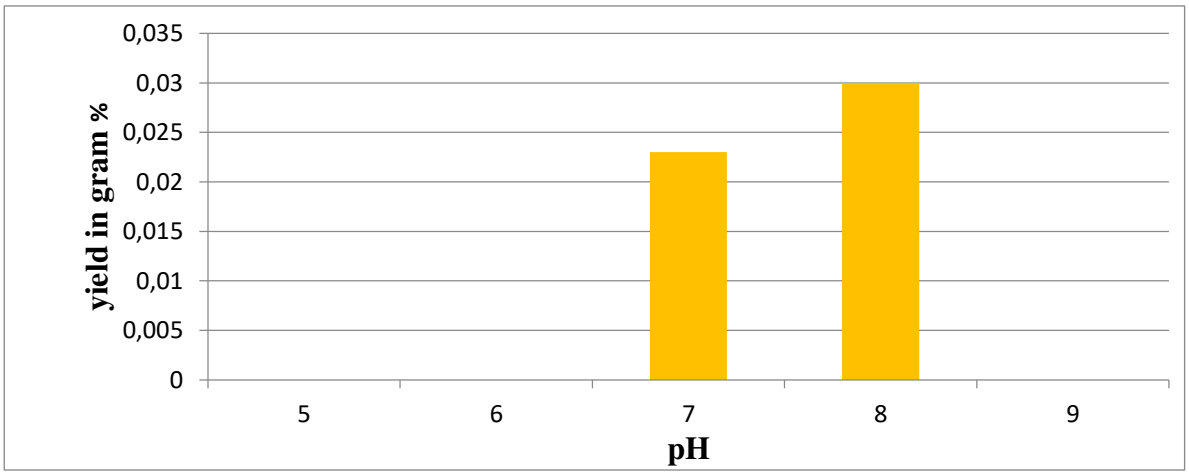
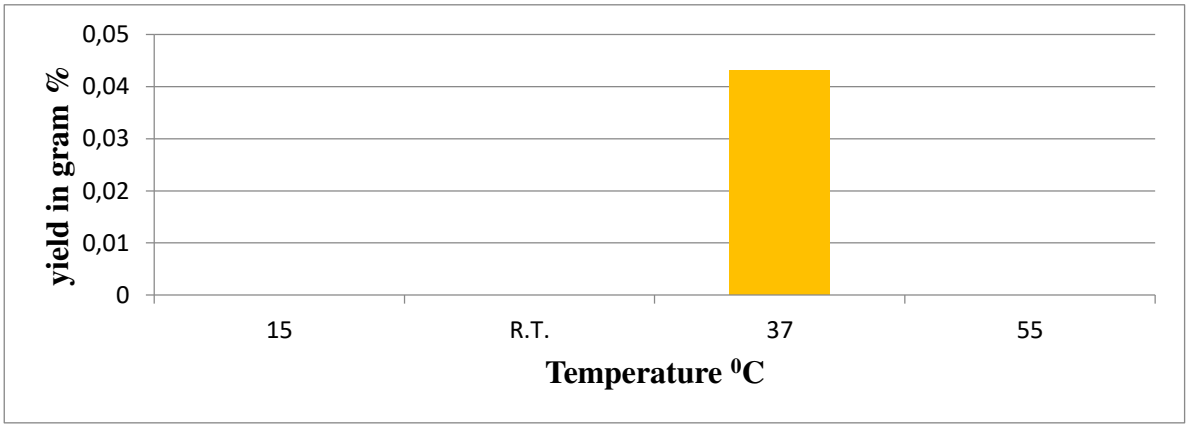


Graph 4: Effect of pH on the yield of violet pigment.

The effect of incubation temperature on the yield of violacein pigment by *Jathinobacterium* sppwas as depicted in **graph 5**. The optimum temperature was 37^0C and the production was totally inhibited above and below that. This may be due to drastic reduction in the rate of metabolic activities at temperature other than optimum. Similar results were reported for *C. vaccinii* CV5 with an optimum temperature of 37°C producting 1358 µg/L of violacein pigment **[5].**



Graph 5: Effect of temperature on the yield of violet pigment.

The violacein production was the best under static condition(**graph 6**)but production of violacein inhibited in presence of excess oxygen. Many researchersalso reported identical results regarding effect of agitation on the yield of pigment. This might possibly because of aggregation was interrupted and aggregates disintegrated at higher agitations because of continuous shearing stress provided by the shaker**[8].**

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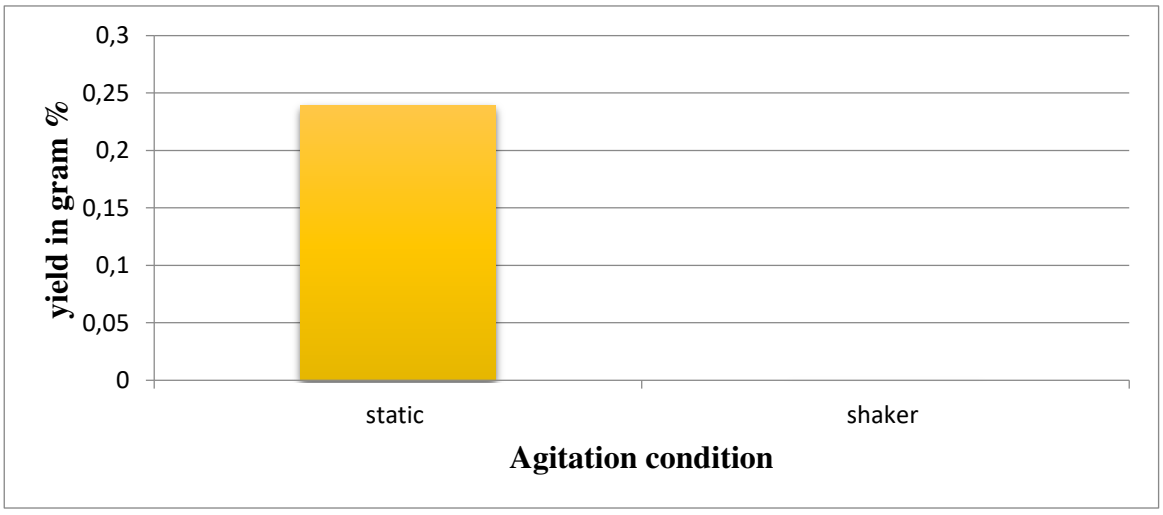
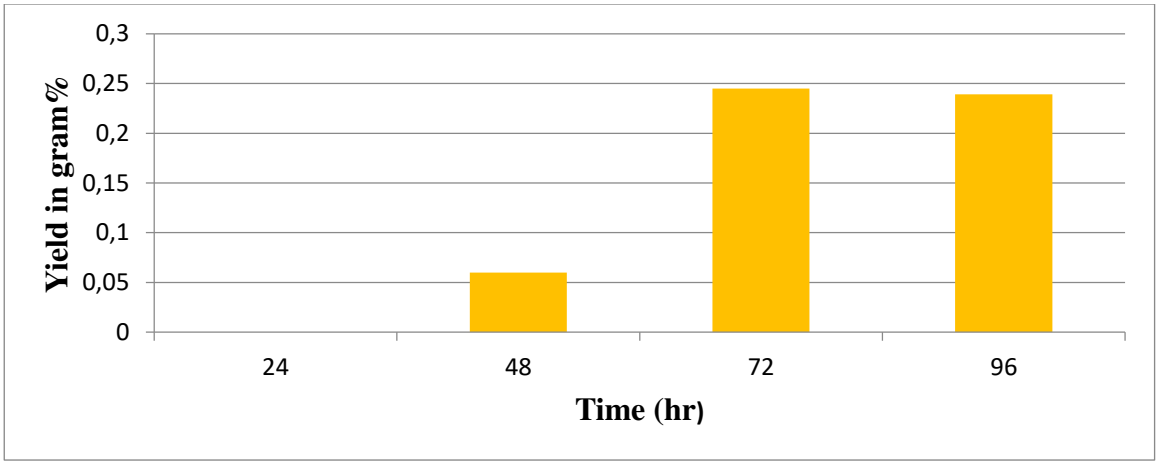


Graph 6: Effect of agitation on the yield of violet pigment.

There was no pigment production up to 24 hr and it increased thereafter but further reduced after 72 hr. The highest yield of violacein (1.42 gm%) was at 72 hr. (**graph7).** Identical resultswere reported by Vishnu T.S and Palaniswamy M in 2017with maximum production of violacein (1752 µg/L) by*C. vaccinii* CV5at 72 hrof incubation with decline in yield thereafter **[5].**



Graph 7: Effect of incubation time on the yield of violet pigment.

CONCLUSION

The violacein pigment producer from soil was *Jathinobacterium* species.For production of natural eco-friendly violaceinpigmentglucose and peptone were the best carbon, energy source and nitrogen source respectively. Wheat was the only better natural carbon and energy source apart from pure sugars. There was absolute inhibition of violacein pigment production at extreme lowand high temperature. Also, acidic pH and excess of oxygen totally inhibited violacein production. Therefore, optimum productionconditions for violacein pigment by *Jathinobacterium* species were 37^0C, pH 8, 72 hr of time period under static condition and the best nutritional requirement was glucose (0.5%) and peptone (1.5%).

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