International Journal of Advanced Biotechnology and Research(IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol5, Issue4, 2014, p619-627
http://www.bipublication.com

Study on Bacterial and Fungal Growth Expending the Supernatant And Pellet Released During The Degradation Of Coir Pith By Cyanobacterium (*Oscillatoria annae*)

Silambuselvi Muthukaruppan[1]., Malini Dhanaraju[2]., Rajakumar Sundaram[2]., and Malliga Perumal[2]*

[1]Dept. of Biotech. and Genetic Engg., Bharathidasan University, Tiruchirappalli -24.
Dept. of Marine Biotechnology, Bharathidasan University, Tiruchirappalli -24.
*Corresponding author: Email: malli62@yahoo.com,
Tel: 914312418591, Mob: 9487549118, Fax: +91431 – 2407084.

[Received 24/08/2014, Accepted-11/10/2014]

ABSTRACT:

The lignocellulosic waste (coir pith) has been discharged during retting process from coconut (*Coccus nucifera* Linn) consists of cellulose, hemicellulose and lignin. Through the action of enzymes, the coir pith was effectively degraded by fresh water cyanobacterium (*Oscillatoria annae*). Degradation of coir pith by cyanobacterium indicated the presence of phenolic compounds acquainted in both supernatant and pellet. In this study, the incurred supernatant and pellet was efficiently utilized for inhibition and induction of bacterial and fungal growth. In addition, more number of peaks was found which could be due to the presence of phenolic compounds in the pellet and supernatant. This was confirmed by HPLC analysis.

Keywords: *Lignocellulosic, coir pith, Oscillatoria annae, bacteria, fungi.*

[I] INTRODUCTION:

Unremarkably, microorganism needs nutritional requirements for their growth, maintenance and reproduction which create the generation of differential and selective media in the microbiological world [1]. The ability of microorganisms to thrive on cellulosic materials was exploited in isolating cellulolytic organisms from wood wastes [2]. The bioconversion of cellulosic materials has been receiving attention in recent years. It is now a subject of intensive research as a contribution to the development of large- scale conversion process [3, 4]. The recycling of the waste material in which it was systematically used and reused to bring about the drastic increase in resource productivity [5, 6]. The waste product of coir yarn industry was coir dust and coir pith or coco peat which constitute about 70% of the husk, which was derived from coconut (*Coccus nucifera* L.). The quantity of coir pith produced was so enormous making its disposal difficult because of its lignocellulosic nature [7]. Lignocellulose, as a chemical complex biopolymer composed of mainly cellulose, hemicellulose and lignin [8]. Biodegradation is increasingly being considered as a less alternative to physical and chemical means of decomposing organic pollutants [9]. Cyanobacteria are capable of abating various organic pollutants [10]. The lignin degrading ability of fresh water cyanobacterium (*O. annae*) by the presence of manganese independent peroxidase, laccase, polyphenol oxidase and other cellulolytic enzymes like endogluconase and xylanase has been reported. Hence, *O.*

annae was selected for this study to degrade coir pith. Degradation of coir pith resulted in release of some organic compounds such as phenol which resulted in change in colour of medium from colourless to brown. The resultant supernatant (cyanospray) brown in colour which has phenolic compounds [11] and the pellet (cyanopith) were used in different concentrations for identifying their effect on bacterial and fungal growth.

[II] MATERIALS AND METHODS

2. 1. Organism and culture conditions

Fresh water cyanobacterium (*O. annae)* was obtained from the germplasm of National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli, Tamil nadu, India. The culture was maintained in BG-11 medium at 1500 lux at 25±2 with 30 days light / dark (10/14 hrs.) cycle.

2. 2. Coir pith

Coir pith was collected from coir industry, near Srirangam, Tiruchirappalli, Tamil nadu, India.

2. 3. Medium and growth conditions

2. 3. 1. Incubation

In a series of 250 ml conical flasks, 100 ml of sterile BG11 medium was poured and 1 g of cyanobacterial culture (*O. annae*) was inoculated, which was considered as positive control and to appropriate flask 1g of lignocellulosic material coir pith was added as negative control and medium was inoculated with the cyanobacterial culture and coir pith in ratio 1:10, which was considered as test**.** After 30 days of incubation, supernatant (cyanospray) and pellet (cyanopith) were filtered, dried and utilized for HPLC analysis**.**

2. 3. 2. Separation of compounds using HPLC

The HPLC analysis (waters model no.2690, USA) of dried material was subjected to HPLC (High Performance Liquid Chromatography) was carried out with C_{18} column (symmetry, 4.6 × 250nm) to find out the compounds present in it. The coir pith (CP), cyanobacterium (CB) (*O. annae*) and the combination of CP with CB were allowed to dry in sun shade after incubation of 30 days. Then the dried pellets (1:10) were grinded with HPLC grade methanol. Then each sample was centrifuged separately and 10 ml of supernatant was extracted with equal volume of methanol. Then the compounds were isolated using HPLC at 25˚C, Eluent- acetonitrile: water (80:20 v/v) flow rate was 20µL for10min[12].

2. 4. Microbial culture

Bacteria and fungi were isolated by serial dilution method from soil and used as stock culture.

2. 5. Determination of bacterial growth

Different types of culture filtrates such as CP - coir pith, CB - Cyanobacterium (*Oscillatoria annae),* and degradative product of coir pith by CB (cyanospray and cyanopith) were filtered on 30^{th} day and were used for bacterial and fungal growth determinations and NB served as control.

2. 6. Determination of bacterial and fungal growth by cyanospray crystals

2. 6. 1. Bacterial and fungal growth in broth

From the dried crystals of cyanospray (cys), 1 - 5% was prepared and bacterial, fungal cultures were inoculated separately and incubated for 24 and 48 hrs. and the optical density (OD) was measured at 625nm and 530nm and compared with control.

2. 6. 1. Bacterial and fungal growth in solid media

Nutrient agar (NA), Rose Bengal Agar (RBA) and agar agar along with cys (1 – 5%) was prepared and bacterial, fungal cultures were inoculated separately in plates and incubated for 24 and 48 hrs. and the growth was compared with controls.

2. 7. Determination of bacterial growth by cyanopith (cyp)

Extraction was taken from cyp and different dilutions (10 – 100%) were prepared and used for bacterial and fungal growth in liquid and solid media as mentioned above. (2. 6 – 2. 6. 1).

3. RESULTS

3. 1. Isolation of compounds from degraded coir pith

HPLC (High Performance Liquid Chromatography) depicts the presence of

compounds (two) in coir pith, CB – (six) and degraded coir pith by *O. annae* (ten) (Fig: 1 - 3).

3. 2. Bacterial and fungal growth determination in pellet and supernatant

Bacterial growth in lower concentrations (0.5%) in cyanopith showed higher optical density and it's similar to Nutrient Broth (NB). Bacterial growth was considerably reduced in cyanospray when compared to cyanopith and nutrient broth control. Complete inhibition was observed in cyanobacterial and coir pith filtrate (Fig - 4). Bacterial growth in NB mixed with cyanospray was found to be reduced from lower to higher concentrations and completely inhibited at higher concentrations (4 and 5%) when compared to control. Whereas, surprisingly the growth was completely inhibited at all the concentrations alone with cyanospray treatment (Fig - 5). Fungal growth in Dextrose Broth (DB) with cyanospray showed moderate growth in lowest concentrations and thereafter gradual decrease occurs with increased concentrations. Complete inhibition was observed in cyanospray treatment alone at all the concentrations (Fig - 6). Bacterial growth in combination of nutrient broth with cyanopith filtrate evidenced gradual increase from lower concentrations (10%) to higher concentrations (50 and 60%) and declined phase was ascertained from 70 – 100% concentrations. However, bacterial growth was also completely inhibited in all the concentrations of cyanopith filtrate (Fig - 7). In the treatment of DB with cyanopith filtrate, fungal growth was found to be gradually increased from lower to higher concentrations and showed gradual reduction with increasing concentrations, on the other hand complete inhibition with cyanopith treatment alone was testified. Nevertheless, growth in combined treatment was not similar to control (Fig - 8). Maximum growth was observed in nutrient broth with cyanospray at lowest concentrations (1%) which was similar to control but slightly reduced growth was observed in other concentrations (2 and 3%) and complete inhibition was observed in higher concentrations (4 and 5%). There was no growth found in agar agar with cyanospray treatment (Fig - 9). Normal fungal growth was obtained in Rose Bengal Agar (RBA) plates at the same time slight inhibition of fungal colonies were observed in RBA with cyanospray treated plates when compared to control. Indeed, no growth was obtained in the fungal colonies inoculated with cyanospray alone (Fig – 10). Moderate bacterial growth level was established at lower concentration (10%) and there was gradual decrease while increasing the concentration till 100%. In cyanopith mixed with agar agar, no growth was observed (Fig - 11). Identical results were obtained with fungal plates treated with cyanopith (Fig - 12).

[IV] DISCUSSION

Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial [13], antifungal [14]. Many investigations mentioned that methanol extracts of cyanobacterium - *Nostoc muscorum* revealed antibacterial activity on *Sclerotinia sclerotiorum* stated by [15]. Also, the methanolic extract of blue green algae has been investigated by [16] for in vitro antimicrobial activity against *Proteus vulgaris, Bacillus cereus.* Antimicrobial activity of cyanobacteria refers almost to filamentous strains belonging to a wide range of genera [17 - 21]. Most anti-microbial active components that have been identified are not water soluble and organic solvents extracts have been found to more potent [22]. Extracts of cyanobacterium *Spirulina platensis* obtained by different solvents exhibited different degrees of antimicrobial activity on both gram positive and gram negative organisms [23]. The recent investigations with cyanobacteria have demonstrated the antimicrobial effects of *Oscillatoria* sp. [24]. Fractionated material of the culture filtrate exhibited pronounced antifungal activity because of the presence of phenolics specially chlorogenic, ferulic, caffeic, coumaric and protocatechuic acids which are reported to be antifungal [25]. [26] and [27] stated that the result of cultural studies in which the effects of micafungin on the growth and

viability of *C. albicans* in osmotic stabilizer-supplemented medium were compared with those in supplemented medium showed that in the conventional isotonic medium micafungin exhibits a fungicidal action. Antimicrobial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in the leakage of the cytoplasm of the organism and its coagulation [28]. The fresh water cyanobacterium used in this study possessed twofold capacity of about antimicrobial activity and degradation of lignocellulosic material – coir pith. The resultant degraded materials like pellet (cyp) and supernatant (cys) when used in lower concentrations showed induction of bacterial and fungal growth but the growth was inhibited when it was used in higher concentrations while mixed with other media, but complete inhibition was observed in cyanopith and cyanospray treatment alone. The study also provided the HPLC analysis which was found to be exhibited more number of compounds (nine) suggesting the antimicrobial activity on bacteria and fungi. HPLC determination indicates that the observed biological activity of the treated samples parallel the concentration [29]. [30] stated that HPLC analysis of non- volatile species allow us to establish definitive structures. Several authors have analyzed polyphenolic compounds in propolis of diverse origins using different extraction systems [31 - 35]. Degradation of curcumin under various conditions such as alkaline or photoradiated conditions was examined for compounds in HPLC analysis [36 - 38]. Further investigation of compounds will be conducted in future studies.

[V] CONCLUSION

Fresh water cyanobacterium (*O. annae*) has the ability to degrade the coir pith and the resultant pellet and supernatant in different concentrations showed different activities. Further, the resultant degraded materials like pellet (cyp) and supernatant (cys) when used in lower concentrations showed induction of bacterial and fungal growth but the growth was inhibited when it was used in higher concentrations while mixed with other media, but complete inhibition was observed in cyanopith and cyanospray treatment alone.

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Oscillatoria annae BDU 6 and its lignolytic enzyme activity in response to coir pith degradation. *Int. J. Curr. Sci*, 78-86.

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Fig: 1. Coir pith

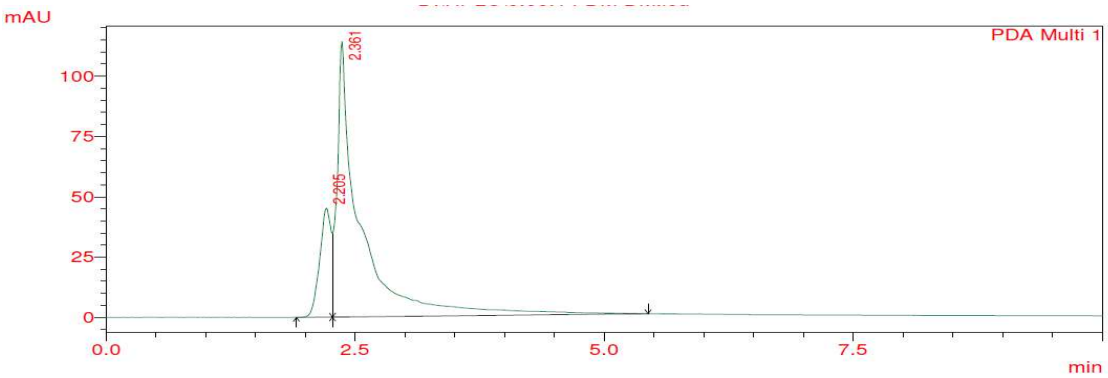


Fig: 2. Cyanobacterium (*Oscillatoria annae*)

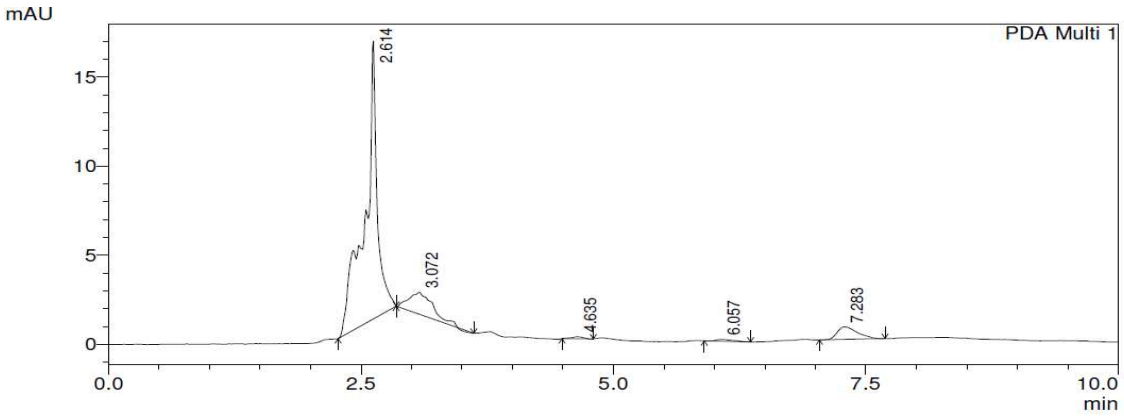
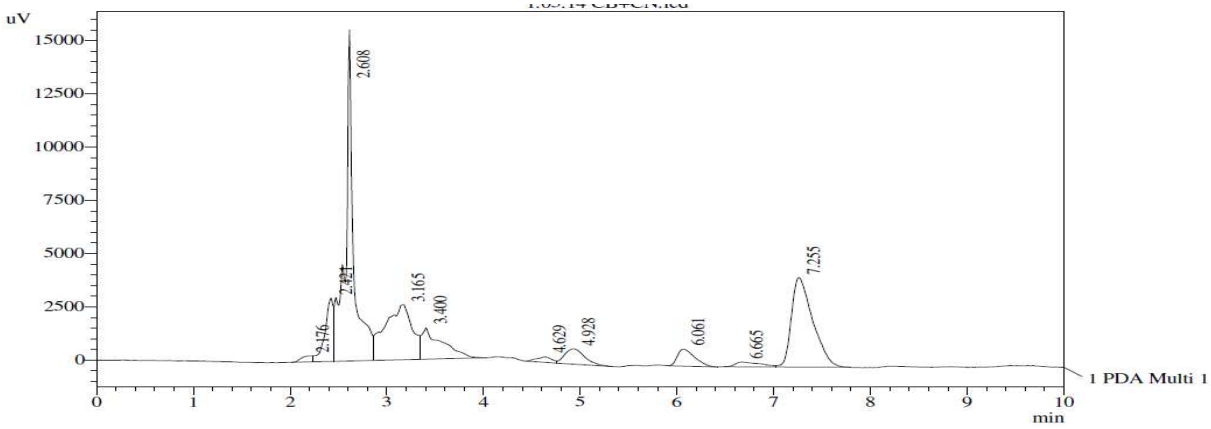


Fig: 3. Cyanobacterium + coir pith



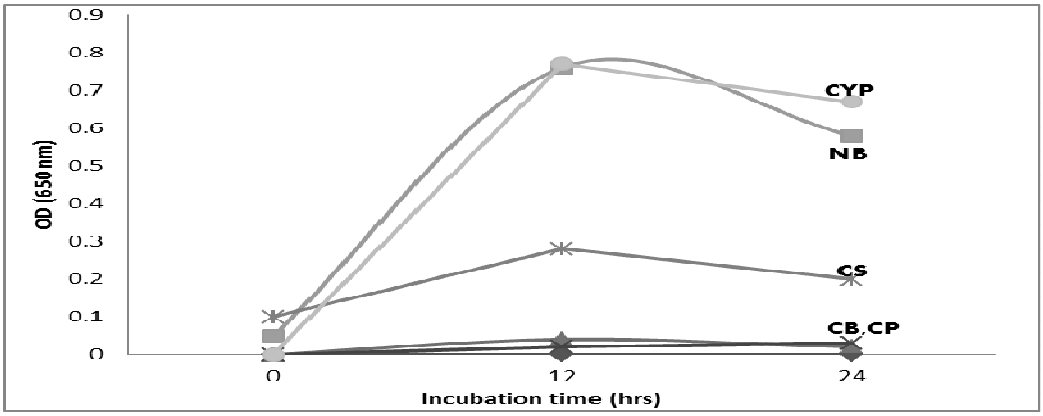


Fig: 4. Effect of different culture filtrates (30th day) on the growth of bacteria**:**
CP- Coir pith;CB-Cyanobacterium (*Oscillatoria annae*); N.B- Nutrient Broth, CS- Cyanospray (0.5%); CYP- Cyanopith (0.5%)

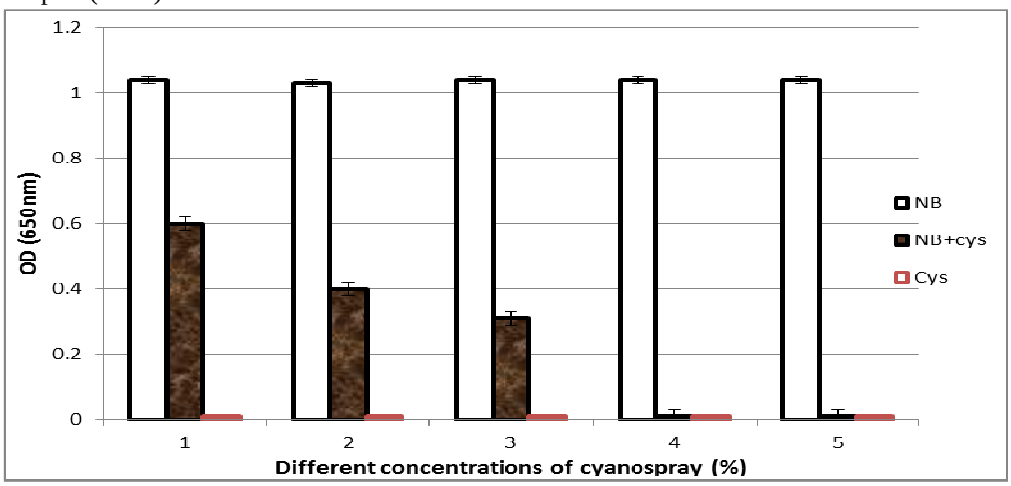


Fig: 5. Effect of cyanospray (1-5%) on bacterial growth in broth:
NB- Nutrient Broth; Cys–Cyanospray

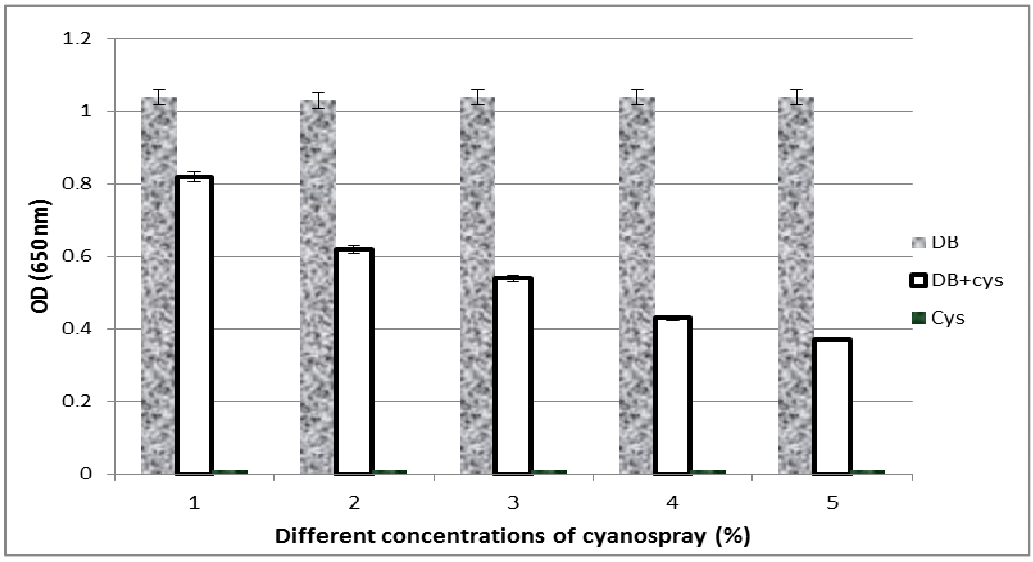


Fig: 6. Effect of cyanospray (1-5%) on fungal growth in broth: DB- Dextrose Broth; Cys–Cyanospray

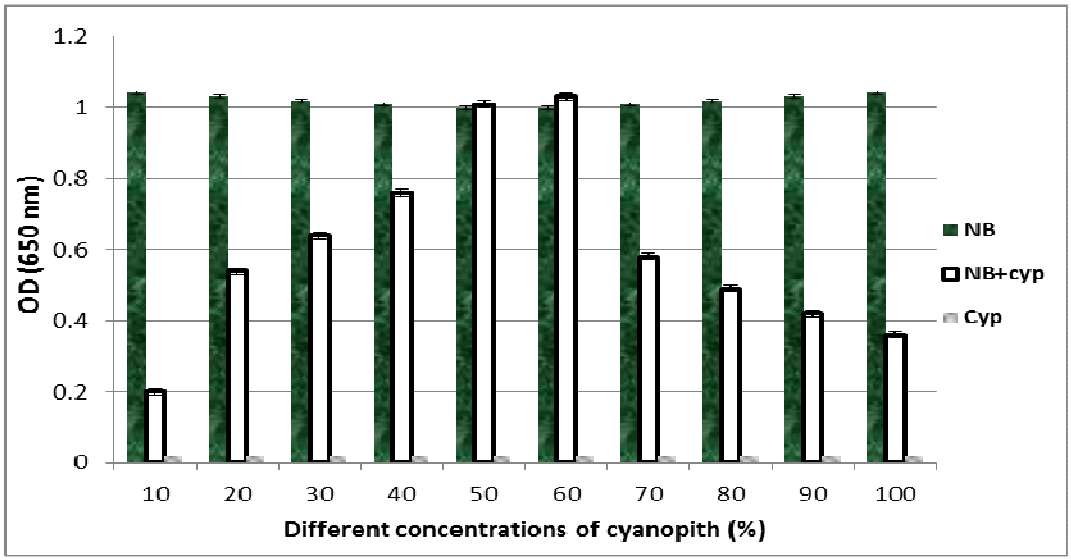


Fig: 7. Effect of cyanopith (10-100%) on bacterial growth in broth:
NB- Nutrient Broth; Cyp –Cyanopith

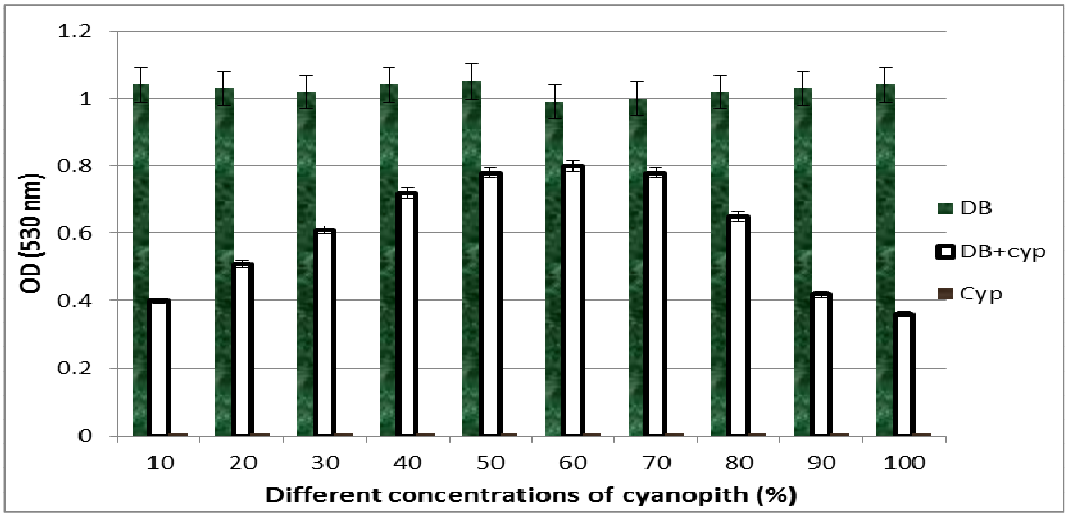


Fig: 8. Effect of cyanopith (10-100%) on fungal growth in broth:
DB- Dextrose Broth; Cyp –Cyanopith.

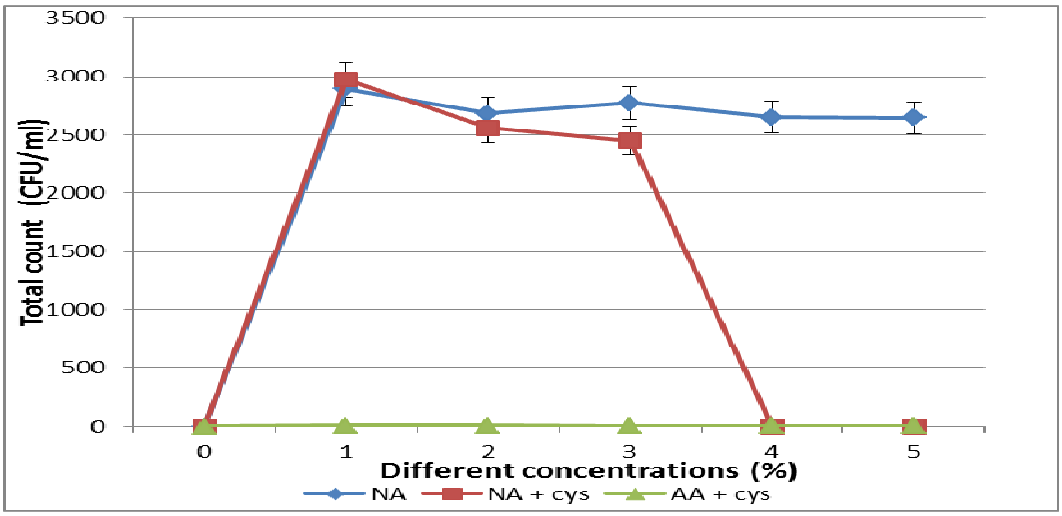


Fig: 9. Effect of cyanospray (1-5%) on bacterial growth in solid media:
NA- Nutrient Agar; AA- Agar agar; Cys- Cyanospray; CFU- Colony Forming Units.

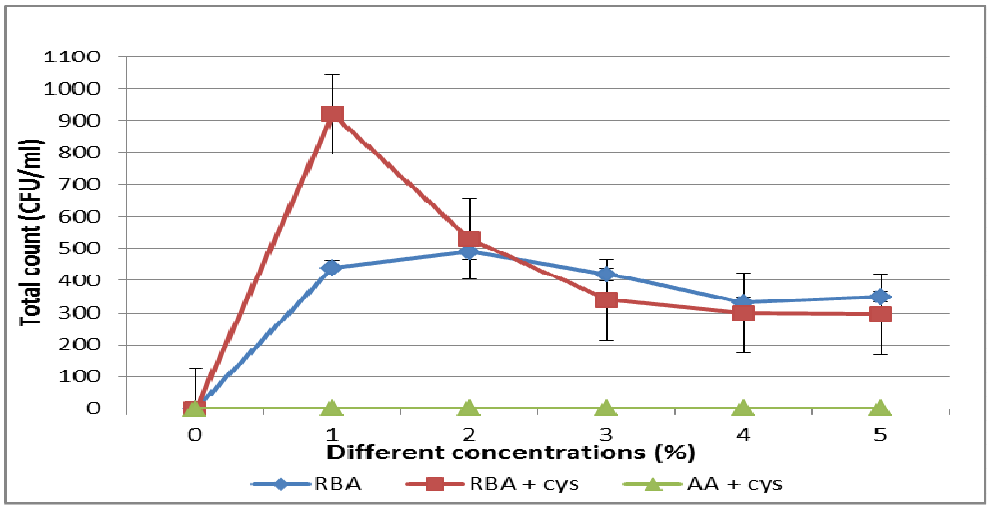


Fig: 10. Effect of cyanospray (1-5%) on fungal growth in solid media:
RBA – Rose Bengal agar; Cys - Cyanospray; AA- Agar agar; CFU- Colony Forming Units.

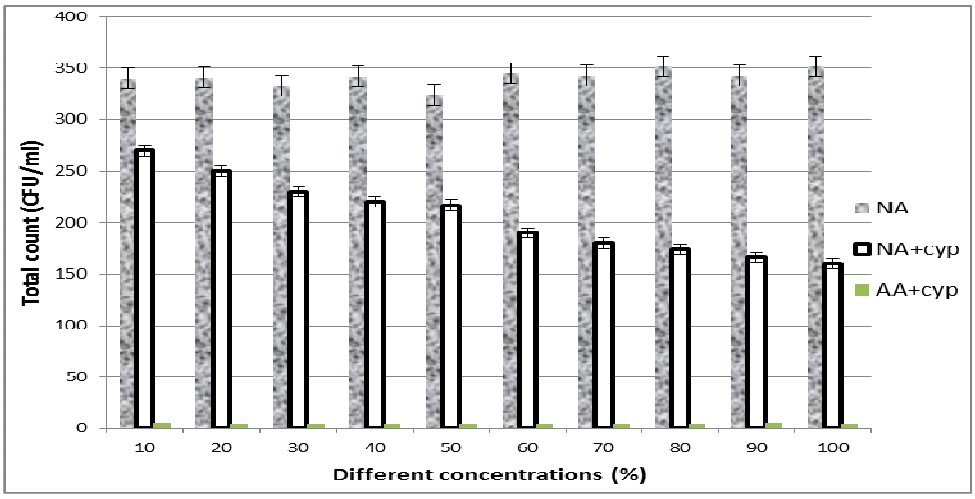


Fig: 11. Effect of cyanopith (10-100%) on bacterial growth in solid media:
NA- Nutrient Agar; Cyp- Cyanopith; AA- Agar agar; CFU- Colony Forming Units.

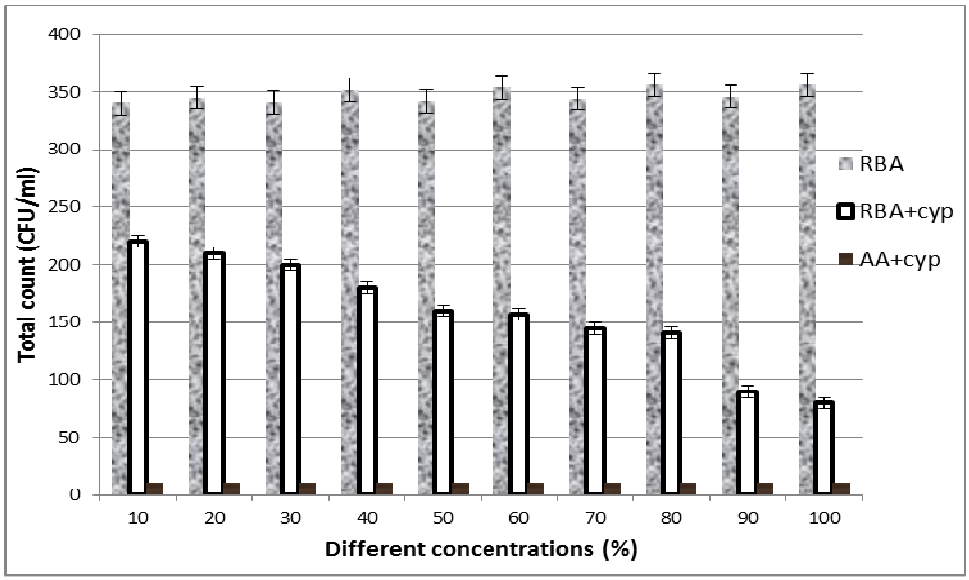


Fig: 12. Effect of cyanopith (10-100%) on fungal growth in solid media:
RBA- Rose Bengal agar; Cyp –Cyanopith; AA- Agar agar; CFU- Colony Forming Units.